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Genetic Mechanisms of RBM20-Controlled Titin Splicing in Familial Dilated Cardiomyopathy

The Interplay Between EMT and Cancer Stem Cells in Tumor Expansion and Promising Antitumor Strategies

TikTok's algorithm: Does customer personalization come at the expense of magnifying filter bubbles, echo chambers and how may it foster ethnocentrism?

Raman Spectroscopy: Basics, Physics and Cancer detection analysis

Stem Cells in the Regeneration of Major Visceral Organs: Reduction in Xenotransplantation-associated Medical and Ethical Complications

Automated system to detect and solve water parameters issues

Editors Piece

This year, the journal has had a transformational makeover. We have exceeded records. We have expanded our team by 143 members. It is through the generosity of their efforts that we can celebrate such feats.

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Cayman Osei-Bonsu
EDITOR IN CHIEF 2023-2024

Young Scientists Journal

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Young Scientists Journal (YSJ) is a peer-reviewed scientific journal written and produced by 12-20 year olds across the world with an interest in science, technology, engineering, and mathematics (STEM).

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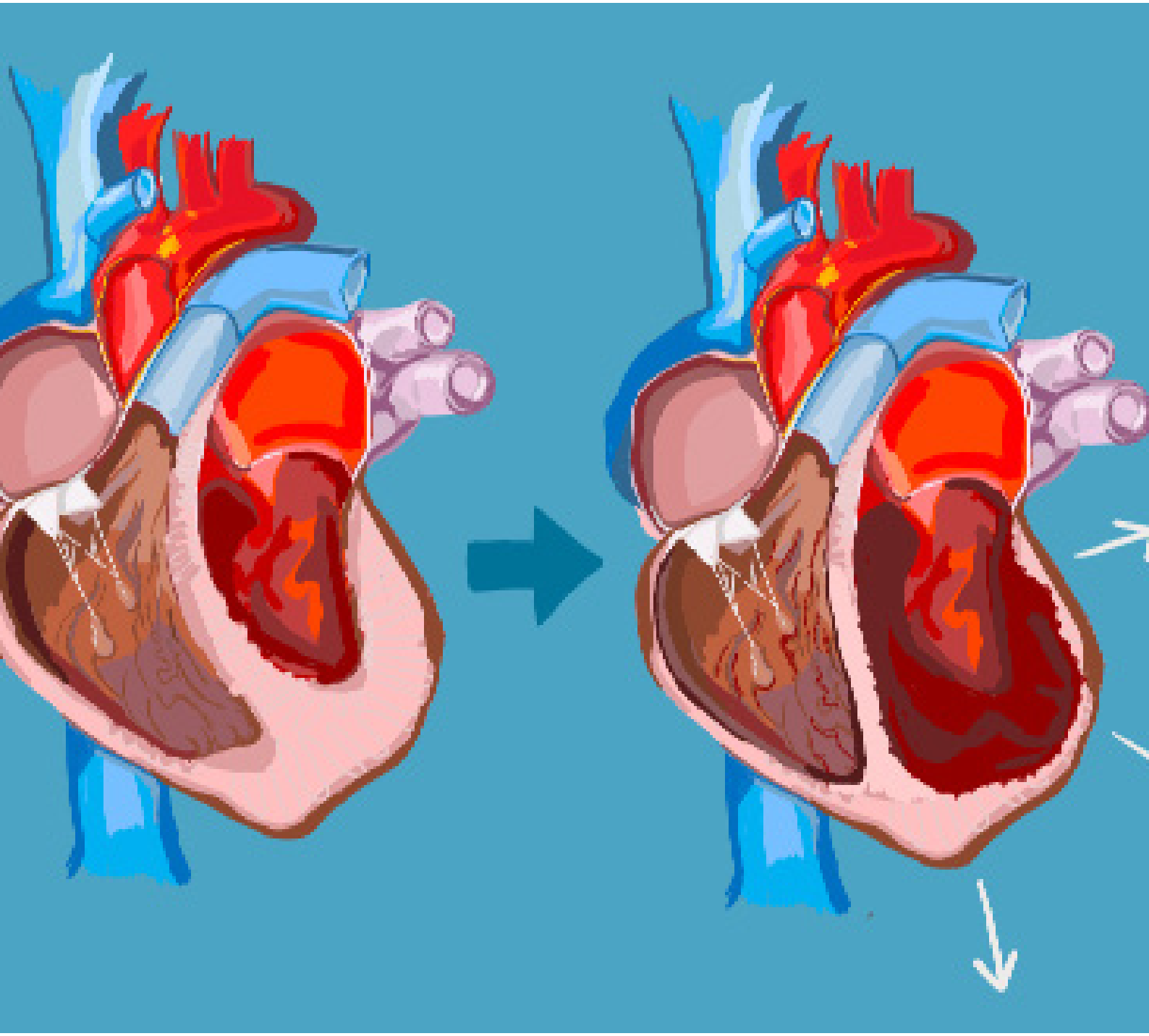


Abstract

The RBM20 gene encodes RNA-Binding Motif Protein 20 (RBM20), an alternative splicing factor that targets multiple genes and proteins involved in cardiac development, such as the sarcomeric protein titin (TTN). Nearly 40 RBM20 gene mutation variants have been reported in the past 10 years, and heterozygous missense mutations in the RBM20 gene controlling TTN splicing, which disrupts protein production, are among the most common genetic causes of dilated cardiomyopathy (DCM), seen in up to 25% of DCM patients. As TTN is a giant sarcomeric protein, RBM20-controlled TTN splicing may result in transcription errors linked to sarcomere assembly and diastolic function, thereby rendering a high arrhythmia burden that includes atrial and ventricular tachyarrhythmias in RBM20 and DCM phenotype carrier patients. DCM is the most common form of cardiomyopathy and the second most frequent cause of heart failure. It is one of the leading causes of death worldwide, with a 30-50% chance of being passed down to the next generation. Therefore, this article will present an in-depth review addressing the genetic mechanisms of RBM20-controlled TTN splicing, and the crucial role of genetic testing in the early diagnosis, evaluation, and possible treatment of DCM to prevent death from sudden cardiac arrest.

Dilated Cardiomyopathy

Dilated cardiomyopathy (DCM) is the most frequent form of cardiomyopathy (CM), characterized by left ventricular or biventricular dilatation and systolic dysfunction in the absence of coronary artery disease pressure or volume overload.[1] As a result of a stretched or 'baggy' left ventricle, the cardiac muscle weakens and may thin or flop, and is therefore unable to pump blood throughout the body efficiently (Figure 1). DCM accounts for 30-40% of all CM cases with high rates of morbidity and mortality; it is the second most common etiology of heart failure after ischemic cardiomyopathy (ICM).[2] Consequently, DCM is one of the leading causes of heart transplantation (HT) and thereby, death, worldwide.[3, 4] However, due to the limited availability of donor organs and complicated clinical course management, only a number of DCM patients are listed for HT as a last resort. In the United States, 26% of patients listed for HT in the United Network Sharing registry were diagnosed with DCM.[5] Out of those who were listed, nearly 5% of DCM patients died while waiting for HT, 3% were delisted due to deterioration, and <70% were transplanted. [6] The life expectancy of DCM patients is limited and varies according to other underlying medical conditions, but the median survival time is only five years after being diagnosed.[7]

The global prevalence of DCM was estimated to be 1:2500 in 1989, [8] but recent studies show that it now ranges between 1:250 to 1:500 among the general population.[9, 10] DCM is also known to be three times more frequent in males than females, and only 0.057% prevalent in the paediatric population.[11, 12] Additionally, DCM clinically presents itself in a highly gender-specific manner as male patients have been reported to suffer from an earlier disease onset along with a more severe disease progression.[13, 14] It is important to note, however, that these numbers may be underestimated due to geographic and ethnic variations, patient selection, and changes in diagnostic criteria. Diagnostic criteria, according to the guidelines for DCM from the British Society of Echocardiography, include the presence of 1) <25% of fractional shortening or 2) <45% of ejection fraction and 3) >117% of left ventricle end-diastolic diameter shown through Cardiac Magnetic Resonance (CMR) testing results.[14] CMR also helps distinguish primary from secondary forms of DCM as it allows an integral evaluation of the cardiac biventricular geometry, volumes, mass, function, tissue characterization (myocardial fat and edema), as well as the identification and quantification of focal or diffuse fibrosis.[15]

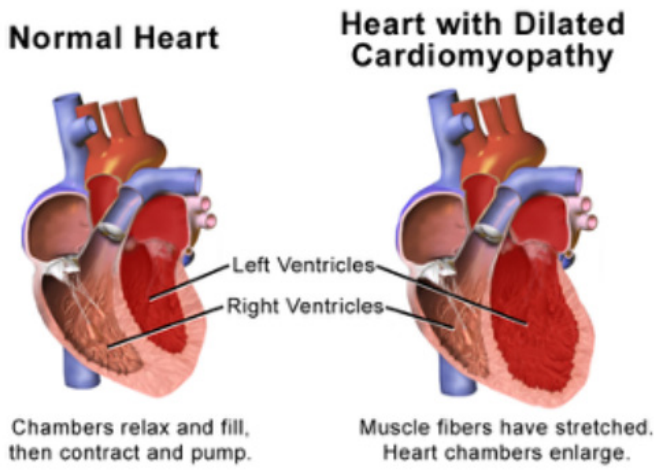


Figure 1. Illustration and comparison of a normal heart and a heart with dilated cardiomyopathy. The left ventricle in the heart with dilated cardiomyopathy appears dilated with stretched muscle fibers. [16]

1.1. Familial Dilated Cardiomyopathy

According to The European Society of Cardiology, DCM can be identified as either familial (genetic) or nonfamilial (non-genetic). Familial DCMs (FDCM) account for 30-50% of all DCM cases with 40% reported having identifiable genetic abnormalities.[17] According to the UCLA Cardiovascular Genetics Clinic, approximately 80-90% of FDCM cases were inherited in a Mendelian autosomal dominant pattern while the remaining percentage was composed of an autosomal recessive or X-linked transmission.[18]

FDCM is associated with early-onset, end-stage heart failure, and an increased risk of sudden death by cardiac arrest due to molecular defects caused by gene mutations that pattern maladaptive genetic and cellular mechanisms. [19] Over the past decade, more than 100 genes have been identified as DCM-causative mutations in the Human Gene Mutation Database and the Online Mendelian Inheritance in Man Database (Figure 2).[20] Core genes associated with the arrhythmogenic DCM type include MHY7, TNNT2, TPM1, LMNA, and RBM20.[21] The first pathogenic variant in the RBM20 gene which is now known to lead to the mis-splicing of genes such as TTN, LDB3, CAMK2D, and RYR2 was reported in 2009.[22, 23] Now, truncating variants in the titin gene (TTNtv) have been revealed to be the most common DCM-causing mutation. TTNtv have been found in an estimated 20-25% of DCM patients,[24-26] and sudden cardiac death was reported in >50% of RBM20 gene mutation-carrying patients.[27]

References	Domain	Mutation	Origin	Type	Effect on RBM20	Splicing regulation[a]	AF	VA	HF or SD
Brauch et al., 2009	RS-rich	R634Q	Familial	Hetero	Unknown	Defective	0/10	1/10	0/10
		R636S	Familial	Hetero	Unknown	Defective	1/13	0/13	2/13
		R636H	Familial	Hetero	Unknown	Unknown	0/2	0/2	0/2
		S637G	Familial	Hetero	Unknown	Defective	0/4	1/4	1/4
		P638L	Familial	Hetero	Unknown	Defective	2/16	6/16	5/16
Li et al., 2010	RRM	V535I	Sporadic[b]	Hetero	Unknown	Unaffected	1/1	0/1	1/1
	RS-rich	R634Q	Sporadic	Hetero	Unknown	Defective	0/1	0/1	1/1
		R634W	Familial	Hetero	Exclusion from nucleus[c]	Defective	0/1	0/1	0/1
		R636C	Familial	Hetero	Unknown	Unknown	1/2	1/2	1/2
		R636H	Familial	Hetero	Unknown	Unknown	0/3	1/3	1/3

Figure 2. RBM20 mutants identified in DCM patients. [28]

1.1.1. Recent FDMC Case Reports

1.1.1.1. Patient AAM19

Mr. A is a 19-year-old adopted African American male with underlying medical conditions such as diabetes mellitus type 2 and morbid obesity who was admitted to the MC cardiac intensive care unit (ICU) due to presentations of cardiogenic shock and acute respiratory failure requiring intubation. Mr. A developed recurrent atrial arrhythmias and eventually underwent atrial flutter ablation.[29]

Transthoracic echocardiogram (TEC) revealed a severely enlarged left ventricle and left ventricular dilatation with a biplane ejection fraction of 20% and severe global hypokinesis. Mild right ventricular chamber enlargement with mildly decreased systolic function was also noted. Cardiac magnetic resonance (CMR) imaging demonstrated a severely enlarged left ventricular chamber size with a left ventricular ejection fraction of 32% and global hypokinesis. [29]

Genetic testing was also conducted due to the severe degree of systolic dysfunction despite an extremely young age. Sequencing through a cardiomyopathy-specific gene panel, which tests for variants within a total of 67 genes, demonstrated heterozygosity for a rare and likely pathogenic variant involving the RBM20 gene.[29] Therefore, doctors from the Department of Cardiovascular and General Internal Medicine at Mayo Clinic, Rochester, USA, diagnosed Mr. A with DCM.

1.1.1.2. Patient I19M

Anon is a 19-year-old Indian male with a history of coeliac disease (CD) who was admitted to AIIMS ICU 12 months after being diagnosed with DCM. His blood pressure was 94/52 mmHg with a heart rate of 73 beats per minute during his admission, indicating systolic dysfunction. Echocardiographic screening showed that anon suffered from a severe left ventricular systolic dysfunction with an ejection fraction of 14% along with a left bundle branch block.[30]

Genetic testing was also conducted using next-generation sequencing which showed a heterozygous missense variant in the RBM20 gene. Detailed family history revealed that one of his cousins had also been diagnosed with DCM and died from sudden cardiac death at the age of 26 years and that his 60-year-old aunt was also affected by DCM. Meanwhile, the 50-year-old father of his deceased cousin and his 26-year-old sister also showed clinical features of DCM through echocardiography screening but were both asymptomatic.[29] Anon was readmitted after a month and listed for heart transplantation due to indications of heart failure but had a sudden cardiac death after 3 months.

2. RBM20 Structure, Function, and Localisation

The RBM20 gene is located on the long arm of chromosome 10 and has 14 exons. It is highly expressed as a splicing regulator in cardiac and skeletal muscle during the 11th to 20th week of human fetal development.[31] The RBM20 protein contains 1227 amino acids and is composed of two zinc finger (ZnF) domains, an RNA recognition motif (RRM), a serine- and arginine-rich region (RS region), a leucine-rich region, and a glutamate-rich region.[28]

RBM20 is predominantly located in the nucleus. Its nuclear localization is regulated through phosphorylation of the RS region.[32] Mutation of phosphorylation sites in the RS region leads to translocation out of the nucleus, thereby negating the function of RBM20 (Figure 3).[32] This region also mediates protein-to-protein interactions with other splicing factors such as U2AFG5 and U2AF35, functioning as a domain that plays the most critical role in splicing activities. Consequently, the most frequent disease-causing RBM20 mutations occur in the RS region.[33]

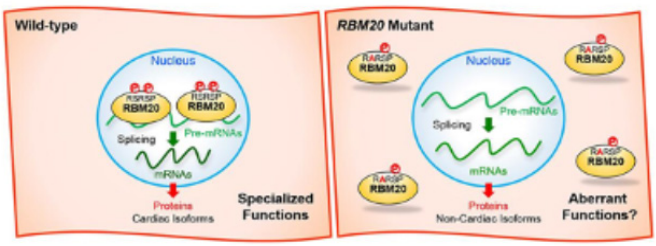


Figure 3. (Left) Wild-type RBM20 capable of regulating pre-mRNA splicing of its target genes to produce cardiac isoforms of mRNAs which will then be translated into cardiac protein isoforms with specialized functions. (Right) Missense mutation with a substitution in the RSRSP stretch disrupts the normal functions of RBM20 as the mutant RBM20 proteins no longer get imported into the nucleus. Consequently, pre-mRNAs of the RBM20-target genes are processed into non-cardiac isoforms of mRNA which will be translated into non-cardiac protein isoforms but most likely lack the specialized functions and/or exert aberrant functions. Moreover, the mutant RBM20 proteins retained in the cytoplasm may also exert aberrant functions. P: phosphorylation.[28]

RBM20 is a splicing factor targeting multiple cardiac genes involved in cardiac development including the sarcomeric protein titin (TTN).[34] Loss of function in pathogenic RBM20 gene variants leads to the mis-splicing of TTN genes in both human and murine models. [35, 36] The RBM20 gene specifically controls post-transcriptional splicing of sarcomeric and calcium handling genes in the cardiomyocyte.[37] Based on data retrieved from isolated cardiomyocytes in murine models carrying pathogenic RBM20 variants, mice have developed both dilated cardiomyopathy and ventricular arrhythmia as well as pro-arrhythmic calcium release from the sarcoplasmic reticulum.[27]

Pathogenic RBM20-controlled TTN splicing is a single nucleotide polymorphism that replaces a cytosine nucleotide with a thymine nucleotide, resulting in a premature translational stop codon and thereby, absent or disrupted protein production.[29]Intriguingly, close to 40 RBM20 gene mutation variants have been reported in the past 12 years, and heterozygous missense mutations in the RBM20 gene involved in TTN splicing have been identified as one of the most common genetic causes of DCM, seen in up to 25% of DCM patients.[28] These mutations are enriched in a hot-spot composed of an arginine-serine-arginine-serine-proline (RSRSP) stretch at p.634 to p.638 amino acids, which contain crucial amino acids for the protein structure and function (Figure 4).[28]

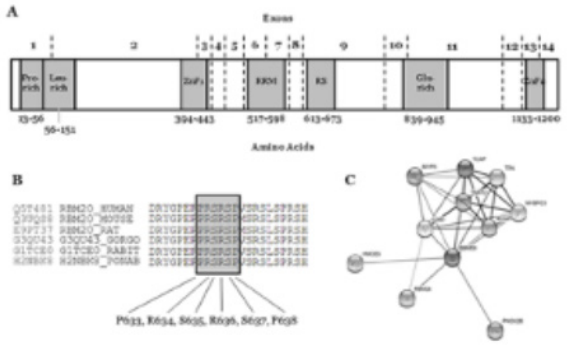


Figure 4. Structure and network of the RBM20 protein.[10] (A) Glu-rich: Glutamate rich region; Leu-rich: Leucine rich region; Pro-rich: Proline rich region; RRM: RNA Recognition Motif; RS: Arginine-Serine Domain; ZnF1: Zinc Finger region 1; ZnF2: Zinc Finger region 2. (B) Conservation between species of RS region (amino acids 634-638) (C) Network of the ten closest proteins to RBM20.

3. Titin Protein

TTN is an essential component of the sarcomere, the basic unit that facilitates striated muscle contractions. TTN is a giant filament protein with a molecular weight ranging between 2,900 to 3,800 kDa. This protein acts as a molecular spring in the sarcomere with scaffold and signaling functions which define the passive stiffness as well as diastolic function of the cardiomyocyte.[38, 39] As a giant sarcomeric protein, TTN is composed of four structural subunits which span half of the sarcomere, connecting the Z-disk to the M-line (Figure 5).[40] The Z-line is responsible for embedding and anchoring TTN to the Z-disk. The I-band is composed of repetitive immunoglobulin (Ig) regions in charge of providing the extensible "spring-like" function of the TTN due to its ability to extend when mechanical force is applied. The A-band consists of Ig regions alternating with fibronectin, serving as a stable anchor for myosin binding during muscle contraction due to its characteristic as a non-extensible and rigid region. Lastly, the M-band contains serine or threonine kinase domains which form a scaffold with myomesin to link myosin to thick filaments found at the M-line of the sarcomere. Aside from its structural role, TTN is also essential for sarcomere formation, mechanosensing, and signal transduction.[41]

TTN is comprised of 364 exons which produce an estimated

27,000 to 33,000 amino acids, the largest number of exons in a single human gene. Alternative splicing of the TTN gene by RBM20 represses over 160 consecutive exons.[41] Specific exons such as exons 9 and 10, are inextricably linked to sarcomere assembly, ion transport, and diastolic function, along with intracellular calcium handling and expression of calcium rendering,[42, 43] thereby rendering high arrhythmia burden that includes atrial and ventricular tachyarrhythmias in RBM20 carrier patients.[44]

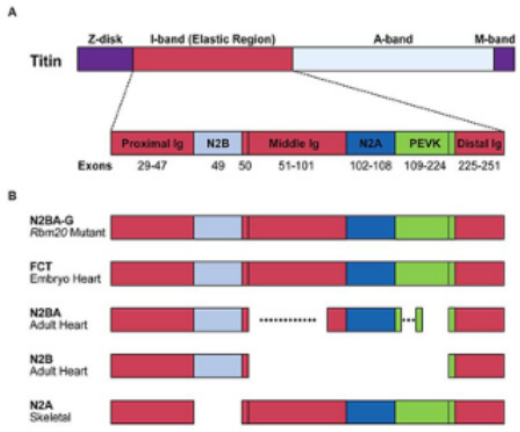
3.1. Role of RBM20 in Alternative TTN Splicing

RBM20 has been reported to affect TTN alternative splicing through the regulation of circular RNA, otherwise known as circRNA.[45] CircRNA is formed during transcription when the spliceosome covalently binds the 5' and 3' ends of an exon, forming a stable RNA molecule.[46] CircRNAs co-generate with mRNAs to regulate gene expression by competing with mRNA transcription to decrease the availability of linear mRNAs. According to a study by Ashwal-Fluss et al. (2014), murine models carrying pathogenic RBM20 gene variants lacked TTN circRNA.[47] Therefore, it can be concluded that RBM20 is essential in forming TTN circRNA as a pathway by which RBM20 modulates the alternative splicing of crucial genes such as TTN.

3.2. Alternative TTN Splicing—Correlation to DCM

Gene splicing is a post-transcriptional event that enables a single gene to increase its coding capacity as the pre-mRNA transcribed from one gene can lead to various mature mRNA molecules which generate multiple functional proteins.[48] This definition is in line with a statement given by Beqqali (2018), that alternative splicing (AS) is a molecular mechanism utilized by the cell to generate greater transcriptomic and proteomic diversity compared to the genome.[37] According to Zhiguo et al. (2013), an estimated 95% of genes found in human cells have been reported to exhibit AS,[49] exponentially increasing biological information flow at the molecular level as approximately 90,000 protein species are encoded by only 25,000 genes in humans.[50, 51] TTN is alternatively spliced and has three major isoforms: N2A, N2B, and N2BA. N2B and N2BA are isoforms expressed within adult cardiomyocytes with different lengths and extensibility (Figure 5).[38-41]

Passive stiffness, which occupies a major proportion of the myocardium,[52] is modulated in cardiomyocytes through the alternative expression of TTN isoforms with TTN N2B predominance.[53] The N2B protein is comparatively shorter with fewer exons expressed from the I-band as well as less elasticity due to the lack of Ig-like domains. Due to its larger size and increased elasticity, N2BA has less rigidness and thereby, less passive stiffness. Experimentally, cardiac tissue extracted from DCM patients exhibit decreased passive tension and increased ratio of N2BA:N2B isoforms. Therefore, the ratio of expression of different TTN isoforms influences myocardial passive stiffness, a common feature of DCM.[53] Additionally, reduced systolic function and the enlargement of ventricles in DCM may be partially explained by the decrease of passive stiffness which leads to lower diastolic pressure. The increased N2BA:N2B ratios also correlate to increased end diastolic volume, increased systolic volume, and decreased systolic ejection fraction—all symptoms commonly found in DCM.[41]



isoforms.[26] (A) Schematic domain structure of TTN protein with indicated names and positions as well as corresponding exons provided below each domain. (B) Schematic structure of TTN isoforms with names of the isoforms and the tissues responsible for the main expression of the indicated isoforms. Dotted lines indicate highly variable alternatively spliced regions.

4. Molecular Mechanisms of TTNtv DCM Pathogenicity

Although TTNtv are highly associated with the development of DCM, 2-3% of the asymptomatic general population are also TTNtv carriers.[55] According to the results of a complete TTN sequencing in a diverse cohort (>5,000 people) whose cardiac phenotypes were known, Roberts et al. (2015) concluded that the key predictor of TTNtv pathogenicity relies on whether or not the affected exon is expressed in cardiac tissue.[56] TTNtv located in constitutive exons were more often pathogenic whereas those located in exons that are minimally expressed in cardiac tissue can be "bypassed" through differential splicing and hence, had a lower risk of DCM.[41] The pathogenicity of a truncation mutation in TTN can be calculated using the percentage of TTN transcripts in which a given exon is spliced into an expressed transcript based on RNA sequencing data from left-ventricular human cardiac tissue. A percentage over 0.9% suggests that a high proportion of the total transcripts included a given exon in cardiac tissue. Results associate these exons with a 93% probability of pathogenicity in DCM phenotype patients.[56]

4.1. Genetic Modification of TTN

As a giant protein that bridges half of the sarcomere, TTN plays a crucial role in sarcomere stabilization and maintenance of passive and active tension.[41, 57] TTNtv have been reported to abnormally shorten TTN proteins and thereby result in sarcomere and cardiomyocyte dysfunction through a "poison peptide" mechanism, although the exact

molecular mechanisms of how TTNtv lead to DCM have not yet been demonstrated.[29, 41] RBM20-controlled TTNtv have been specifically reported to replace a cytosine nucleotide with a thymine nucleotide, resulting in a premature translational stop codon and hence, disrupting protein production.[29]

The suggestion of the "poison peptide" mechanism stemmed from the discovery of a specific missense mutation in TTN that led to a truncated protein, specifically CMD1G mutation, discovered in two separate human families which inherited the DCM phenotype through an autosomal dominant manner.[58] The CMD1G mutation affected a highly conserved immunoglobulin fold located at the Z-disc I-band transition,[58] directly altering sarcomere assembly due to the loss of a binding site. Induced pluripotent stem cell-derived cardiomyocytes (iPSC) made from a DCM patient also showed that cells with heterozygous A-band TTNtv were not capable of effectively forming sarcomeres, specifically the formation of protocostameres and Z-disks due to loss of binding site for β-cardiac myosin on TTN, further leading to a risk of reduced diastolic tension in the patient.[59]

It is also important to note that A-band and I-band TTNtv do not affect the amount of TTN protein encoded, but rather increase nonsense-mediated mRNA decay (NMD), leading to the degradation of abnormal mRNA transcripts by NMD which then reduces TTN allelic expression.[60] This reduction increases metabolic stress which then causes compensatory changes in the cardiomyocyte and eventually produces DCM phenotypes.[61, 62] Moreover, these compensatory changes include affected cardiac metabolism such as the activation of mTOR complex 1 signaling which causes pathogenic responses related to protein synthesis.[63, 64]

4.2. Post-Translational TTN Modification

According to Tharp et al. (2019), TTN function is highly regulated by post-translational modifications which involve phosphorylation and dephosphorylation at unique sites within the protein.[41] Due to its gigantic size and results of proteomic analysis as listed on online databases such as Phosphomouse and Phosphosite, TTN is predicted to have the most phosphorylation sites of any protein.[65, 66] However, only a small number of these phosphosites have been directly linked to structural and functional TTN alterations.[67] Phosphorylation on TTN can be categorized based on which domain is being altered while noting the predominance of "spring-like" I-domains due to its dynamic properties. Phosphorylation on an I-domain has the most significant and negative effects on passive and active sarcomere tension as well as the length and tension of cardiomyocytes.[41]

5. Clinical Diagnosis Methods for RBM20-Associated DCM

In the field of cardiology, genetics is a key tool for the prevention and treatment of cardiovascular diseases such as FDCM through risk predictions and personalized medicine. For instance, family cascade screening strategy and refined prognosis stratification can help locate genetic determinants to identify members at risk or with early stage diseases, providing an opportunity for early intervention. Advanced genetic panelling can also assess arrythmia risk for certain subtypes, increasing the accuracy of offered treatments.[68, 69, 70] RBM20-controlled TTNtv have been associated with high penetrance, an early-onset diagnosis of FDCM, and end-stage heart failure due to an increased risk of sudden cardiac death (SDC).[71] The European Society of Cardiology (ESC) recommended genetic testing (GT) to enable predictive diagnosis in suspected patients and first-degree relatives who fulfill the diagnostic criteria of cardiomyopathy according to a cascade screening strategy.[72]GT should also be followed by genetic sequencing (GS) using a cardiomyopathy-specific gene panel to confirm the pathogenic gene variants carried by patients. It is important to note, however, that genetic testing is only diagnostic, not predictive. Furthermore, genetic counselling (GC) is also offered to two-thirds of patients, 85% of the time by cardiologists, as recommended by the European guidelines in 2010.[73]

However, it has been reported that GT and GC for DCM have been performed in a substantial proportion (one-third) of CM patients but less often when compared to other subtypes and recommendations stated in the European guidelines despite clear evidence for genetic background.[74] Moreover, male patients who are younger and had more history of familial disease were prioritized for GT and GC, hence contributing to the under-investigation of DCM through the restriction of diagnosis and treatment opportunities to a specific subpopulation.[74] Thus, further efforts are needed to offer both GT and GC to a larger proportion of patients with DCM regardless of age, sex, familial status, and co-morbidities. Large regional differences have also been observed in GT. Regions such as Eastern Europe and North Africa, which are less economically developed, have the lowest testing rates compared to other nations in the same region, reflecting the lack of insurance modalities to cover the costs of GT.[74] Therefore, national and regional initiatives dedicated to funding life-saving researches as well as promoting healthcare systems (i.e. British Heart Foundation and European Reference Network for Rare and Low Prevalence Complex Diseases of the Heart) for under-investigated hereditary diseases such as DCM should be supported.

6. Clinical Treatment Options for RBM20-Associated DCM

Current guidelines from the ESC recommend the implantation of an implantable cardioverter-defibrillator (ICD) or HT in efforts to treat DCM patients who show an left ventricular ejection fraction below 35% and symptomatic heart failure.[75]Unfortunately, ICDs have a high rate of complications including infection, lead dysfunction, lead malfunction, lead displacement, and inappropriate discharges, or specific mechanical complications such as pneumothorax or bleeding.[76] ICDs are also incapable of treating specific symptoms or reverting mutational causes or consequences according to the genetic background of

patients who may suffer from different cardiomyopathy subtypes. In other words, the risk stratification in DCM is inadequate and ineffective as ICDs do not improve the overall survival in primary prevention of DCM.[77] There is a significant opportunity to delay or stop progression to heart failure and sudden cardiac death with early interventions.[78]Thus, there is a need to innovate a preventive and personalized clinical treatment method to reduce lethal arrhythmic burdens and properly manage DCM patients, specifically those carrying RBM20-spliced TTNtv.

7.The Upregulation of RBM20 As Potential TTNtv Therapy

RBM20 mutations are inextricably linked to a clear DCM phenotype in humans. Recent studies done by Guo et al. (2012) using rat models have shown that a knockout of RBM20 may lead to an increased ratio of N2BA:N2B TTN isoforms, suggesting that RBM20 regulates isoform expression of TTN and causes DCM.[36] However, in the same RBM20 knockout rats, a decreased N2BA:N2B ratio was observed as a direct result of a viral expression of RBM20,[36] suggesting that the upregulation of RBM20 favors N2B, the shorter and stiffer isoform production, and could possibly rescue a DCM phenotype. These results were recapitulated in iPSC from a DCM patient carrying missense RBM20. Recent advances in iPSC technology and direct differentiation of iPSCs into cardiomyocytes (iPSC-CMs) make it possible to model genetic heart disease in-vitro. By applying CRISPR/Cas9 genome editing technology to introduce three RBM20 mutations in iPSCs and differentiating them into iPSC-CMs, Briganti et al. (2020) successfully established an in-vitro model of RBM20 mutant DCM.[78] The splicing of RBM20 cardiac-specific genes, along with abnormal intracellular calcium handling and calcium rendering caused by TTNtv have been associated with the disease manifestation of DCM in patients.[79] Both increased and decreased calcium release have been reported to impair heart contractions.[80] However, all-trans retinoic acid have been reported to upregulate RBM20 expression and revert the splicing, calcium handling, and contractility defects in iPSC-CMs with different causal RBM20 mutations.[54, 79] Thus, it can be concluded that the pharmacological upregulation of RBM20 to suppress the expression N2BA:N2B ratios is a promising therapeutic target for treating DCM patients.

8.Conclusion

The RBM20 gene encodes the splicing factor RNA Binding Motif Protein-20 which regulates alternative splicing, targeting numerous cardiac genes such as titin. As a result, truncation variants of the titin gene are created and may cause RBM20 mutant dilated cardiomyopathy. This literary review addressed how RBM20 gene mutations pattern maladaptive genetic and cellular mechanisms, along with how these molecular defects affect cardiac and sarcomere function, thereby rendering diastolic dysfunction and high arrhythmia burden in inherited dilated cardiomyopathy patients.

Further research is needed to demonstrate the molecular mechanisms of RBM20-controlled TTN splicing and its effects on cardiac physiology at genetic, transcriptional, and post-translational levels in order to catalyze an adequate and personalized treatment option. Genetic testing, sequencing, and counseling for patients are recommended for early diagnosis and treatment as genetic dilated cardiomyopathy is a highly penetrative cardiac disease. With the current advancements in technology, the pharmacological upregulation of RBM20 poses as the most promising therapeutic target for treating DCM patients.

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References

1. Schultheiss, Heinz-Peter, DeLisa Fairweather, Alida L. P. Caforio, Felicitas Escher, Ray E. Hershberger, Steven E. Lipshultz, Peter P. Liu, et al. 2019. "Dilated Cardiomyopathy." Nature Reviews Disease Primers 5 (1). https://doi.org/10.1038/s41572-019-0084-1.

2. McNally, Elizabeth M., and Luisa Mestroni. 2017. "Dilated Cardiomyopathy." Circulation Research 121 (7). https://doi.org/10.1161/CIRCRESAHA.116.309396.

3. Japp, Alan G., Ankur Gulati, Stuart A. Cook, Martin R. Cowie, and Sanjay K. Prasad. 2016. "The Diagnosis and Evaluation of Dilated Cardiomyopathy." Journal of the American College of Cardiology 67 (25). https://doi.org/10.1016/j.jacc.2016.03.590.

4. Pinto, Yigal M., Perry M. Elliott, Eloisa Arbustini, Yehuda Adler, Aris Anastasakis, Michael Böhm, Denis Duboc, et al. 2016. "Proposal for a Revised Definition of Dilated Cardiomyopathy, Hypokinetic Non-Dilated Cardiomyopathy, and Its Implications for Clinical Practice: A Position Statement of the ESC Working Group on Myocardial and Pericardial Diseases." European Heart Journal 37 (23). https://doi.org/10.1093/eurheartj/ehv727.

5. Seidelmann, Sara B., Olga Laur, John Hwa, Eugene Depasquale, Lavanya Bellumkonda, Lissa Sugeng, Pawel Pomianowski, et al. 2016. "Familial Dilated Cardiomyopathy Diagnosis Is Commonly Overlooked at the Time of Transplant Listing." The Journal of Heart and Lung Transplantation 35 (4). https://doi.org/10.1016/j.healun.2015.12.002.

6. Khayata, Mohamed, Sadeer G Al-Kindi, and Guilherme H Oliveira. 2019. "Contemporary Characteristics and Outcomes of Adults with Familial Dilated Cardiomyopathy Listed for Heart Transplantation." World Journal of Cardiology 11 (1). https://doi.org/10.4330/wjc.v11.i1.38.

7. Mahmaljy, Hadi, Varun S. Yelamanchili, and Mayank Singhal. 2021. "Dilated Cardiomyopathy." StatPearls, January.

8. Codd, M B, D D Sugrue, B J Gersh, and L J Melton. 1989. "Epidemiology of Idiopathic Dilated and Hypertrophic Cardiomyopathy. A Population-Based Study in Olmsted County, Minnesota, 1975-1984." Circulation 80 (3). https://doi.org/10.1161/01.CIR.80.3.564.

9. Hershberger, Ray E., Dale J. Hedges, and Ana Morales. 2013. "Dilated Cardiomyopathy: The Complexity of a Diverse Genetic Architecture." Nature Reviews Cardiology 10 (9). https://doi.org/10.1038/

nrcardio.2013.105.

10. Jordà, Paloma, Rocío Toro, Carles Diez, Joel Salazar-Mendiguchía, Anna Fernandez-Falgueras, Alexandra Perez-Serra, Monica Coll, et al. 2021. "Malignant Arrhythmogenic Role Associated with RBM20: A Comprehensive Interpretation Focused on a Personalized Approach." Journal of Personalized Medicine 11 (2). https://doi.org/10.3390/jpm11020130.

11. Boer, Susanna L. den, M. (Lennie) van Osch-Gevers, Gijs van Ingen, Gideon J. du Marchie Sarvaas, Gabriëlle G. van Iperen, Ronald B. Tanke, Ad P.C.M. Backx, et al. 2015. "Management of Children with Dilated Cardiomyopathy in The Netherlands: Implications of a Low Early Transplantation Rate." The Journal of Heart and Lung Transplantation 34 (7). https://doi.org/10.1016/j.healun.2015.01.980.

12. Kirk, Richard, David Naftel, Timothy M. Hoffman, Christopher Almond, Gerard Boyle, Randall L. Caldwell, James K. Kirklin, Kirstie White, and Anne I. Dipchand. 2009. "Outcome of Pediatric Patients With Dilated Cardiomyopathy Listed for Transplant: A Multi-Institutional Study." The Journal of Heart and Lung Transplantation 28 (12). https://doi.org/10.1016/j.healun.2009.05.027.

13. Hey, Thomas Morris, Torsten B. Rasmussen, Trine Madsen, Mads Malik Aagaard, Maria Harbo, Henning Mølgaard, Jacob E. Møller, Hans Eiskjær, and Jens Mogensen. 2019. "Pathogenic RBM20 -Variants Are Associated With a Severe Disease Expression in Male Patients With Dilated Cardiomyopathy." Circulation: Heart Failure 12 (3). https://doi.org/10.1161/CIRCHEARTFAILURE.118.005700.

14. Mathew, Thomas, Lynne Williams, Govardhan Navaratnam, Bushra Rana, Richard Wheeler, Katherine Collins, Allan Harkness, et al. 2017. "Diagnosis and Assessment of Dilated Cardiomyopathy: A Guideline Protocol from the British Society of Echocardiography." Echo Research and Practice 4 (2). https://doi.org/10.1530/ERP-16-0037.

15. Karamitsos, Theodoros D., Jane M. Francis, Saul Myerson, Joseph B. Selvanayagam, and Stefan Neubauer. 2009. "The Role of Cardiovascular Magnetic Resonance Imaging in Heart Failure." Journal of the American College of Cardiology54 (15). https://doi.org/10.1016/j.jacc.2009.04.094.

16. Blausen.com staff. 2014. "Medical gallery of Blausen Medical 2014." WikiJournal of Medicine 1 (2). https://doi.org/10.15347/wjm/2014.010.

17. Elliott, P., B. Andersson, E. Arbustini, Z. Bilinska, F. Cecchi, P. Charron, O. Dubourg, et al. 2007. "Classification of the Cardiomyopathies: A Position Statement from the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases." European Heart Journal 29 (2). https://doi.org/10.1093/eurheartj/ehm342.

18. Skrzynia, Cecile, Jonathan Berg, Monte Willis, and Brian Jensen. 2014. "Genetics and Heart Failure: A Concise Guide for the Clinician." Current Cardiology Reviews 11 (1). https://doi.org/10.2174/1573403X09666131117170446.

19. Fernández-Ruiz, Irene. 2020. "Upregulating RBM20 as a Therapy for DCM." Nature Reviews Cardiology 17 (11). https://doi.org/10.1038/s41569-020-00458-4.

20. Fatkin, Diane, Inken G. Huttner, Jason C. Kovacic, J.G. Seidman, and Christine E. Seidman. 2019. "Precision Medicine in the Management of Dilated Cardiomyopathy." Journal of the American College of Cardiology 74 (23). https://doi.org/10.1016/j.jacc.2019.10.011.

21. Maggi, Lorenzo, Nicola Carboni, and Pia Bernasconi. 2016. "Skeletal Muscle Laminopathies: A Review of Clinical and Molecular Features." Cells 5 (3). https://doi.org/10.3390/cells5030033.

22. Parikh, Victoria N., Colleen Caleshu, Chloe Reuter, Laura C. Lazzeroni, Jodie Ingles, John Garcia, Kristen McCaleb, et al. 2019. "Regional Variation in RBM20 Causes a Highly Penetrant Arrhythmogenic Cardiomyopathy." Circulation: Heart Failure 12 (3). https://doi.org/10.1161/CIRCHEARTFAILURE.118.005371.

23. Brauch, Katharine M., Margaret L. Karst, Kathleen J. Herron, Mariza de Andrade, Patricia A. Pellikka, Richard J. Rodeheffer, Virginia v. Michels, and Timothy M. Olson. 2009. "Mutations in Ribonucleic Acid Binding Protein Gene Cause Familial Dilated Cardiomyopathy." Journal of the American College of Cardiology 54 (10). https://doi.org/10.1016/j.jacc.2009.05.038.

24. Herman, Daniel S., Lien Lam, Matthew R.G. Taylor, Libin Wang, Polakit Teekakirikul, Danos Christodoulou, Lauren Conner, et al. 2012. "Truncations of Titin Causing Dilated Cardiomyopathy." New England Journal of Medicine 366 (7). https://doi.org/10.1056/NEJMoa1110186.

25. LeWinter, Martin M., and Henk L. Granzier. 2013. "Titin Is a Major Human Disease Gene." Circulation 127 (8). https://doi.org/10.1161/CIRCULATIONAHA.112.139717.

26. Fatkin, Diane, and Inken G. Huttner. 2017. "Titin-Truncating Mutations in Dilated Cardiomyopathy." Current Opinion in Cardiology 32 (3). https://doi.org/10.1097/HCO.0000000000000382.

27. Hoogenhof, Maarten M.G. van den, Abdelaziz Beqqali, Ahmad S. Amin, Ingeborg van der Made, Simona Aufiero, Mohsin A.F. Khan, Cees A. Schumacher, et al. 2018. "RBM20 Mutations Induce an Arrhythmogenic Dilated Cardiomyopathy Related to Disturbed Calcium Handling." Circulation 138 (13). https://doi.org/10.1161/CIRCULATIONAHA.117.031947.

28. Watanabe, Takeshi, Akinori Kimura, and Hidehito Kuroyanagi. 2018. "Alternative Splicing Regulator RBM20 and Cardiomyopathy." Frontiers in Molecular Biosciences 5 (November). https://doi.org/10.3389/fmolb.2018.00105.

Agboola, Kolade M., Trevon McGill, Barry A. Boilson, Naveen L. Pereira, and Jacob C. Jentzer. 2021. "The Role of Genetic Testing in the Evaluation of Dilated Cardiomyopathies." Case Reports in Cardiology 2021 (March). https://doi.org/10.1155/2021/6641108.

30. Das, Soumi, and Sandeep Seth. 2021. "Familial Dilated Cardiomyopathy with RBM20 Mutation in an Indian Patient: A Case Report." The Egyptian Heart Journal 73 (1). https://doi.org/10.1186/s43044-021-00165-6.

31. Filippello, Agnese, Pamela Lorenzi, Elisa Bergamo, and Maria Grazia Romanelli. 2013. "Identification of Nuclear Retention Domains in the RBM20 Protein." FEBS Letters 587 (18). https://doi.org/10.1016/j.febslet.2013.07.018.

32. Murayama, Rie, Mariko Kimura-Asami, Marina Togo-Ohno, Yumiko Yamasaki-Kato, Taeko K. Naruse, Takeshi Yamamoto, Takeharu Hayashi, et al. 2018. "Phosphorylation of the RSRSP Stretch Is Critical for Splicing Regulation by RNA-Binding Motif Protein 20 (RBM20) through Nuclear Localization." Scientific Reports 8 (1). https://doi.org/10.1038/s41598-018-26624-w.

33. Maatz, Henrike, Marvin Jens, Martin Liss, Sebastian Schafer, Matthias Heinig, Marieluise Kirchner, Eleonora Adami, et al. 2014. "RNA-Binding Protein RBM20 Represses Splicing to Orchestrate Cardiac Pre-MRNA Processing." Journal of Clinical Investigation 124 (8). https://doi.org/10.1172/JCI74523.

34. Lennermann, D., J. Backs, and M. M. G. van den Hoogenhof. 2020. "New Insights in RBM20 Cardiomyopathy." Current Heart Failure Reports 17 (5). https://doi.org/10.1007/s11897-020-00475-x.

35. Li, Shijun, Wei Guo, Colin N. Dewey, and Marion L. Greaser. 2013. "Rbm20 Regulates Titin Alternative Splicing as a Splicing Repressor." Nucleic Acids Research 41 (4). https://doi.org/10.1093/nar/gks1362.

36. Guo, Wei, Sebastian Schafer, Marion L Greaser, Michael H Radke, Martin Liss, Thirupugal Govindarajan, Henrike Maatz, et al. 2012. "RBM20, a Gene for Hereditary Cardiomyopathy, Regulates Titin Splicing." Nature Medicine 18 (5). https://doi.org/10.1038/nm.2693.

37. Beqqali, A. 2018. "Alternative Splicing in Cardiomyopathy." Biophysical Reviews 10 (4). https://doi.org/10.1007/s12551-018-0439-y.

38. Freiburg, Alexandra, Karoly Trombitas, Wolfgang Hell, Olivier Cazorla, Francoise Fougerousse, Thomas Centner, Bernhard Kolmerer, et al. 2000. "Series of Exon-Skipping Events in the Elastic Spring Region of Titin as the Structural Basis for Myofibrillar Elastic Diversity." Circulation Research 86 (11). https://doi.org/10.1161/01.RES.86.11.1114.

39. LeWinter, Martin M., Yiming Wu, Siegfried Labeit, and Henk Granzier. 2007. "Cardiac Titin: Structure, Functions and Role in Disease." Clinica Chimica Acta 375 (1–2). https://doi.org/10.1016/j.cca.2006.06.035.

40. Shih, Yu-Huan, Alexey v. Dvornikov, Ping Zhu, Xiao Ma, Maeng Jo Kim, Yonghe Ding, and Xiaolei Xu. 2016. "Exon- and Contraction-Dependent Functions of Titin in Sarcomere Assembly." Development, January. https://doi.org/10.1242/dev.139246.

41. Tharp, Charles A., Mary E. Haywood, Orfeo Sbaizero, Matthew R. G. Taylor, and Luisa Mestroni. 2019. "The Giant Protein Titin's Role in Cardiomyopathy: Genetic, Transcriptional, and Post-Translational Modifications of TTN and Their Contribution to Cardiac Disease." Frontiers in Physiology 10 (November). https://doi.org/10.3389/fphys.2019.01436.

42. Wyles, Saranya P., Xing Li, Sybil C. Hrstka, Santiago Reyes, Saji Oommen, Rosanna Beraldi, Jessica Edwards, Andre Terzic, Timothy M. Olson, and Timothy J. Nelson. 2016. "Modeling Structural and Functional Deficiencies of RBM20Familial Dilated Cardiomyopathy Using Human Induced Pluripotent Stem Cells." Human Molecular Genetics 25 (2). https://doi.org/10.1093/hmg/ddv468.

43. Sedaghat-Hamedani, Farbod, Jan Haas, Feng Zhu, Christian Geier, Elham Kayvanpour, Martin Liss, Alan Lai, et al. 2017. "Clinical Genetics and Outcome of Left Ventricular Non-Compaction Cardiomyopathy." European Heart Journal 38 (46). https://doi.org/10.1093/eurheartj/ehx545.

44. Wyles, SP, SC Hrstka, S Reyes, A Terzic, TM Olson, and TJ Nelson. 2016. "Pharmacological Modulation of Calcium Homeostasis in Familial Dilated Cardiomyopathy: An In Vitro Analysis From an RBM20 Patient-Derived IPSC Model." Clinical and Translational Science 9 (3). https://doi.org/10.1111/cts.12393.

45. Khan, Mohsin A.F., Yolan J. Reckman, Simona Aufiero, Maarten M.G. van den Hoogenhof, Ingeborg van der Made, Abdelaziz Beqqali, Dave R. Koolbergen, et al. 2016. "RBM20 Regulates Circular RNA Production From the Titin Gene." Circulation Research 119 (9). https://doi.org/10.1161/CIRCRESAHA.116.309568.

46. Memczak, Sebastian, Marvin Jens, Antigoni Elefsinioti, Francesca Torti, Janna Krueger, Agnieszka Rybak, Luisa Maier, et al. 2013. "Circular RNAs Are a Large Class of Animal RNAs with Regulatory Potency." Nature 495 (7441). https://doi.org/10.1038/nature11928.

47. Ashwal-Fluss, Reut, Markus Meyer, Nagarjuna Reddy Pamudurti, Andranik Ivanov, Osnat Bartok, Mor Hanan, Naveh Evantal, Sebastian Memczak, Nikolaus Rajewsky, and Sebastian Kadener. 2014. "CircRNA Biogenesis Competes with Pre-MRNA Splicing." Molecular Cell 56 (1). https://doi.org/10.1016/j.molcel.2014.08.019.

48. Tazi, Jamal, Nadia Bakkour, and Stefan Stamm. 2009. "Alternative Splicing and Disease." Biochimica et Biophysica Acta (BBA) - Molecular Basis of Disease 1792 (1). https://doi.org/10.1016/j.bbadis.2008.09.017.

49. E, Zhiguo, Lei Wang, and Jianhua Zhou. 2013. "Splicing and Alternative Splicing in Rice and Humans." BMB Reports 46 (9). https://doi.org/10.5483/BMBRep.2013.46.9.161.

50. Pan, Qun, Ofer Shai, Leo J Lee, Brendan J Frey, and Benjamin J Blencowe. 2008. "Deep Surveying of Alternative Splicing Complexity in the Human Transcriptome by High-Throughput Sequencing." Nature Genetics 40 (12). https://doi.org/10.1038/ng.259.

51. Kim, Eddo, Alon Magen, and Gil Ast. 2007. "Different Levels of Alternative Splicing among Eukaryotes." Nucleic Acids Research 35 (1). https://doi.org/10.1093/nar/gkl924.

52. Rivas-Pardo JA, Eckels EC, Popa I, Kosuri P, Linke WA, Fernández JM. 2016. :Work Done by Titin Protein Folding Assists Muscle Contraction". Cell Rep 14 (6). https://doi.org/10.1016/j.celrep.2016.01.025.

53. Nagueh, Sherif F., Gopi Shah, Yiming Wu, Guillermo Torre-Amione, Nicholas M.P. King, Sunshine Lahmers, Christian C. Witt, Katy Becker, Siegfried Labeit, and Henk L. Granzier. 2004. "Altered Titin Expression, Myocardial Stiffness, and Left Ventricular Function in Patients With Dilated Cardiomyopathy." Circulation 110 (2). https://doi.org/10.1161/01.CIR.0000135591.37759.AF.

54. Streckfuss-Bömeke, Katrin, Malte Tiburcy, Andrey Fomin, Xiaojing Luo, Wener Li, Claudia Fischer, Cemil Özcelik, et al. 2017. "Severe DCM Phenotype of Patient Harboring RBM20 Mutation S635A Can Be Modeled by Patient-Specific Induced Pluripotent Stem Cell-Derived Cardiomyocytes." Journal of Molecular and Cellular Cardiology 113 (December). https://doi.org/10.1016/j.yjmcc.2017.09.008.

55. Pan, Stephen, Colleen A. Caleshu, Kyla E. Dunn, Marcia J. Foti, Maura K. Moran, Oretunlewa Soyinka, and Euan A. Ashley. 2012. "Cardiac Structural and Sarcomere Genes Associated With Cardiomyopathy Exhibit Marked Intolerance of Genetic Variation." Circulation: Cardiovascular Genetics 5 (6). https://doi.org/10.1161/CIRCGENETICS.112.963421.

56. Roberts, Angharad M., James S. Ware, Daniel S. Herman, Sebastian Schafer, John Baksi, Alexander G. Bick, Rachel J. Buchan, et al. 2015. "Integrated Allelic, Transcriptional, and Phenomic Dissection of the Cardiac Effects of Titin Truncations in Health and Disease." Science Translational Medicine 7 (270). https://doi.org/10.1126/scitranslmed.3010134.

57. Matsuo, Masafumi, Hiroyuki Awano, Nobuhiro Maruyama, and Hisahide Nishio. 2019. "Titin Fragment in Urine: A Noninvasive Biomarker of Muscle Degradation." In . https://doi.org/10.1016/bs.acc.2019.01.001.

58. Gerull, Brenda, Michael Gramlich, John Atherton, Mark McNabb, Karoly Trombitás, Sabine Sasse-Klaassen, J.G. Seidman, et al. 2002. "Mutations of TTN, Encoding the Giant Muscle Filament Titin, Cause Familial Dilated Cardiomyopathy." Nature Genetics 30 (2). https://doi.org/10.1038/ng815.

59. Chopra, Anant, Matthew L. Kutys, Kehan Zhang, William J. Polacheck, Calvin C. Sheng, Rebeccah J. Luu, Jeroen Eyckmans, et al. 2018. "Force Generation via β-Cardiac Myosin, Titin, and α-Actinin Drives Cardiac Sarcomere Assembly from Cell-Matrix Adhesions." Developmental Cell 44 (1). https://doi.org/10.1016/j.devcel.2017.12.012.

60. Schafer, Sebastian, Antonio de Marvao, Eleonora Adami, Lorna R Fiedler, Benjamin Ng, Ester Khin, Owen J L Rackham, et al. 2017. "Titin-Truncating Variants Affect Heart Function in Disease Cohorts and the General Population." Nature Genetics 49 (1). https://doi.org/10.1038/ng.3719.

61. Tabish, Ali M., Valerio Azzimato, Aris Alexiadis, Byambajav Buyandelger, and Ralph Knöll. 2017. "Genetic Epidemiology of Titin-Truncating Variants in the Etiology of Dilated Cardiomyopathy." Biophysical Reviews 9 (3). https://doi.org/10.1007/s12551-017-0265-7.

62. Ware, James S., and Stuart A. Cook. 2018. "Role of Titin in Cardiomyopathy: From DNA Variants to Patient Stratification." Nature Reviews Cardiology 15 (4). https://doi.org/10.1038/nrcardio.2017.190.

63. Ramos, F. J., S. C. Chen, M. G. Garelick, D.-F. Dai, C.-Y. Liao, K. H. Schreiber, V. L. MacKay, et al. 2012. "Rapamycin Reverses Elevated MTORC1 Signaling in Lamin A/C-Deficient Mice, Rescues Cardiac and Skeletal Muscle Function, and Extends Survival." Science Translational Medicine 4 (144). https://doi.org/10.1126/scitranslmed.3003802.

64. Yano, Toshiyuki, Shinya Shimoshige, Takayuki Miki, Masaya Tanno, Atsushi Mochizuki, Takefumi Fujito, Satoshi Yuda, et al. 2016. "Clinical Impact of Myocardial MTORC1 Activation in Nonischemic Dilated Cardiomyopathy." Journal of Molecular and Cellular Cardiology 91 (February). https://doi.org/10.1016/j.yjmcc.2015.12.022.

65. Huttlin, Edward L., Mark P. Jedrychowski, Joshua E. Elias, Tapasree Goswami, Ramin Rad, Sean A. Beausoleil, Judit Villén, Wilhelm Haas, Mathew E. Sowa, and Steven P. Gygi. 2010. "A Tissue-Specific Atlas of Mouse Protein Phosphorylation and Expression." Cell 143 (7). https://doi.org/10.1016/j.cell.2010.12.001.

66. Hornbeck, Peter v., Bin Zhang, Beth Murray, Jon M. Kornhauser, Vaughan Latham, and Elzbieta Skrzypek. 2015. "PhosphoSitePlus, 2014: Mutations, PTMs and Recalibrations." Nucleic Acids Research 43 (D1). https://doi.org/10.1093/nar/gku1267.

67. Krysiak, Judith, Andreas Unger, Lisa Beckendorf, Nazha Hamdani, Marion von Frieling-Salewsky, Margaret M. Redfield, Cris G. dos Remedios, et al. 2018. "Protein Phosphatase 5 Regulates Titin Phosphorylation and Function at a Sarcomere-Associated Mechanosensor Complex in Cardiomyocytes." Nature Communications 9 (1). https://doi.org/10.1038/s41467-017-02483-3.

68. Lee, Teresa M., and Stephanie M. Ware. 2015. "Toward Personalized Medicine: Does Genetic Diagnosis of Pediatric Cardiomyopathy Influence Patient Management?" Progress in Pediatric Cardiology 39 (1). https://doi.org/10.1016/j.ppedcard.2015.01.008.

69. Leopold, Jane A., and Joseph Loscalzo. 2018. "Emerging Role of Precision Medicine in Cardiovascular Disease." Circulation Research 122 (9). https://doi.org/10.1161/CIRCRESAHA.117.310782.

70. McNally, Elizabeth M., and Luisa Mestroni. 2017. "Dilated Cardiomyopathy: Genetic Determinants and Mechanisms." Circulation Research 121 (7). https://doi.org/10.1161/CIRCRESAHA.116.309396.

71. Li, Duanxiang, Ana Morales, Jorge Gonzalez-Quintana, Nadine Norton, Jill D. Siegfried, Mark Hofmeyer, and Ray E. Hershberger. 2010. "Identification of Novel Mutations in RBM20 in Patients with Dilated Cardiomyopathy." Clinical and Translational Science 3 (3). https://doi.org/10.1111/j.1752-8062.2010.00198.x.

72. Sousa, Alexandra, Oana Moldovan, Ana Lebreiro, Mafalda Bourbon, Natália António, Quitéria Rato, Patrícia Rodrigues, et al. 2020. "Recommendations for Genetic Testing in Cardiology: Review of Major International Guidelines." Revista Portuguesa de Cardiologia (English

Edition) 39 (10). https://doi.org/10.1016/j.repce.2020.03.008.
73. Charron, P., M. Arad, E. Arbustini, C. Basso, Z. Bilinska, P. Elliott, T. Helio, et al. 2010. "Genetic Counselling and Testing in Cardiomyopathies: A Position Statement of the European Society of Cardiology Working Group on Myocardial and Pericardial Diseases." European Heart Journal 31 (22). https://doi.org/10.1093/eurheartj/ehq271.
74. Heliö, Tiina, Perry Elliott, Juha W. Koskenvuo, Juan R. Gimeno, Luigi Tavazzi, Michal Tendera, Juan Pablo Kaski, et al. 2020. "ESC EORP Cardiomyopathy Registry: Real-life Practice of Genetic Counselling and Testing in Adult Cardiomyopathy Patients." ESC Heart Failure 7 (5). https://doi.org/10.1002/ehf2.12925.
75. Ramakumar, Venkatakrishnan, and Nitish Naik. 2018. "Current Status of the ICD in Nonischemic Cardiomyopathy." Journal of the Practice of Cardiovascular Sciences 4 (1). https://doi.org/10.4103/jpcs.jpcs_16_18.
76. Schinkel, Arend F.L., Pieter A. Vriesendorp, Eric J.G. Sijbrands, Luc J.L.M. Jordaens, Folkert J. ten Cate, and Michelle Michels. 2012. "Outcome and Complications After Implantable Cardioverter Defibrillator Therapy in Hypertrophic Cardiomyopathy." Circulation: Heart Failure 5 (5). https://doi.org/10.1161/CIRCHEARTFAILURE.112.969626.
77. Akhtar, M., and P. M. Elliott. 2019. "Risk Stratification for Sudden Cardiac Death in Non-Ischaemic Dilated Cardiomyopathy." Current Cardiology Reports 21 (12). https://doi.org/10.1007/s11886-019-1236-3.
78. Kinnamon, Daniel D., Ana Morales, Deborah J. Bowen, Wylie Burke, Ray E. Hershberger, Ray E. Hershberger, Daniel D. Kinnamon, et al. 2017. "Toward Genetics-Driven Early Intervention in Dilated Cardiomyopathy." Circulation: Cardiovascular Genetics 10 (6). https://doi.org/10.1161/CIRCGENETICS.117.001826.
79. Briganti, Francesca, Han Sun, Wu Wei, Jingyan Wu, Chenchen Zhu, Martin Liss, Ioannis Karakikes, et al. 2020. "IPSC Modeling of RBM20-Deficient DCM Identifies Upregulation of RBM20 as a Therapeutic Strategy." Cell Reports 32 (10). https://doi.org/10.1016/j.celrep.2020.108117.
80. Marks, Andrew R. 2003. "Calcium and the Heart: A Question of Life and Death." Journal of Clinical Investigation 111 (5). https://doi.org/10.1172/JCI18067.

The Interplay Between EMT and Cancer Stem Cells in Tumor Expansion and Promising Antitumor Strategies

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Abstract

The statistically significant correlation between cancer-related deaths and metastasis makes tumor metastasis prevention crucial in the improvement of therapeutic and clinical treatments. With its ability to confer cancer cells more mobility, invasive capability, and resistance to apoptosis, the epithelial-mesenchymal transition (EMT) has been suspected for its role in driving cancer progression from carcinogenesis to metastasis. More importantly, tumor cells, after undergoing the EMT, have shown acquired traits of stem cells, which makes them even stronger in therapeutic resistance. Scientists are increasingly targeting the EMT pathways as their complex biological steps have been seen as promising opportunities to find cancer treatments or even cures. However, experiments testing the EMT's influence on metastasis in vivo have been technically challenging and generated unexpected results. So, having a clear-cut definition of and understanding how the EMT worsens cancer metastasis remains an unachieved mission. To accomplish this mission, many studies have started to use in vivo imaging, advanced lineage tracing systems, and in vivo models. These tools could efficiently help uncover the intricate driving mechanism of EMT in metastasis. This review discusses the recent advances scientists have made regarding the biological concepts of EMT in boosting metastasis and future clinical or therapeutic innovations.

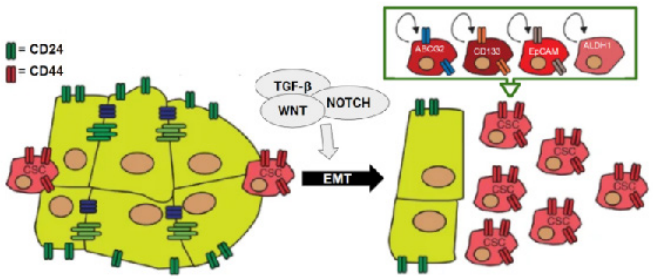
Introduction

Dilated cardiomyopathy (DCM) is the most frequent form First observed in the development of embryos, the EMT is a process in which cells lose their epithelial features and gain mesenchymal features.[1] EMT results in spindle-shaped cells that have removed cellular polarity.[1] These traits are often characterised by mesenchymal cells which have more motility and invasive tendencies.1 This transition is a transformation between two morphologically different states and types of cells.1 The epithelial cells lose their E-cadherin and adopt more vimentin, a mesenchymal cell marker.2 The loss of E-cadherin in epithelial cells is a fundamental process in the EMT because it triggers a cascade of morphologic alterations that allow for a full transition.2 More notably, cancer cells have shown expression of EMT-related molecular pathways that plays the same role in embryonic development.3

Many growth factors, including hepatocyte, transforming growth factor-β2, and epidermal growth factor, are key EMT initiators.4 When these factors are activated, intracellular signaling cascades are triggered to downregulate E-cadherin.4 Besides, the signaling cascades also alter the cellular cytoskeletal matrix and certain pattern of gene expressions, all leading to a clear-cut transformation.4 After epithelial cells lose their E-cadherin, their cell-cell adhesions also break down, making it easier for them to migrate.4 A transcriptional repressor of E-cadherin called SNAI1 (snail) is one key element to understanding driving mechanisms of EMT.5

The discovery of this zinc finger molecule provides opportunities for investigation of the link between intracellular signaling and downregulation of E-cadherin.5 Signaling pathways activating SNAI1 silence the gene expression of E-cadherin through binding the critical E2 boxes to the transcriptional site of the E-cadherin promoter.6 Alternatively, SNAI2 (slug), zeb1, zeb2, SMAD-interacting protein 1, and TWIST1 are other E-cadherin transcriptional repressors that have been discovered that also serve similar functions to SNAI1.7

Figure 1: The ability to initiate and expand a tumor has long been known as a unique hallmark of cancer stem cells (CSCs), otherwise regarded as the source of cancer progression.8 Scientists can use a set of marker proteins such as ABCG2 (a member of the ABC family transporter), CD133, EpCAM (an epithelial cell adhesion molecule), and ALDH1 (aldehyde dehydrogenase 1), to mark the cancer stem cells, so that they could be separated from the other cancer cells.9 Cancer stem cells can both indefinitely self-renew, which ensures the long-term survival of cancer cells, and differentiate, which ensures that the tumor heterogeneity is maintained, making treatments less effective.9 The EMT can induce a CSC-like phenotype to original cancer cells.10 TGF-β, Wnt or Notch are examples of EMT inducers that result in cells acquiring a CD44 high CD24 low phenotype, which closely resembles that of CSCs.1



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Epithelial-Mesenchymal Transition

Epithelial-mesenchymal transition (EMT) is a process, as the name implies, in which epithelial cells turn into mesenchymal cells through the acquisition of certain characteristics.12 This EMT process can be observed in many biological processes such as tissue fibrosis, embryonic evolution, tissue formation, and wound healing.12 Moreover, EMT can be key in tumor growth, drug resistance, and metastasis.13 Since this transition plays a major role in cancer development, it has been targeted in many therapeutic and clinical treatments.13 The EMT is a result of the synergization of many different signaling pathways such as the transforming growth factor beta (TGF-β) signaling pathway, the receptor tyrosine kinase (RTK) signaling pathway, the Wnt/β-catenin signaling pathway, the signal transducer and activator of transcription 3 (STAT3) signaling pathway, the extracellular matrix (ECM)-mediated signaling pathway, and many more.13 Many of these signaling pathways initiate EMT by mediating the three main transcription factors: Snail, Twist, and ZEB.14 Additionally, these pathways also upregulate mesenchymal cell markers, downregulate epithelial cell markers, and thus alter the overall property of epithelial cells.

Snails are members of the family of the ZINC finger transcription factors.5 There are three types of Snails: Snail1 (Snail), Snail2 (Slug), and Snail3 (Smuc).5 Out of the three, Snail1 has a significant role in the modulation of cancer cell migration and metastasis through the epithelial-mesenchymal transition.5

Many different Snail1 transcription factors can bind to promoters to activate expression of metastasis-associated 1 family member 3 (MTA3), hypoxia-inducible factor 1-alpha (HIF-1a), glioma-associated oncogene homolog 1 (Gli1), lysyl oxidase-like 2 (LOXL2), and many more.15 Besides, many of these factors can bind to the E-cadherin gene, the gene responsible for alterations of E-cadherin. The sudden shifts in expressed E-cadherin levels are strongly correlated with EMT and cancer metastasis.16 Also, Snail1 can regulate proteins involved in extracellular cell-cell interaction and intracellular signaling pathways such as Claudin (CLDN), Occludin (OCLN), Zona occludens 1 (ZO-1), Cytokeratin 18, and Mucin 1.17 Some discoveries have even proven that Snail1 modulates the expression of two types of matrix metalloproteinases (MMP): MMP-2 and MMP-9.18 More importantly, Snail1 mediates the gene expression of other transcription factors involved in enhancing EMT, namely ZEB-1 and ZEB-2.19

The second main transcription factor in EMT is called Twist, which is classified as a BASIC HELIX-LOOP-HELIX (BHLH) and often plays many key roles in many physiological pathways.20 There are two types of Twist, Twist-1 (Twist) and Twist-2 (Dermo-1), which help cells form their mesodermal layer.21 Mutated Twist in drosophila causes a mutated phenotype in which they lack internal organs.22 This Twist mutation, in humans, can lead to Saethre-Chotzen syndrome, where patients have craniosynostosis and mild limb anomalies.22 Twist-1 plays a pivotal role in many progressive steps that result in cancer metastasis, angiogenesis, and stemness.23 There is a high correlation between Twist-1 and Twist-2 expression and cancer cell properties, including frequent invasion, migration, and anoikis resistance.24,25 Therefore, increased Twist-1 and Twist-2 expression can theoretically aid the EMT process, which aids tumor metastasis.24,25

The third key transcription factor of EMT is Zinc finger E-box-binding homeobox ZEB.26 These factors, affecting gene expression of many proteins that affect embryogenesis, differentiation, tumorigenesis, and metastasis, can be found in two forms: ZEB1 and ZEB2.26 ZEB1 and ZEB2 are fundamental to the EMT process, because they restrict E-cadherin expression by binding to the E-cadherin promoters' E-BOX sequences.27 When E-cadherin is downregulated, cancer cells get induced traits of mesenchymal cells, which will then metastasize.27 ZEB1, by binding to the promoter of other genes that makes important proteins in cell-cell interaction, tight junctions (TJ), desmosomes, and cell polarity, reduces epithelial cell properties and enhances metastasis.28

Cancer cells, undergoing the EMT process, often express anoikis resistance.29 Anoikis is simply the programmed cell death (apoptosis) in which epithelial cells self-degrade from breaking their extracellular matrix and neighboring cells.30 Anoikis is a process that could prevent metastasis.30

However, cancer cells, wanting to metastasize, tend to avoid anoikis by separating from the original tumor and hitchhiking around the body through the circulatory and lymphatic system.31 Many EMT-promoting proteins are responsible for cancer cells' anoikis resistance.32 These EMT-promoting proteins decrease E-cadherin expression, increase N-cadherin expression, and therefore further strengthen anoikis resistance.33 Similarly, Twist, Snail, and Zeb1 (the three main EMT transcription factors) also alter the expression of E-cadherin and N-cadherin, thereby enhancing anoikis resistance and metastasis.33

Ankyrin-G protein is another important factor in regulating E-cadherin, because it uses E-cadherin as a bridge from the cytoskeleton to the cell membrane.34 Ankyrin-G protein pushes the NRAGE protein, often found in the plasma membranes, to migrate to the nucleus.35 In the EMT process, which has low levels of E-cadmium and ankyrin-G, the NRAGE protein is translocated to the nucleus and reduces transcription of the tumor suppressor p14ARF gene.36 This directly induces anoikis resistance, because the cells now produce fewer tumor suppressor proteins.36 All of this shows that the Ankyrin-G protein plays a critical role in maintaining the cancer cells' induced anoikis by hemophilically binding to two neighboring cells. On the other hand, altered YAP phosphorylation of E-cadherin and β-catenin further supports anoikis resistance.37 N-cadherin activated by the Akt or the PI3K/Akt signaling pathway mediates tumor anoikis resistance.38

The EMT has attracted many researchers' attention as the power it holds in regulating cancer cell metastasis is a potential target. For example, simvastatin, a drug treating hyperlipidemia, has inhibited EMT.39 Simvastatin works by preventing the expression of EMT factors such as cadherin, vimentin, and β-catenin.39 Therefore, simvastatin can successfully prevent cancer metastasis.39 Many different Phase II clinical trials have utilized simvastatin to treat advanced-stage carcinomas.40 Alternatively, LY2157299 (galunisertib) also prevents cancer metastasis by inhibiting the TGF - β pathway, one of the main inducing pathways of EMT.41

Cancer stem cells are a vital component of tumors as their mechanism ensures cancer survival and progression.42 This EMT process primarily leads to stemness in cancer cells, a state in which cancer cells can self-renew and generate many differentiated cells.42 The stem cells' interaction with the environment is also fundamental to their growth and proliferation.42 Stem cells utilize these properties for the maintenance of tissue homeostasis. Cancer stem cells use these features to survive and advance their malignancy. Cancer cells are usually killed at an early stage using chemotherapy or radiation, whereas certain tumor cells, namely cancer stem cells, might cause tumor relapse.43 Cancer stem cells are extremely treatment-resistant and express certain traits similar to stem cells.43 These cancer stem cells allow for tumor growth, because they give rise to other cancer cells.43 In short, they are the driving cells behind tumor growth and development. CSCs can be identified in many tumors including liver, breast, prostate, pancreas, leukemia, melanoma, and many more by identification of certain cell surface markers.44 Frequently used CSC surface markers such as CD24, CD29, CD44, CD90, CD133, epithelial-specific antigen (ESA), and aldehyde dehydrogenase 1 (ALDH1) can be used to separate the smaller population of cancer stem cells, which will be eventually targeted.45

EMT-induced Cancer Stem Cells

Rare tumor cells (cancer stem cells) possess the ability to self-renew and give birth to normal cancer cells to grow the tumor.46 Scientists are currently investigating the root source of CSCs. Although many different factors contribute to the rise of CSC in a tumor, the EMT has been suspected for its cause in giving cancer cells the capability of cancer stem cells.47 Mesenchymal cells, a product of the EMT, can differentiate into multiple lineages.48 More cancer-associated EMTs lead to an increased amount of migratory cells that can form new tissues and metastasize. CSCs, in squamous cell carcinoma and breast cancer, exhibit both epithelial and mesenchymal characteristics in a shifting manner.49 They can be proliferative like epithelial cells and migratory like mesenchymal cells.50 Cells, after experiencing the EMT, increase their tumor size by at least 10-fold, which further supports the notion that the EMT gives cells characteristic of stem cells.51

Twist thwarts the expression of CD24, which will eventually lead to cells with CSC's phenotypes.52 Snail triggers the dedifferentiation of epithelial cells in colorectal cancer.53 ZEB1, an EMT transcriptional factor, can restrict many epithelial determinants and consequently dedifferentiate cancer cells.54 In gastric, breast, liver, and colon cancer, the existence of Snail influences the dedifferentiated phenotype of cancer cells, conveying that differentiated cancer cells transform into less differentiated cancer cells after undergoing EMT.55 These less differentiated cancer cells exhibit a more CSC-like phenotype.

The EMT confers cancer cells not only stemness properties but also mesenchymal properties.56 For instance, breast cancer cell lines with a high population of CD44+/CD24- cells exhibit both stem/progenitor cell properties and mesenchymal markers.57 Similar findings were also observed in nontumorigenic immortalized human mammary epithelial cells (HMLEs).58 Ectopic expression of Twist or Snail induces EMT, a process in which mesenchymal-like cells with a CD44 high/CD24 low pattern are created.59 This unsurprisingly indicates that HMLEs become more stem-like as a result of undergoing the EMT. These acquired stem cell properties can also be demonstrated using functional assays.60 Scientists had long been testing cancer cells' ability to form tumorspheres to determine their state of stemness. When Snail or Twist is upregulated, HMLEs form more than 30-fold the amount of its current tumorspheres.61 Additionally, the number of CSCs increases two-fold as Twist or Snail, EMT-inducing transcription factors, are overexpressed.61

Researchers have already evaluated breast cancer cells' state by examining their CD24 expression.62 They found that CD24-negative mesenchymal-like cells, unlike CD24-positive epithelial-like cells, were able to form tumorspheres and therefore expand their tumor.62 Cells transitioned from CD24-positive to CD24-negative through EMT, which

Mechanism of EMT-induced Cancer Stemness

How the EMT mechanism gives cancer cells acquired stemness traits is still not totally understood.68 However scientists can approach this by examining the EMT transcription factors, such as Twist, Snail, and Zeb, which can modulate non-coding RNA expression.69 miRNAs are small non-coding RNA molecules significantly affecting cell differentiation as they function in the post-transcriptional period of gene expression regulation.70 Therefore, the factors that affect the expression and processing of miRNA could play a pivotal role in the cells' stemness or differentiation.

miRNAs that promote differentiation include the let-7 family and miR-34. The let-7 family can be observed in all somatic cells, so they can be seen as an obstacle to maintaining cells' stemness.71 Scientists have classified the let-7 family as a group of tumor suppressors due to their pluripotency and various oncogenic traits.71 For instance, let-7 can inhibit Lin28, a gene responsible for self-renewal within cells.72 As a result, decreased expression of Myc and Sall4 occurs.72 Scientists are still understanding the mechanism in which let-7 loss happens. Let-7 genes can be removed through the reprogramming of somatic cells using additional transcription factors such as Oct4, Sox2, Klf4, and c-Myc, which induce pluripotency into differentiated cells.73

In cellular reprogramming, the inhibition of let-7 allows for the pluripotency targets to be expressed, thus increasing efficiency.74 When Snail binds to the let-7 promoters, its expression increases while that of let-7 decreases.75 In pluripotent stem cells, low levels of let-7 are maintained by Lin28, which prevents the processing and production of let-7.76 Additionally, Twist, another one of three main EMT transcription factors, also represses let-7 expression.77 Thus, it can be concluded that the EMT factors are a major repression source of let-7 and other tumor-suppressor miRNAs.

Like let-7, miR-34 inhibits pluripotency factors so that cells don't acquire stemness traits.78 As a p53 target, its expression is likely dysregulated in p53-mutated cancers.79 When Snail represses miR-34 expression by binding to the miR-34 promoter, cells in EMT develop their stemness phenotype.80

Aside from let-7 and miR-34, many alternative miRNAs could influence the establishment of the stem cells in tumors. Despite the power let-7 has in differentiating cells, it lacks in comparison to the power of miRNAs that activate self-renewal, called ESCC (ESC-specific cell cycle) miRNAs.81 The miR290 cluster is an example of ESCC miRNAs.82 Therefore when let-7 is downregulated, cell self-renewal is more unlikely, especially in conjunction with the expression of ESCC miRNAs. This shows how the path that cells take to acquire stemness traits is complicated as there are "checks and balances" that keep the cell in its differentiated state.

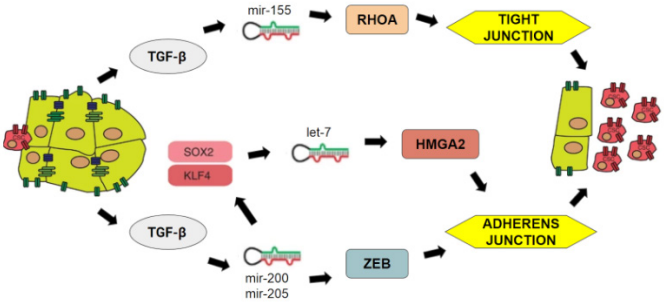

Figure 2: microRNAs can be seen as a mediator of EMT, which causes cancer cells' stemness. EMT induced by TGF-β is activated by mir-155, a microRNA that could target RHOA to break down tight junctions, which ultimately results in the creation of more cancer stem cells.83 TGF-β can also modulate restrictive actions against mir-200 and mir-205, which increases levels of Zeb protein, which decreases levels of E-cadherin, which causes adherens junctions breakdown.84 Increased levels of Zeb can also come back and repress mir-200 and mir-205 by promoting Sox2 and Klf4, two attackers of mir-200.85 This triggers the renewal of cancer stem cells. TGF-β can also cause downregulation of E-cadherin by increasing HMGA2 activity.86 The let-7 microRNA could block CSC's self-renewal and repress HMGA2, a molecule that helps in E-cadherin repression. Therefore, it can be inferred that there is a correlation between TGF-β signaling, EMT, and the emergence of CSCs.

Since 1991, the association between the loss of E-cadherin and the EMT causing tumor dedifferentiation has been observed. Recent discoveries show that the expression of individual EMT-inducing transcription factors such as Snail, Twist, or Zeb, can transform the cancer cells into cancer stem cells. ZEB1, an EMT transcription factor in carcinoma cells, is unusually overexpressed in pancreatic cancer, causing the stem-like phenotypes in previously non-stem-like cells.87 ZEB1 has also been shown to be a significant player in the stage of tumor initiation in a xenograft model.88 Similar to how Snail dedifferentiates cells by inducing expression of let-7 and miR-34, the EMT transcription factor ZEB1 inhibits many miRNAs such as miR-203, miR-200, and miR-183 so that stemness factors such as Sox2, KLF4, and BMI1, get upregulated.89 Hence, ZEB1 can be linked to the EMT and cancer cell dedifferentiation (stemness). EMT in ovarian cancer development turns the epithelial cells into stem-like cells called mesenchymal cells. EMT factors inhibit pathways and proteins including NF-kB, tumor necrosis factor alpha, β-catenin, and p53 so that cells don't differentiate and stay in their pluripotent state.90

Another way EMT factors can give cells stemness traits

is through the prevention of senescence.91 Somatic cells' life span is limited and when differentiation ceases, their dividing and self-renewing ability also cease. However, both stem and cancer cells are able to overcome this limit, which allows them to proliferate at a nonrestrictive pace. The EMT transcriptional factors Snail and Twist could suppress tumor suppressors, say, cycle regulator p16, which enhances the cancer cell's ability to self-renew and grow.92 Thus, it can be concluded that there are many different molecular mechanisms at play in the process of cells gaining EMT-driven stemness. The EMT correlation with stemness can also be seen at a clinical level. Researchers use gene expression profiling to design a system that could quantitatively score tumors on their EMT state.93 This system can identify the distinct EMT states across different tumor types.94 The reported gene expression, given by the system's molecular subtypes, shows that they closely represent the EMT status in ovarian cancer. The prognosis of ovarian cancer patients is defined by clinicopathological parameters, such as the extent of metastasis damage and cell resistance to chemotherapy.95 The higher the EMT score, the worse the prognosis. A higher level of pluripotency and stemness also indicates a higher level of chemoresistance and more chance of metastasis. It can therefore be logically inferred that subtypes with higher EMT scores are associated with the dedifferentiated state. These findings sum up to the conclusion that cancer cells acquire potency or stemness after undergoing the EMT, which shows how the EMT can cause dedifferentiation in cancer cells.

EMT-induced Therapeutic Resistance

Mediators of EMT enhanced not only cellular motility but also cellular survival. In an environment of serum starvation and TNF-α treatment, the expression of Snail in Madin–Darby Canine Kidney (MDCK) cells strengthens its resistance.96 This anti-apoptotic cellular reaction was correlated with Snail expression, through the activation of both the MAPK and the PI3K pathways.97 Through inhibiting pro-apoptotic factors such as p53, DNA Fragmentation Factor 40, and BH3-Interacting Domain Death Agonist, Slug that was transfected into MCF7 breast cancer cells causes cells' resistance to programmed cell death (due to DNA damage).98 After scientists found that EMT links to enhanced survival pathways and anti-apoptotic behaviors, they are interested in exploring how EMT causes cells' resistance to anti-neoplastic therapeutic strategies.

Recent studies have investigated the cancer cells' acquired chemotherapy resistance due to EMT upregulation causing molecular alterations in gastrointestinal malignancies. Pancreatic cancer cells' acquired gemcitabine resistance showed changed behaviour and phenotypes that aligned with EMT.99 For example, the resistant pancreatic cancer cells experienced upregulated vimentin, lack of E-cadherin expression, and β-catenin nuclear translocation.100 Applying chronic oxaliplatin exposure to CRC cells can trigger resistant EMT-correlated phenotypic mutations including loss of polarity, spindle shape, and increased mobility.101 The oxaliplatin-induced resistant cells, as predicted, showed decreased levels of E-cadherin in sync with increased levels of snail and vimentin, all hallmarks distinctive of EMT.

These studies demonstrate that cancer cells undergo EMT to adopt a new anti-apoptotic and pro-survival state when they are induced with stress from chemotherapy. Alternatively, rather than chemotherapy inducing EMT, chemotherapy may result in clonal selection and propagation of cells with enhanced pro-survival pathway activation as observed with EMT.102 Therefore, EMT pathways can also be seen as a direct mechanism or mediator of chemotherapy resistance.103 For instance, Panc-1 cancer cells with transfection of Snail adopted unusual EMT traits that make them sensitive to chemotherapy treatment such as 5-fluorouracil and gemcitabine.104 More recently, manually-programmed expression of Snail in colon cancer cells increases the CSC population and phenotype that explains its oxaliplatin resistance.105 Specifically, snail-expressing HCT116 and HT29 cells, two types of colorectal cancer stem cells, demonstrated EMT-linked morphological, functional, and molecular characteristics such as having a 10-fold resistance to oxaliplatin.105

While anti-gastrointestinal-malignancy drugs have shown partial success in compromising vascular endothelial growth factor and epidermal growth factor receptors, a fully successful treatment is still far away due to roadblocks such as therapeutics resistance. More importantly, scientists have examined the influence of EMT in these molecule-targeting therapy's outcomes. It was discovered that there was erlotinib resistance in head and neck squamous cell carcinoma.106 Erlotinib is a tyrosine kinase inhibitor of epidermal growth factor receptors.107 An upregulation of vimentin contrasting to the downregulation of claudin-4, E-cadherin, and claudin-7 that supported this resistance is what microassay and western blot analysis shows.108 Conclusively, this change in protein expression pattern resembles distinguishable traits of cells undergoing EMT.

To further understand how EMT relates to drug resistance, researchers have used the tumor from patients, whose cancer cells prove the erlotinib to be ineffective, for investigation. What they found was that after being treated with erlotinib, tumors with E-cadherin depletion take a shorter time to progress compared to tumors with E-cadherin bulges. Unsurprisingly, non-small lung cancer cells that were treated with gefitinib (another tyrosine kinase inhibitor of epidermal growth factor receptor) also show the same pattern of low E-cadherin results in short time to progression and high E-cadherin results in long time to progression.109 Levels of E-cadherin were measured by immunohistochemical staining.110 Applying vascular endothelial growth factor receptor 1 and bevacizumab, an angiogenesis inhibitor targeting vascular endothelial growth factors, strengthen the multiple colon cancer cell lines' migratoratory and invasive properties.111 These recent discoveries demonstrating EMT's pivotal role in acquired chemoresistance show that it is necessary for the development of novel drugs or therapies that could successfully inhibit EMT pathways, which would predictably improve patient outcomes when used in sync with traditional therapies such as chemotherapy and radiation.

Conclusion

During cancer progression, the synergization between EMT and CSCs contributes a major part to the survival, aggressiveness, and mobility of a tumor. EMT and CSCs can be seen as mediators of cancer development since they guard cancer cells against harmful environments or drugs and promote metastasis for long-term survival and worsened malignancy. Even though scientists are currently in a period where neoadjuvant treatment is commonly used, it should be carefully considered before use because these treatments could trigger more aggressive cancer phenotypes by conferring cancer cells the capability of metastasis and resistance. This concern suggests that researchers need to spend more time to further investigate the impact clinical therapy has on the pathobiological advancement of cancer. As discussed previously, methods blocking EMT pathways and subsequently canceling the maintenance of CSC prove to be future endeavors worthy of attention and trials. Luckily, this path is feasible as there have already been studies conducted to identify certain pharmacological agents capable of regulating the tumor's state of differentiation. CSCs, cancer cells in the dedifferentiated or pluripotent state, can be compromised through the promotion of their differentiating ability.47

Therefore, agents that could induce cancer stem cells to differentiate into a more niche, less pluripotent state, namely salinomycin or HDAC inhibitors, may hold significant therapeutic potential.112 Alternatively, both the TGF-β and Wnt pathways could be targeted as a strategy to decelerate the rate of EMT, which will lead to downregulation of CSCs.113 These pathways activate not only EMT but also anti-apoptotic signaling, including the ones involving PI3K and nuclear factor-κB. Since PI3K and Akt play a major role in EMT, inhibiting them with the purpose of eliminating EMT and the rise of CSCs may show promising results. Lastly, since microRNAs contribute to the modulation of EMT and CSC emergence, injections of microRNAs manually designed to decrease EMT can also be seen as another bright path towards disrupting cancer progression.

To sum up, researchers are having a much better understanding of how EMT carries out functions to improve cellular mobility and survival, which ultimately aids in tumor malignancy. It is well understood that EMT plays a pivotal role in the development of aggressive CSCs, no matter which organ the tumor originates from. Aside from being an effective marker of cancer aggressiveness and therapeutic resistance, EMT and CSC molecular pathways can also be targeted therapeutically to advance the fight against cancer.

References

1. Kalluri, Raghu, and Robert A Weinberg. "The basics of epithelial-mesenchymal transition." The Journal of clinical investigation vol. 119,6 (2009): 1420-8. doi:10.1172/JCI39104
2. Lamouille, Samy et al. "Molecular mechanisms of epithelial-mesenchymal transition." Nature reviews. Molecular cell biology vol. 15,3 (2014): 178-96. doi:10.1038/nrm3758
3. Roche, Joëlle. "The Epithelial-to-Mesenchymal Transition in Cancer." Cancers vol. 10,2 52. 16 Feb. 2018, doi:10.3390/cancers10020052
4. Lee, Jonathan M et al. "The epithelial-mesenchymal transition: new insights in signaling, development, and disease." The Journal of cell biology vol. 172,7 (2006): 973-81. doi:10.1083/jcb.200601018
5. Wang, Yifan et al. "The Role of Snail in EMT and Tumorigenesis." Current cancer drug targets vol. 13,9 (2013): 963-972. doi:10.2174/15680096113136660102
6. Kaufhold, S., Bonavida, B. Central role of Snail1 in the regulation of EMT and resistance in cancer: a target for therapeutic intervention. J Exp Clin Cancer Res 33, 62 (2014). https://doi.org/10.1186/s13046-014-0062-0
7. Garg, Minal. "Epithelial-mesenchymal transition - activating transcription factors - multifunctional regulators in cancer." World journal of stem cells vol. 5,4 (2013): 188-95. doi:10.4252/wjsc.v5.i4.188
8. Yu, Zuoren et al. "Cancer stem cells." The international journal of biochemistry & cell biology vol. 44,12 (2012): 2144-51. doi:10.1016/j.biocel.2012.08.022
9. Mohan, Amrutha et al. "Reporters of Cancer Stem Cells as a Tool for Drug Discovery." Frontiers in oncology vol. 11 669250. 22 Apr. 2021, doi:10.3389/fonc.2021.669250
10. Wang, Sha-Sha et al. "Links between cancer stem cells and epithelial-mesenchymal transition." OncoTargets and therapy vol. 8 2973-80. 16 Oct. 2015, doi:10.2147/OTT.S91863
11. Zhang, Jingyu et al. "Signal Transduction Pathways of EMT Induced by TGF-β, SHH, and WNT and Their Crosstalks." Journal of clinical medicine vol. 5,4 41. 28 Mar. 2016, doi:10.3390/jcm5040041
12. Kalluri R. (2009). EMT: when epithelial cells decide to become mesenchymal-like cells. The Journal of clinical investigation, 119(6), 1417–1419. https://doi.org/10.1172/JCI39675
13. Gonzalez, David M, and Damian Medici. "Signaling mechanisms of the epithelial-mesenchymal transition." Science signaling vol. 7,344 re8. 23 Sep. 2014, doi:10.1126/scisignal.2005189
14. Sadlecki, P., Jóźwicki, J., Antosik, P., & Grabiec, M. (2018). Expression of selected epithelial-mesenchymal transition transcription factors in serous borderline ovarian tumors and type I ovarian cancers. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, 40(6), 1010428318784807. https://doi.org/10.1177/1010428318784807
15. Tam, Shing Yau et al. "Hypoxia-Induced Epithelial-Mesenchymal Transition in Cancers: HIF-1α and Beyond." Frontiers in oncology vol. 10 486. 8 Apr. 2020, doi:10.3389/fonc.2020.00486
16. Hollestelle, A., Peeters, J. K., Smid, M., Timmermans, M., Verhoog, L. C., Westenend, P. J., Heine, A. A., Chan, A., Sieuwerts, A. M., Wiemer, E. A., Klijn, J. G., van der Spek, P. J., Foekens, J. A., Schutte, M., den Bakker, M. A., & Martens, J. W. (2013). Loss of E-cadherin is not a necessity for epithelial to mesenchymal transition in human breast cancer. Breast cancer research and treatment, 138(1), 47–57. https://doi.org/10.1007/s10549-013-2415-3
17. Ohkubo, T., & Ozawa, M. (2004). The transcription factor Snail downregulates the tight junction components independently of E-cadherin downregulation. Journal of cell science, 117(Pt 9), 1675–1685. https://doi.org/10.1242/jcs.01004
18. Lin, C. Y., Tsai, P. H., Kandaswami, C. C., Lee, P. P., Huang, C. J., Hwang, J. J., & Lee, M. T. (2011). Matrix metalloproteinase-9 cooperates with transcription factor Snail to induce epithelial-mesenchymal transition. Cancer science, 102(4), 815–827. https://doi.org/10.1111/j.1349-7006.2011.01861.x
19. Galván, J. A., Zlobec, I., Wartenberg, M., Lugli, A., Gloor, B., Perren, A., & Karamitopoulou, E. (2015). Expression of E-cadherin repressors SNAIL, ZEB1 and ZEB2 by tumour and stromal cells influences tumour-budding phenotype and suggests heterogeneity of stromal cells in pancreatic cancer. British journal of cancer, 112(12), 1944–1950. https://doi.org/10.1038/bjc.2015.177
20. Lee, M. S., Lowe, G. N., Strong, D. D., Wergedal, J. E., & Glackin, C. A. (1999). TWIST, a basic helix-loop-helix transcription factor, can regulate the human osteogenic lineage. Journal of cellular biochemistry, 75(4), 566–577. https://doi.org/10.1002/(sici)1097-4644(19991215)75:4<566::aid-jcb3>3.0.co;2-0
21. Li, L., Cserjesi, P., & Olson, E. N. (1995). Dermo-1: a novel twist-related bHLH protein expressed in the developing dermis. Developmental biology, 172(1), 280–292. https://doi.org/10.1006/dbio.1995.0023
22. Kress, W., Schropp, C., Lieb, G., Petersen, B., Büsse-Ratzka, M., Kunz, J., Reinhart, E., Schäfer, W. D., Sold, J., Hoppe, F., Pahnke, J., Trusen, A., Sörensen, N., Krauss, J., & Collmann, H. (2006). Saethre-Chotzen syndrome caused by TWIST 1 gene mutations: functional differentiation from Muenke coronal synostosis syndrome. European journal of human genetics : EJHG, 14(1), 39–48. https://doi.org/10.1038/sj.ejhg.5201507
23. Zhu, Q. Q., Ma, C., Wang, Q., Song, Y., & Lv, T. (2016). The role of TWIST1 in epithelial-mesenchymal transition and cancers. Tumour biology : the journal of the International Society for

Oncodevelopmental Biology and Medicine, 37(1), 185–197. https://doi.org/10.1007/s13277-015-4450-7
24. Oh, Bo Young et al. "Twist1-induced epithelial-mesenchymal transition according to microsatellite instability status in colon cancer cells." Oncotarget vol. 7,35 (2016): 57066-57076. doi:10.18632/oncotarget.10974
25. Li, Y., Wang, W., Wang, W., Yang, R., Wang, T., Su, T., Weng, D., Tao, T., Li, W., Ma, D., & Wang, S. (2012). Correlation of TWIST2 up-regulation and epithelial-mesenchymal transition during tumorigenesis and progression of cervical carcinoma. Gynecologic oncology, 124(1), 112–118. https://doi.org/10.1016/j.ygyno.2011.09.003
26. Zhang, P., Sun, Y., & Ma, L. (2015). ZEB1: at the crossroads of epithelial-mesenchymal transition, metastasis and therapy resistance. Cell cycle (Georgetown, Tex.), 14(4), 481–487. https://doi.org/10.1080/15384101.2015.1006048
27. Xavier, P., Cordeiro, Y. G., Rochetti, A. L., Sangalli, J. R., Zuccari, D., Silveira, J. C., Bressan, F. F., & Fukumasu, H. (2018). ZEB1 and ZEB2 transcription factors are potential therapeutic targets of canine mammary cancer cells. Veterinary and comparative oncology, 16(4), 596–605. https://doi.org/10.1111/vco.12427
28. Bhat, Ajaz A et al. "Tight Junction Proteins and Signaling Pathways in Cancer and Inflammation: A Functional Crosstalk." Frontiers in physiology vol. 9 1942. 23 Jan. 2019, doi:10.3389/fphys.2018.01942
29. Cao, Zheng et al. "Anoikis and EMT: Lethal "Liaisons" during Cancer Progression." Critical reviews in oncogenesis vol. 21,3-4 (2016): 155-168. doi:10.1615/CritRevOncog.2016016955
30. Gilmore, A. Anoikis. Cell Death Differ 12, 1473–1477 (2005). https://doi.org/10.1038/sj.cdd.4401723
31. Kim, Yong-Nyun et al. "Anoikis resistance: an essential prerequisite for tumor metastasis." International journal of cell biology vol. 2012 (2012): 306879. doi:10.1155/2012/306879
32. Simpson, C. D., Anyiwe, K., & Schimmer, A. D. (2008). Anoikis resistance and tumor metastasis. Cancer letters, 272(2), 177–185. https://doi.org/10.1016/j.canlet.2008.05.029
33. Frisch, Steven M et al. "Mechanisms that link the oncogenic epithelial-mesenchymal transition to suppression of anoikis." Journal of cell science vol. 126,Pt 1 (2013): 21-9. doi:10.1242/jcs.120907
34. Kizhatil, K., Davis, J. Q., Davis, L., Hoffman, J., Hogan, B. L., & Bennett, V. (2007). Ankyrin-G is a molecular partner of E-cadherin in epithelial cells and early embryos. The Journal of biological chemistry, 282(36), 26552–26561. https://doi.org/10.1074/jbc.M703158200
35. Yang, Q., Pan, Q., Li, C. et al. NRAGE is involved in homologous recombination repair to resist the DNA-damaging chemotherapy and composes a ternary complex with RNF8–BARD1 to promote cell survival in squamous esophageal tumorigenesis. Cell Death Differ 23, 1406–1416 (2016). https://doi.org/10.1038/cdd.2016.29
36. Kumar, S., Park, S. H., Cieply, B., Schupp, J., Killiam, E., Zhang, F., Rimm, D. L., & Frisch, S. M. (2011). A pathway for the control of anoikis sensitivity by E-cadherin and epithelial-to-mesenchymal transition. Molecular and cellular biology, 31(19), 4036–4051. https://doi.org/10.1128/MCB.01342-10
37. Morciano, G.; Vezzani, B.; Missiroli, S.; Boncompagni, C.; Pinton, P.; Giorgi, C. An Updated Understanding of the Role of YAP in Driving Oncogenic Responses. Cancers 2021, 13, 3100. https://doi.org/10.3390/cancers13123100
38. Li, P., Liang, Z., Hou, G., Song, L., Zhang, R., Gan, Y., Zhang, C., Xu, Y., & Zhou, Q. (2017). N-Cadherin-Mediated Activation of PI3K/Akt-GSK-3β Signaling Attenuates Nucleus Pulposus Cell Apoptosis Under High-Magnitude Compression. Cellular physiology and biochemistry : international journal of experimental cellular physiology, biochemistry, and pharmacology, 44(1), 229–239. https://doi.org/10.1159/000484649
39. Xie, Feng et al. "Simvastatin blocks TGF-β1-induced epithelial-mesenchymal transition in human prostate cancer cells." Oncology letters vol. 11,5 (2016): 3377-3383. doi:10.3892/ol.2016.4404
40. Arildsen, N. S., & Hedenfalk, I. (2020). Simvastatin is a potential candidate drug in ovarian clear cell carcinomas. Oncotarget, 11(40), 3660–3674. https://doi.org/10.18632/oncotarget.27747
41. Herbertz, S., Sawyer, J. S., Stauber, A. J., Gueorguieva, I., Driscoll, K. E., Estrem, S. T., Cleverly, A. L., Desaiah, D., Guba, S. C., Benhadji, K. A., Slapak, C. A., & Lahn, M. M. (2015). Clinical development of galunisertib (LY2157299 monohydrate), a small molecule inhibitor of transforming growth factor-beta signaling pathway. Drug design, development and therapy, 9, 4479–4499. https://doi.org/10.2147/DDDT.S86621
42. Zheng, X., Dai, F., Feng, L., Zou, H., Feng, L., & Xu, M. (2021). Communication Between Epithelial-Mesenchymal Plasticity and Cancer Stem Cells: New Insights Into Cancer Progression. Frontiers in oncology, 11, 617597. https://doi.org/10.3389/fonc.2021.617597
43. Castillo, V., Valenzuela, R., Huidobro, C., Contreras, H. R., & Castellon, E. A. (2014). Functional characteristics of cancer stem cells and their role in drug resistance of prostate cancer. International journal of oncology, 45(3), 985–994. https://doi.org/10.3892/ijo.2014.2529
44. Yang, L., Shi, P., Zhao, G. et al. Targeting cancer stem cell pathways for cancer therapy. Sig Transduct Target Ther 5, 8 (2020). https://doi.org/10.1038/s41392-020-0110-5
45. Bahnassy, A. A., Fawzy, M., El-Wakil, M., Zekri, A. R., Abdel-Sayed, A., & Sheta, M. (2015). Aberrant expression of cancer stem cell markers (CD44, CD90, and CD133) contributes to disease progression and reduced survival in hepatoblastoma patients: 4-year survival data. Translational research : the journal of laboratory and clinical medicine, 165(3), 396–406. https://doi.org/10.1016/j.trsl.2014.07.009
46. O'Brien, C. A., Kreso, A., & Jamieson, C. H. (2010). Cancer stem cells and self-renewal. Clinical cancer research : an official journal of the American Association for Cancer Research, 16(12), 3113–3120. https://doi.org/10.1158/1078-0432.CCR-09-2824
47. Wang, H., & Unternaehrer, J. J. (2019). Epithelial-mesenchymal Transition and Cancer Stem Cells: At the Crossroads of Differentiation and Dedifferentiation. Developmental dynamics : an official publication of the American Association of Anatomists, 248(1), 10–20. https://doi.org/10.1002/dvdy.24678
48. Visweswaran, M., Pohl, S., Arfuso, F., Newsholme, P., Dilley, R., Pervaiz, S., & Dharmarajan, A. (2015). Multi-lineage differentiation of mesenchymal stem cells - To Wnt, or not Wnt. The international journal of biochemistry & cell biology, 68, 139–147. https://doi.org/10.1016/j.biocel.2015.09.008
49. González-González, Rogelio et al. "Epithelial-Mesenchymal Transition Associated with Head and Neck Squamous Cell Carcinomas: A Review." Cancers vol. 13,12 3027. 17 Jun. 2021, doi:10.3390/cancers13123027
50. Shao, Jing-Zhi et al. "In vitro inhibition of proliferation, migration and epithelial-mesenchymal transition of human lens epithelial cells by fasudil." International journal of ophthalmology vol. 11,8 1253-1257. 18 Aug. 2018, doi:10.18240/ijo.2018.08.02
51. Mani, Sendurai A et al. "The epithelial-mesenchymal transition generates cells with properties of stem cells." Cell vol. 133,4 (2008): 704-15. doi:10.1016/j.cell.2008.03.027
52. Vesuna, F., Lisok, A., Kimble, B., & Raman, V. (2009). Twist modulates breast cancer stem cells by transcriptional regulation of CD24 expression. Neoplasia (New York, N.Y.), 11(12), 1318–1328. https://doi.org/10.1593/neo.91084
53. Kroepil, Feride et al. "Snail1 expression in colorectal cancer and its correlation with clinical and pathological parameters." BMC cancer vol. 13 145. 22 Mar. 2013, doi:10.1186/1471-2407-13-145
54. Aigner, K., Dampier, B., Descovich, L., Mikula, M., Sultan, A., Schreiber, M., Mikulits, W., Brabletz, T., Strand, D., Obrist, P., Sommergruber, W., Schweifer, N., Wernitznig, A., Beug, H., Foisner, R., & Eger, A. (2007). The transcription factor ZEB1 (deltaEF1) promotes tumour cell dedifferentiation by repressing master regulators of epithelial polarity. Oncogene, 26(49), 6979–6988. https://doi.org/10.1038/sj.onc.1210508
55. Yasui, K., Shimamura, M., Mitsutake, N., & Nagayama, Y. (2013). SNAIL induces epithelial-to-mesenchymal transition and cancer stem cell-like properties in aldehyde dehydroghenase-negative thyroid cancer cells. Thyroid : official journal of the American Thyroid Association, 23(8), 989–996. https://doi.org/10.1089/thy.2012.0319
56. Ricciardi, M., Zanotto, M., Malpeli, G. et al. Epithelial-to-mesenchymal transition (EMT) induced by inflammatory priming elicits mesenchymal stromal cell-like immune-modulatory properties in cancer cells. Br J Cancer 112, 1067–1075 (2015). https://doi.org/10.1038/bjc.2015.29
57. Sheridan, C., Kishimoto, H., Fuchs, R. K., Mehrotra, S., Bhat-Nakshatri, P., Turner, C. H., Goulet, R., Jr, Badve, S., & Nakshatri, H. (2006). CD44+/CD24- breast cancer cells exhibit enhanced invasive properties: an early step necessary for metastasis. Breast cancer research : BCR, 8(5), R59. https://doi.org/10.1186/bcr1610
58. Joshi, P. S., Modur, V., Cheng, J., Robinson, K., & Rao, K. (2017). Characterization of immortalized human mammary epithelial cell line HMEC 2.6. Tumour biology : the journal of the International Society for Oncodevelopmental Biology and Medicine, 39(10), 1010428317724283. https://doi.org/10.1177/1010428317724283
59. Kidan, Noa et al. "Ectopic Expression of Snail and Twist in Ph+ Leukemia Cells Upregulates CD44 Expression and Alters Their Differentiation Potential." Journal of Cancer vol. 8,19 3952-3968. 23 Oct. 2017, doi:10.7150/jca.19633
60. de Lucas, B., Pérez, L. M., & Gálvez, B. G. (2019). Functional Assays of Stem Cell Properties Derived from Different Niches. Methods

in molecular biology (Clifton, N.J.), 2002, 29–38. https://doi.org/10.1007/7651_2018_175
61. Malouf, Gabriel G et al. "Architecture of epigenetic reprogramming following Twist1-mediated epithelial-mesenchymal transition." Genome biology vol. 14,12 R144. 24 Dec. 2013, doi:10.1186/gb-2013-14-12-r144
62. Ortiz-Montero, Paola et al. "CD24 expression and stem-associated features define tumor cell heterogeneity and tumorigenic capacities in a model of carcinogenesis." Cancer management and research vol. 10 5767-5784. 16 Nov. 2018, doi:10.2147/CMAR.S176654
63. Katsuno, Y., Lamouille, S., & Derynck, R. (2013). TGF-β signaling and epithelial-mesenchymal transition in cancer progression. Current opinion in oncology, 25(1), 76–84. https://doi.org/10.1097/CCO.0b013e32835b6371
64. Lim, S., Becker, A., Zimmer, A., Lu, J., Buettner, R., & Kirfel, J. (2013). SNAI1-mediated epithelial-mesenchymal transition confers chemoresistance and cellular plasticity by regulating genes involved in cell death and stem cell maintenance. PloS one, 8(6), e66558. https://doi.org/10.1371/journal.pone.0066558
65. Olmeda, D., Montes, A., Moreno-Bueno, G. et al. Snai1 and Snai2 collaborate on tumor growth and metastasis properties of mouse skin carcinoma cell lines. Oncogene 27, 4690–4701 (2008). https://doi.org/10.1038/onc.2008.118
66. Chui M. H. (2013). Insights into cancer metastasis from a clinicopathologic perspective: Epithelial-Mesenchymal Transition is not a necessary step. International journal of cancer, 132(7), 1487–1495. https://doi.org/10.1002/ijc.27745
67. Prieto-García, E., Díaz-García, C. V., García-Ruiz, I., & Agulló-Ortuño, M. T. (2017). Epithelial-to-mesenchymal transition in tumor progression. Medical oncology (Northwood, London, England), 34(7), 122. https://doi.org/10.1007/s12032-017-0980-8
68. Tiwari, N., Gheldof, A., Tatari, M., & Christofori, G. (2012). EMT as the ultimate survival mechanism of cancer cells. Seminars in cancer biology, 22(3), 194–207. https://doi.org/10.1016/j.semcancer.2012.02.013
69. Garg, Minal. "Epithelial-mesenchymal transition - activating transcription factors - multifunctional regulators in cancer." World journal of stem cells vol. 5,4 (2013): 188-95. doi:10.4252/wjsc.v5.i4.188
70. Ling, Hui et al. "MicroRNAs and other non-coding RNAs as targets for anticancer drug development." Nature reviews. Drug discovery vol. 12,11 (2013): 847-65. doi:10.1038/nrd4140
71. Su, J. L., Chen, P. S., Johansson, G., & Kuo, M. L. (2012). Function and regulation of let-7 family microRNAs. MicroRNA (Shariqah, United Arab Emirates), 1(1), 34–39. https://doi.org/10.2174/2211536611201010034
72. Yermalovich, A.V., Osborne, J.K., Sousa, P. et al. Lin28 and let-7 regulate the timing of cessation of murine nephrogenesis. Nat Commun 10, 168 (2019). https://doi.org/10.1038/s41467-018-08127-4
73. Worringer, K. A., Rand, T. A., Hayashi, Y., Sami, S., Takahashi, K., Tanabe, K., Narita, M., Srivastava, D., & Yamanaka, S. (2014). The let-7/LIN-41 pathway regulates reprogramming to human induced pluripotent stem cells by controlling expression of prodifferentiation genes. Cell stem cell, 14(1), 40–52. https://doi.org/10.1016/j.stem.2013.11.001
74. Akao Y, Nakagawa Y, Naoe T. let-7 microRNA functions as a potential growth suppressor in human colon cancer cells. Biol Pharm Bull. 2006;29:903–6.
75. Unternaehrer, Juli J et al. "The epithelial-mesenchymal transition factor SNAIL paradoxically enhances reprogramming." Stem cell reports vol. 3,5 (2014): 691-8. doi:10.1016/j.stemcr.2014.09.008
76. Thornton, James E, and Richard I Gregory. "How does Lin28 let-7 control development and disease?." Trends in cell biology vol. 22,9 (2012): 474-82. doi:10.1016/j.tcb.2012.06.001
77. Chirshev, Evgeny et al. "Let-7 as biomarker, prognostic indicator, and therapy for precision medicine in cancer." Clinical and translational medicine vol. 8,1 24. 28 Aug. 2019, doi:10.1186/s40169-019-0240-y
78. Choi, Y. J., Lin, C. P., Ho, J. J., He, X., Okada, N., Bu, P., Zhong, Y., Kim, S. Y., Bennett, M. J., Chen, C., Ozturk, A., Hicks, G. G., Hannon, G. J., & He, L. (2011). miR-34 miRNAs provide a barrier for somatic cell reprogramming. Nature cell biology, 13(11), 1353–1360. https://doi.org/10.1038/ncb2366
79. Okada, Nobuhiro et al. "A positive feedback between p53 and miR-34 miRNAs mediates tumor suppression." Genes & development vol. 28,5 (2014): 438-50. doi:10.1101/gad.233585.113
80. Siemens, H., Jackstadt, R., Hünten, S., Kaller, M., Menssen, A., Götz, U., & Hermeking, H. (2011). miR-34 and SNAIL form a double-negative feedback loop to regulate epithelial-mesenchymal transitions. Cell cycle (Georgetown, Tex.), 10(24), 4256–4271. https://doi.org/10.4161/cc.10.24.18552
81. Mens, Michelle M J, and Mohsen Ghanbari. "Cell Cycle Regulation of Stem Cells by MicroRNAs." Stem cell reviews and reports vol. 14,3 (2018): 309-322. doi:10.1007/s12015-018-9808-y
82. Lichner, Z., Páll, E., Kerekes, A., Pállinger, E., Maraghechi, P., Bosze, Z., & Gócza, E. (2011). The miR-290-295 cluster promotes pluripotency maintenance by regulating cell cycle phase distribution in mouse embryonic stem cells. Differentiation; research in biological diversity, 81(1), 11–24. https://doi.org/10.1016/j.diff.2010.08.002
83. Kong, William et al. "MicroRNA-155 is regulated by the transforming growth factor beta/Smad pathway and contributes to epithelial cell plasticity by targeting RhoA." Molecular and cellular biology vol. 28,22 (2008): 6773-84. doi:10.1128/MCB.00941-08
84. Duan, Yingjun, and Qianxue Chen. "TGF-β1 regulating miR-205/miR-195 expression affects the TGF-β signal pathway by respectively targeting SMAD2/SMAD7." Oncology reports vol. 36,4 (2016): 1837-44. doi:10.3892/or.2016.5023
85. Brabletz, Simone, and Thomas Brabletz. "The ZEB/miR-200 feedback loop--a motor of cellular plasticity in development and cancer?." EMBO reports vol. 11,9 (2010): 670-7. doi:10.1038/embor.2010.117
86. Thuault, Sylvie et al. "Transforming growth factor-beta employs HMGA2 to elicit epithelial-mesenchymal transition." The Journal of cell biology vol. 174,2 (2006): 175-83. doi:10.1083/jcb.200512110
87. Krebs, A. M., Mitschke, J., Lasierra Losada, M., Schmalhofer, O., Boerries, M., Busch, H., Boettcher, M., Mougiakakos, D., Reichardt, W., Bronsert, P., Brunton, V. G., Pilarsky, C., Winkler, T. H., Brabletz, S., Stemmler, M. P., & Brabletz, T. (2017). The EMT-activator Zeb1 is a key factor for cell plasticity and promotes metastasis in pancreatic cancer. Nature cell biology, 19(5), 518–529. https://doi.org/10.1038/ncb3513
88. Siebzehnrubl, Florian A et al. "The ZEB1 pathway links glioblastoma initiation, invasion and chemoresistance." EMBO molecular medicine vol. 5,8 (2013): 1196-212. doi:10.1002/emmm.201302827
89. Wellner, U., Schubert, J., Burk, U. C., Schmalhofer, O., Zhu, F., Sonntag, A., Waldvogel, B., Vannier, C., Darling, D., zur Hausen, A., Brunton, V. G., Morton, J., Sansom, O., Schüler, J., Stemmler, M. P., Herzberger, C., Hopt, U., Keck, T., Brabletz, S., & Brabletz, T. (2009). The EMT-activator ZEB1 promotes tumorigenicity by repressing stemness-inhibiting microRNAs. Nature cell biology, 11(12), 1487–1495. https://doi.org/10.1038/ncb1998
90. Rinkenbaugh, Amanda L, and Albert S Baldwin. "The NF-κB Pathway and Cancer Stem Cells." Cells vol. 5,2 16. 6 Apr. 2016, doi:10.3390/cells5020016
91. Smit, Marjon A, and Daniel S Peeper. "Epithelial-mesenchymal transition and senescence: two cancer-related processes are crossing paths." Aging vol. 2,10 (2010): 735-41. doi:10.18632/aging.100209
92. Zhu, P., Zhang, C., Gao, Y., Wu, F., Zhou, Y., & Wu, W. S. (2019). The transcription factor Slug represses p16Ink4a and regulates murine muscle stem cell aging. Nature communications, 10(1), 2568. https://doi.org/10.1038/s41467-019-10479-4
93. Dezső, Z., Oestreicher, J., Weaver, A., Santiago, S., Agoulnik, S., Chow, J., Oda, Y., & Funahashi, Y. (2014). Gene expression profiling reveals epithelial mesenchymal transition (EMT) genes can selectively differentiate eribulin sensitive breast cancer cells. PloS one, 9(8), e106131. https://doi.org/10.1371/journal.pone.0106131
94. Minafra, L., Bravatà, V., Forte, G. I., Cammarata, F. P., Gilardi, M. C., & Messa, C. (2014). Gene expression profiling of epithelial-mesenchymal transition in primary breast cancer cell culture. Anticancer research, 34(5), 2173–2183.
95. Lan, A., & Yang, G. (2019). Clinicopathological parameters and survival of invasive epithelial ovarian cancer by histotype and disease stage. Future oncology (London, England), 15(17), 2029–2039. https://doi.org/10.2217/fon-2018-0886
96. Haraguchi, M., Indo, H. P., Iwasaki, Y., Iwashita, Y., Fukushige, T., Majima, H. J., Izumo, K., Horiuchi, M., Kanekura, T., Furukawa, T., & Ozawa, M. (2013). Snail modulates cell metabolism in MDCK cells. Biochemical and biophysical research communications, 432(4), 618–625. https://doi.org/10.1016/j.bbrc.2013.02.035
97. Henderson, V., Smith, B., Burton, L. J., Randle, D., Morris, M., & Odero-Marah, V. A. (2015). Snail promotes cell migration through PI3K/AKT-dependent Rac1 activation as well as PI3K/AKT-independent pathways during prostate cancer progression. Cell adhesion & migration, 9(4), 255–264. https://doi.org/10.1080/19336918.2015.1013383
98. Elkholi, Rana et al. "The Role of BH3-Only Proteins in Tumor Cell Development, Signaling, and Treatment." Genes & cancer vol. 2,5

(2011): 523-37. doi:10.1177/1947601911417177
99. Amrutkar, Manoj, and Ivar P Gladhaug. "Pancreatic Cancer Chemoresistance to Gemcitabine." Cancers vol. 9,11 157. 16 Nov. 2017, doi:10.3390/cancers9110157
100. Wang, W., Dong, L., Zhao, B., Lu, J., & Zhao, Y. (2018). E-cadherin is downregulated by microenvironmental changes in pancreatic cancer and induces EMT. Oncology reports, 40(3), 1641–1649. https://doi.org/10.3892/or.2018.6528
101. Yang, A. D., Fan, F., Camp, E. R., van Buren, G., Liu, W., Somcio, R., Gray, M. J., Cheng, H., Hoff, P. M., & Ellis, L. M. (2006). Chronic oxaliplatin resistance induces epithelial-to-mesenchymal transition in colorectal cancer cell lines. Clinical cancer research : an official journal of the American Association for Cancer Research, 12(14 Pt 1), 4147–4153. https://doi.org/10.1158/1078-0432.CCR-06-0038
102. Greaves, Mel, and Carlo C Maley. "Clonal evolution in cancer." Nature vol. 481,7381 306-13. 18 Jan. 2012, doi:10.1038/nature10762
103. Smith, Bethany N, and Neil A Bhowmick. "Role of EMT in Metastasis and Therapy Resistance." Journal of clinical medicine vol. 5,2 17. 27 Jan. 2016, doi:10.3390/jcm5020017
104. Halloran, C. M., Ghaneh, P., Shore, S., Greenhalf, W., Zumstein, L., Wilson, D., Neoptolemos, J. P., & Costello, E. (2004). 5-Fluorouracil or gemcitabine combined with adenoviral-mediated reintroduction of p16INK4A greatly enhanced cytotoxicity in Panc-1 pancreatic adenocarcinoma cells. The journal of gene medicine, 6(5), 514–525. https://doi.org/10.1002/jgm.540
105. El Khoury, F., Corcos, L., Durand, S., Simon, B., & Le Jossic-Corcos, C. (2016). Acquisition of anticancer drug resistance is partially associated with cancer stemness in human colon cancer cells. International journal of oncology, 49(6), 2558–2568. https://doi.org/10.3892/ijo.2016.3725
106. Van Allen, Eliezer M et al. "Genomic Correlate of Exceptional Erlotinib Response in Head and Neck Squamous Cell Carcinoma." JAMA oncology vol. 1,2 (2015): 238-44. doi:10.1001/jamaoncol.2015.34
107. Ling, Y. H., Li, T., Yuan, Z., Haigentz, M., Jr, Weber, T. K., & Perez-Soler, R. (2007). Erlotinib, an effective epidermal growth factor receptor tyrosine kinase inhibitor, induces p27KIP1 up-regulation and nuclear translocation in association with cell growth inhibition and G1/S phase arrest in human non-small-cell lung cancer cell lines. Molecular pharmacology, 72(2), 248–258. https://doi.org/10.1124/mol.107.034827 (Retraction published Mol Pharmacol. 2015 Aug;88(2):390)
108. Lin, Xinjian et al. "Regulation of the Epithelial-Mesenchymal Transition by Claudin-3 and Claudin-4." PloS one vol. 8,6 e67496. 21 Jun. 2013, doi:10.1371/journal.pone.0067496
109. Richardson, F., Young, G. D., Sennello, R., Wolf, J., Argast, G. M., Mercado, P., Davies, A., Epstein, D. M., & Wacker, B. (2012). The evaluation of E-Cadherin and vimentin as biomarkers of clinical outcomes among patients with non-small cell lung cancer treated with erlotinib as second- or third-line therapy. Anticancer research, 32(2), 537–552.
110. Harigopal, Malini et al. "Aberrant E-cadherin staining patterns in invasive mammary carcinoma." World journal of surgical oncology vol. 3 73. 14 Nov. 2005, doi:10.1186/1477-7819-3-73
111. Ranieri, G., Patruno, R., Ruggieri, E., Montemurro, S., Valerio, P., & Ribatti, D. (2006). Vascular endothelial growth factor (VEGF) as a target of bevacizumab in cancer: from the biology to the clinic. Current medicinal chemistry, 13(16), 1845–1857. https://doi.org/10.2174/092986706777585059
112. Booth, L., Roberts, J. L., Conley, A., Cruickshanks, N., Ridder, T., Grant, S., Poklepovic, A., & Dent, P. (2014). HDAC inhibitors enhance the lethality of low dose salinomycin in parental and stem-like GBM cells. Cancer biology & therapy, 15(3), 305–316. https://doi.org/10.4161/cbt.27309
113. Katz, Lior H et al. "Targeting TGF-β signaling in cancer." Expert opinion on therapeutic targets vol. 17,7 (2013): 743-60. doi:10.1517/14728222.2013.782287

TikTok's algorithm: Does customer personalization come at the expense of magnifying filter bubbles, echo chambers and how may it foster ethnocentrism?

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Abstract

In the past two decades, the technological world has witnessed multiple developments in social media apps, with Facebook (2004), Youtube (2005), Instagram (2010), and Snapchat (2011), to name just a few. However, with the advent of TikTok, it has itself set unprecedented records. To illustrate, Bytedance's brainchild has become the most downloaded app[1] with over 2.6B times worldwide[2] and attracted over 1.5B monthly active users (compared to 690M monthly active users in 2018)[3]. TikTok users demonstrate a young demographic, with over 60% of them aged below 30[4]. The app, like its predecessors, has constantly been the bone of contention regarding public concerns about security and mass surveillance. While a large body of research has been conducted for social platforms, research on TikTok is still meager and lacks profundity. This research aims to contribute to research by attempting to examine how TikTok is creating echo chambers and whether it fosters ethnocentrism by studying its optimized algorithm, analyzing case studies and conducting a small-scale survey. The finding suggests that TikTok's influence on the formation of echo chambers and biased ideology is amplified by users' behaviors and inborn biases. This research also calls for heightened attention to this new yet underexplored landscape, propelling app developers to take ethical issues into account and alert users on the significance of media literacy.

Introduction

In this era of information decentralization, news comes from various corners. Since the advent of social media platforms, consumption of news has become fractionalized and we can more easily share news via social network platforms (Duffy and Ling 2020). The robust communities of TikTok, a newly-emerged video sharing and creating platform, are not an exception. TikTok is inundated with user-generated contents in which any details of life can be used as materials for creative expression[5]. Like other social media platforms, besides making a name for itself through fulfilling the mission "to inspire creativity and bring joy"[6], TikTok has inevitably been under public scrutiny. One source of controversy is TikTok's associations with the echo-chamber effects, a side effect of its AI algorithm and recommender system. This paper studies TikTok's algorithm, the core of its technology. This research can be utilized by social media scholars to have more insights into potential problems aroused by TikTok. Content browsers and creators can benefit from understanding the platform more deeply to consume and initiate information in a more reasonable manner.

Background

a) Platform overview

TikTok is a short-form, video streaming and sharing app that allows users to create and share videos up to 3 minutes[7] (previously limited to 15 seconds and 60 seconds). TikTok's parent company is ByteDance, a Chinese partially state-owned multinational internet technology company headquartered in Beijing. TikTok's most pervasive contents are music, lip sync, dance videos, and micro-blog content. In 2014, a social media app named Musical.ly increased in popularity among the 13-18 year old demographic[8]. In 2016, Chinese app developer ByteDance rebranded TikTok to Douyin. Within a year, the TikTok app had more than 100 million users[9], and the popularity of its videos continued to rise.

In late 2017, Musical.ly was purchased by ByteDance at $ 1 Billion, as ByteDance want to leverage the U.S. digital platform's young user base[10][11]. In 2018, Bytedance consolidated the user accounts of Musical.ly and TikTok, merging the two apps into one under the name TikTok. With this unified brand and user base, TikTok became a worldwide app.

Like other social media platforms, TikTok's main revenue stream comes from selling advertising[12], which signifies the process of revealing TikTok users to relevant content. This is guaranteed by its AI algorithm, which will be discussed further.

b) Terms definition and clarification

The term "filter bubble" was coined by Pariser[13] in 2011. Although a definition was not provided, the filter bubble

is characterized as "fundamentally alters the way we encounter ideas and information"[13]. A "filter bubble" is described as a state of intellectual isolation[1] that can result from personalized searches when a website algorithm selectively guesses what information a user would like to see based on information about the user, such as location, past click-behavior and search history[14]. As a result, users become separated from information that disagrees with their viewpoints, effectively isolating them in their own cultural or ideological bubbles.

In discussions of news media, an echo chamber refers to situations in which beliefs are amplified or reinforced by communication and repetition inside a closed system and insulated from rebuttal[15]. By participating in an echo chamber, people are able to seek out information that reinforces their existing views without encountering opposing views, potentially resulting in an unintended exercise in confirmation bias.

"Epistemic bubble" was described as a social epistemic structure in which other relevant voices have been left out[16] or an informational network from which relevant voices have been excluded by omission[17].

The three aforementioned terms are oftentimes used interchangeably, which inadvertently blurs their distinguishable features and causes misconceptions and ambiguity. In the scope of this research, it is of necessity to fully comprehend and be able to distinguish between the three terms.

For a simplified distinction between "filter bubble" and "echo chamber", echo chambers could be a result of filtering or they could be the result of other processes, but filter bubbles have to be the result of algorithmic filtering[18].

There is a clear distinction between epistemic bubble and echo chamber: An epistemic bubble is when you do not hear people from the other side. An echo chamber is what happens when you do not trust people from the other side[17]. Likewise, an "epistemic bubble" merely omits contrary views, whereas an "echo chamber" undermines opposing views and brings its members to actively distrust outsiders[17].

Research methodology

Experiment 1: Qualitative survey on users' behavior on social media

A total of 30 participants aged between 16 and 25 currently living in Vietnam (n=24) and other countries (n=6) participated in a qualitative survey on their behavior on social media and perspectives on the content they consume. For participants living in Vietnam, the form was delivered in Vietnamese. For participants living outside Vietnam, the form was delivered in English. Due to the COVID-19, both forms were delivered online. The questionnaire included the following questions:

- Basic information (Name, gender, correspondence,...)
- How frequently do they use social media
- The social media they use most frequently
- Whether they feel irritated/bored when they see posts/videos/images that display content of repetitive topics everyday
- How much they want their newsfeed to display information on other topics
- How much they want their newsfeed to display information on other topics
- Whether they think their culture is superior to (other) ethnic minorities they come across on social media
- Whether they just want to interact (like, share, comment,...) with people who share the same or similar culture with them
- Whether their newsfeed only displays content from people who share the same of similar culture with them

Experiment 2:

The research uses several relevant case studies that illustrate TikTok's AI algorithm bias in support of the arguments in the discussion.

Experiment 3: Personal observation

In order to fully understand TikTok's user interface, user flow and how it recommends videos, the researcher created two separate TikTok accounts. Both of these accounts have their location in Vietnam, yet the researcher deliberately demonstrated interests in distinct sets of topics on the two accounts.
Initially, the researcher determined favourite topics on the two accounts:
Account 1: Science & Education, DIY & Tricks, and Entertainment, GYM & Health.
Account 2: Babies & Family, Dance, Game and Anime.
Then, the researcher intentionally followed accounts that disseminate content on these topics, observed and drew comparisons

Data/Experiment Results

Experiment 1:		
Key Socialdemograp		
		N (%)
Gender	Male	22 (73.33%)
	Female	8 (26.67%)
Age	15	1 (3.33%)
	16	4 (13.33%)
	17	3 (10%)
	18	15 (50%)
	19	1 (3.33%)
	20	0 (0%)
	21	5 (16.67%)
	25	1 (3.33%)
Place of residence	Vietnam	24 (80%)
	Tokyo, Japan	1 (3.33%)
	Indonesia	1 (3.33%)
	Dubai	1 (3.33%)
	Myanmar	1 (3.33%)

	Australia	1 (3.33%)
	Egypt	1 (3.33%)
Total time spent on social media usage (per day)	0-3 hours	6 (20%)
	3-5 hours	6 (20%)
	5-7 hours	11 (36.67%)
	7-9 hours	6 (20%)
	9-11 hours	1 (3.33%)
	Over 11 hours	0 (0%)
Most frequently social media app used	Facebook	16 (53.33%)
	Instagram	7 (23.33%)
	Twitter	0 (0%)
	Snapchat	1 (3.33%)
	Tiktok	0 (0%)
	Youtube	6 (20%)

Table 1: Key socialdemographic characteristics

Answers to multiple choice questions

		N (%)
Do you feel irritated/bored when you see posts/videos/ images that display content of repetitive topics everyday? (For example: For You Page of TikTok only shows cooking videos/ Your Facebook newsfeed only shows content related to football)	Yes	14 (46.67%)
	No, I feel really comfortable	3 (10%)
How would you react if you find out the homogeneous content/opinions you see everyday on the fanpages, groups or accounts that you frequently follow get disputed?	I would turn a blind eye to it	3 (10%)
	I would be bothered to an extent, but I'll just ignore it anyway	15 (50%)
	I will read closely about contradicting points of view, analyze and compare them	12 (40%)
	No	26 (87.77%)

Table 2: Answers to multiple choice questions

Answers to linear scale questions

		N (%)
You want your newsfeed to display information on other topics	"Strongly disagree	
(No, I am satisfied that these social media display content of my favorite topics		
"	1 (3.33%)	
	Disagree	2 (6.67%)
	Neutral	7 (23.33%)
	Agree	16 (53.33%)
	Strongly agree (I want to learn more about other topics as well)	4 (13.33%)
	Completely disagree	3 (10%)
You just want to interact (like, share, comment,...) with people who share the same or similar culture with you	Disagree	8 (26.67%)
	Neutral	11 (36.67%)
	Agree	7 (23.33%)
	Completely agree	1 (3.33%)
Your newsfeed only displays content from people who share the same of similar culture with you	Completely disagree	5 (16.67%)

	Disagree	5 (16.67%)
	Neutral	11 (36.67%)
	Agree	4 (13.33%)
	Completely agree	5 (16.67%)

Table 3: Answers to linear scale questions

Participants' answers
Based on the information gathered, potential bias should be noticed because the majority of participants reside in Viet Nam. According to the data, the participants are frequent social media users. Their ages range from 15 to 25, which is also the largest age group present on TikTok.

Experiment 3:

On starting to use the app, the user flow was identical for both 2 accounts: Registration, Information Confirmation, and Topics Selection.

From the researcher's observation, when first entering the app (post-registration), TikTok automatically showed a video, and then an endless stream of videos. The videos' content were not necessarily related to the user's interests indicated previously. Those unfavourable contents were actually not outweighed by the favourable content. Noteworthily, the first videos shown in #For You Page in both two accounts were identical based on the hashtags. On keeping strolling, the content gradually became more related to the user's interests, revealing a conspicuous difference between the two accounts. However, content from other topics continued to show up from time to time, mainly trendy videos in that specific location (in this case Viet Nam).

Discussion

How TikTok works and how users navigate TikTok
In order to attain a successful relationship with its users, TikTok has to maintain their stickiness to a certain extent first. To accomplish this, TikTok's user interface design plays an important role. When the user starts browsing videos after registration, a video is immediately presented to them. This video takes up virtually the whole screen on any device (with an exception of laptop or PCs). The app then instructs the user to scroll down to view another video, and then a stream of videos will flow continuously. The user will only cease to see those videos once quitting the apps.

That user interface design suggests the main task of users is to keep strolling to see more videos. As they proceed to browse more videos, the TikTok's algorithm has the chance to gather more data to study and understand their personas better, for audience members predominantly select what media messages they attend to and thus shape their individual information environments[19]. Gradually, TikTok's recommended videos will increase in accuracy and relevance to the users. Users then become satisfied and at ease with the videos and develop a belief that some good yet unknown videos await them. With this anticipating mindset, they would browse more videos than they thought they would. A vicious circle ensues, and TikTok's algorithm manages to gain more inputs for its dataset to deliver even more personalized recommendations.

One feature worth taking into consideration is that TikTok helps users alleviate the task of having to search for content or contacts. Unlike Facebook[20], this social media platform does not emphasize on connecting with friends and families or joining groups, building a transparent, recognizable profile or actively seeking a community. TikTok serves its users by learning about them and delivering recommendations.

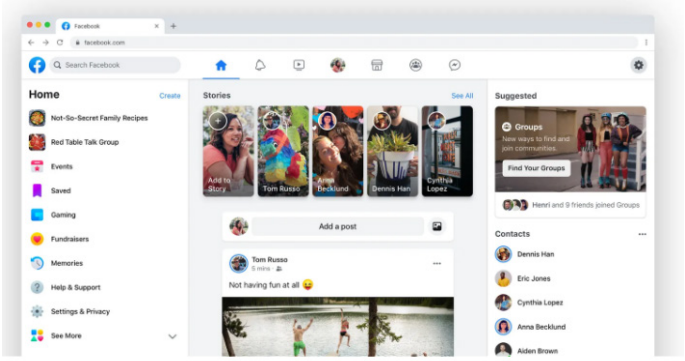


Figure 1: Facebook's interface (source: https://www.theverge.com/2020/8/21/21395079/facebook-new-design-default-september-classic-interface-disappearing)

Figure 2: TikTok's interface (source: self-taken)

TikTok's unprecedented success and capability to distribute the right content to the right user emanates from its algorithm. TikTok's recommendation system works to determine which videos will appear on your "For You Page" (FYP). The stream of videos on each FYP is identical, unique and curated to the user's interests[21].

These machine learning algorithms closely study users' behaviours and interpret them as indicators of interests: the number of clicks, whether a user finishes watching a video from beginning to end... Other factors taken into account are user interactions (such as the videos they share, the accounts they follow,...), video information (which includes details like captions, sounds, and hashtags), device and account settings (language preference, country, location, device type,...).

To be more specific, TikTok's recommender system[22] employs collaborative filtering and content-based filtering[23]. Collaborative filtering is the process employed by recommender systems which entails filtering or evaluating items using the opinions of other people[24]. Collaborative filtering uses similarities between users and items simultaneously to provide recommendations[25].

Psychologists Joseph Luft and Harrington Ingham created the Johari window, a technique and heuristic exercise that helps people better understand their relationship with themselves and others[26]. The theory is divided into 4 quadrants:

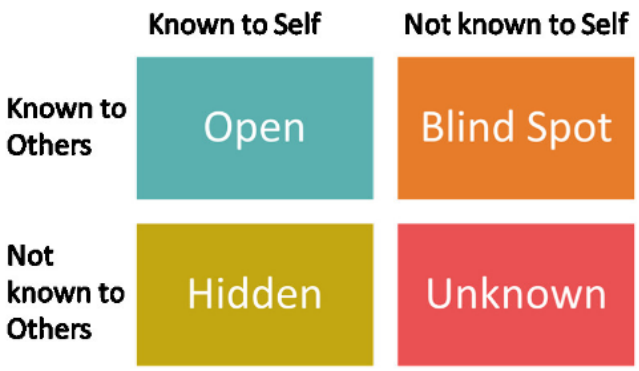


Figure 3: The Johari window (source: https://www.healthynumbers.com.au/using-the-johari-window-to-really-get-to-know-yourself/)

It is also suggested that TikTok's collaborative filtering algorithm may inadvertently help users discover their latent, or hidden interests[5]. Moreover, in a platform where users are not exposed to their acquaintances and other users, they may express their interests more explicitly (e.g. by liking, commenting, following,... hashtags or accounts) without fear of being judged. Due to this, they have more freedom to customize their own feed that is perfectly curated to their personal interests. These behaviors are inevitably monitored and captured by TikTok's algorithm and serve as a valuable source for the algorithm to learn more deeply about the users.

TikTok also adopts the content classification algorithm and the user algorithm that follow a hierarchy classification architecture[27] for content analysis described in the model below:

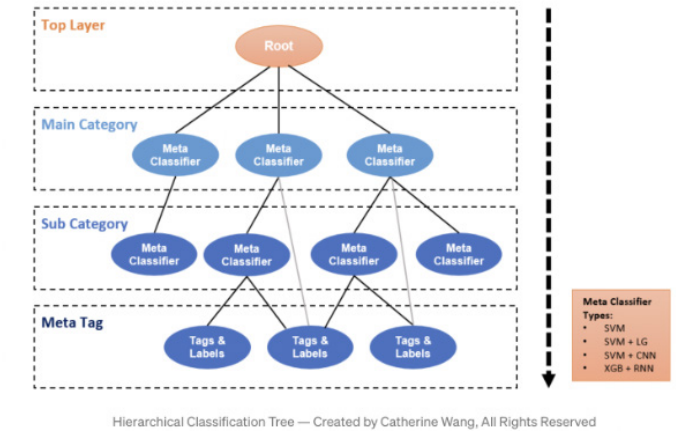


Figure 4: Hierarchical Classification Tree

This is also mentioned in[5] that they build a global algorithm to "construct a hierarchical interest label set based on the theme of the contents, and calculate the relevant degree of each interest label in the set.

Content-based filtering guesses the features and behavior of a user based on the item's features that user positively reacts to[28]. This method revolves completely around comparing user interests to product features. The products that have the most overlapping features with user interests are what's recommended[29]. The model recommends items relevant to a user by picking a similarity metric and setting up the system to score each candidate item according to that metric. The model uses no information about other users[30].

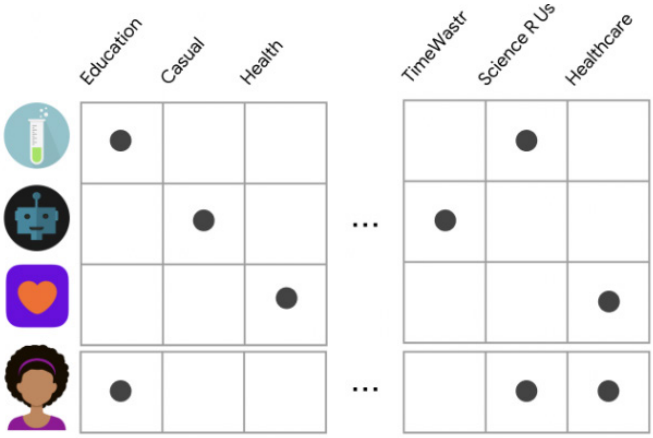


Figure 5: Using dot as a similarity metric (source: https://developers.google.com/machine-learning/recommendation/content-based/basics)

These two filtering techniques differ from each other in that while collaborative filtering uses the assumption that people with similar tastes will rate things similarly, content-based filtering uses the assumption that items with similar objective features will be rated similarly[28].

As TikTok maintains the role of distributing content and tailoring videos to users' specific interests, which relieves its users from deliberately searching for content, it can be seen that TikTok may as well introduce users to content they did not think they would be interested in. By hindering this deliberate process, TikTok is actually lessening the effect of the epistemic bubble or filter bubble, compared to social media platforms like Facebook where users intentionally curate their networks and news feeds in the first place. However, when the users become complacent with their curated #FYP and constantly expect to browse more similar content, that may be the inception of the echo-chamber effect.

The whole narrative so far has examined how TikTok tailors content distribution to its users, but this is lopsidedly focused on video browsers. On a social media that focuses on user-generated content, video creators are worth taking into consideration. As observed from the user interface design of TikTok, it neither implicitly nor explicitly implies the formation of groups, so users will not search for a group that discusses a topic of their interests. Conversely, Facebook groups' admins and moderators[31] or Reddit Subreddit's moderators[32] who have the right to decide which information to appear on their groups. In other words, they have the ability to dictate the flow of information that targets a certain group of audience. Meanwhile, in TikTok, the user's news feeds are at the caprice of TikTok's algorithm, meaning TikTok exhibits more information decentralization. To be more specific, TikTok video creators do not have to be famous nor accredited to draw massive attention. This is corroborated by the fact that even videos with low reaction (a small number of people clicking the "heart" symbol) can appear on video browsers' For You Page quite often.

2) Attempted explanations from a cognitive and psychological angle

In this section, the researcher aims to provide explanations on how filter bubbles and echo chambers are created on TikTok.

a) Sources of bias

According to the results, TikTok users feel comfortable experiencing a wide scope of content, yet are not likely to accept and study opposing viewpoints. Naturally, people are easily prone to confirmation bias[33]. The bias can be in the brain: when dealing with information overload while it is only capable of handling a finite amount of information, the brain uses a number of tricks or shortcuts in the decision-making process[34].

In addition, In [35], the authors list sources of bias in machine learning, one of which is algorithmic bias, defined as bias added by the algorithm itself and not present in the input data.

It is proposed that biases may come from the deeply entrenched dramatic instincts and overdramatic worldview and make the brains jump to swift conclusions without much thinking. Specifically, there are 10 instincts that may distort human's perceptions, of which negativity, size and generalization instincts fit the scope of this research. Negativity instinct is defined as the tendency to notice the bad more than the good, leading to the mega misconception that "The World Is Getting Worse". Generalization instinct leads everyone to automatically categorize and generalize all the time unconsciously, which leads humans to mistakenly group together things, or people, or countries that are actually very different and assume everything or everyone in one category is similar and lastly, jump to conclusions about a whole category based on a few, or even just one, unusual example[36]. Moreover, these misleading generalizations are unscrupulously leveraged by social media as a shorthand to deliver information faster. In TikTok's context, "point-of-view videos", "lip syncing" and "cosplaying" videos easily become trendy by stereotyping certain groups of people.

Users' pre-existing bias, coupled with possible bias in the AI algorithm offered by TikTok, would increasingly distort users' perception of the content they consume, both latently and overtly.

b) The spread of TikTok content

Unlike other social media platforms which many other researchers have devoted to, content dissemination in a video-oriented and content-centric platform like TikTok may differ. People are more likely to believe fake news in a video format compared to text and audio forms of the same story, according to a team of researchers. People are also more willing to share these videos with people in their network[37]. The appeal to video form is characterized by the fact that video contains many streams of information, including audio, visuals, moving images, graphics and text. When people take in all this information, they are mentally overloaded, making them less likely to scrutinize the details. This, along with the seeming realism of what is portrayed, makes videos more likely to be believed and shared. In addition, video content has a higher retention rate compared to text because humans respond to and process visual data better than any other type of data[38]. Besides, recognition memory for information that supported the participants' viewpoint was higher than that for opposing information[39]. For this reason, information, especially pieces that consolidate users' confirmation bias will be lodged in users' memory for a longer period. On a video-based platform, such information is more likely to be recalled then spread, thus magnifying the echo-chamber effect.

3) Concerns regarding ethnocentrism

As suggested above, TikTok exposes its users to different sets of topics in the #ForYouPage, even those deemed unfavourable by a specific user. However, as the innate search for confirmation bias dictates their course of action is supervised by AI, the echo-chamber effect begins to permeate. As users have a tendency to follow and browse content from people of the same race, culture or ethnicity (as suggested in Experiment 1), this poses an imminent problem: TikTok's recommendation system does not have sufficient data of images or videos from underrepresented communities and ethnic minorities. The dataset is ever-growing, but as algorithm bias reflects human bias, the

researcher doubts whether the recommendation system would ever suffice to distribute such content to a wider set of users. In an experiment conducted by Marc Faddoul, an AI researcher at UC Berkeley School of Information, Following the account of a black woman led to recommendations for three more black women. It gets weirdly specific – Faddoul found that hitting the "follow" button on an Asian man with dyed hair gave him more Asian men with dyed hair, and the same thing happened for men with visible disabilities[40].

In another case study, Ziggi Tyler, a popular Black TikTok creator posted several videos demonstrating how he could not include phrases in his bio including the word "Black" without being immediately flagged for "inappropriate content." Other words and phrases, including ones declaring his support for "Black Lives Matter," "black people," "black voices" and "black success," or "I am a black man" would immediately trigger a pop-up message prompting him to "remove any inappropriate content." Conversely, putting "supporting white supremacy", "supporting white success" or even "I am a neo-Nazi" did not prompt the alert message. There are other cases in which black content creators accuse TikTok of suppressing "Black lives matter" content[41][42]. These cases just strengthen the statement that AI algorithm bias may reflect deep-rooted social and human bias. Given that context, fixing AI bias can be really challenging due to the unknown fallacies, potentially flawed processes and lack of social context[43].

As datasets, intentionally or unintentionally, often disregard the presence of minorities, the issues should not be overlooked and regarded as a secondary concern. In a multiracial community, for example, members of a majority group tend to hold negative views of individuals who espouse a dual identity for fear of disloyalty[44].

Besides, users, who deprive a sense of gratification and are acquainted with comfort from browsing TikTok's recommended content, may as well not even bother to learn more about some fragmented culture content they come across. Social identity theory[45] proposes that the groups which people belonged to were an important source of pride and self-esteem and provided them a sense of belonging. It is also suggested that stereotyping is based on a normal cognitive process: the tendency to group things together, wherein the differences between groups and the similarities of things in the same group are exaggerated[45].

Human's innate bias, fueled by the complicatedness of the AI algorithm, may suggest that concerns about ethnocentrism, and even worse, racism is not unsubstantiated.

4) Researcher's Recommendations

As TikTok's algorithm and recommendation system increases its accuracy, and users may reap more gratification as a result, both sides must be cautious of TikTok's not yet conspicuous side effects. The researcher suggests that TikTok, while maintaining and upgrading the AI algorithm as the backbone of its technology, should pour more resources into stricter regulations of content, heightened attention to AI fairness and ethical issues in technology. For example, TikTok can employ frameworks to detect and mitigate algorithmic bias or maintain multifaceted communications with users. On the other hand, users, who have the irrefutable right to determine which content to consume and what use they make of it, must be more conscious of their power to influence not only TikTok's policy but also its powerful AI algorithm. It is high time media literacy and social media's codes of conduct were preached broadly and implemented instead of being neglected. While these measures can be offputting, time-consuming, and deprive users of the comfort they are relishing, social media platforms like TikTok should invest in mitigating the paradoxical effects to demonstrate its responsibility.

Conclusion

In this paper, the researcher reviews some basic principles of TikTok's powerful AI algorithm with an aim to investigating this social media platform's roles in creating the echo chambers. On the one hand, TikTok mitigates the epistemic and filter bubble effects by exhibiting a diverse set of content to study its users, which may reveal even users' latent interests by closely supervising their behaviors and comparing them to indicators of interest. However, once users get to be more familiarized with outstanding comfort and personalization, they may sacrifice exploring new content and opposing views for the sake of convenience. This complementary interaction between users and TikTok's AI algorithm offers an easily achievable sense of satisfaction and escapism, yet inadvertently confines users to their own echo chambers. These procedures occur at an exceptional speed and TikTok users may not be able to fully comprehend the imminent issues posed by this personalization technique. Most importantly, as TikTok serves a young group of users who will soon become the world's leading force, more comprehensive research should be conducted to acquire a more profound understanding of this newly-emerged platform. TikTok, which makes enormous profit from its top-notch AI algorithm, should take into consideration their ethical responsibility of distributing content and preventing unwanted conflicts and disputes. AI's unfavorable outcomes that wreak havoc on minor communities may be easily overlooked and not treated as utmost emergency, and there are insufficient metrics to mathematically measure their level of impact, but constant reevaluation of the datasets and algorithms should be conducted to refine their functions and mitigate undesirable circumstances. Meanwhile, users, who make the decision whether to stay or leave the app, have the power to teach themselves media literacy and consume any information presented to them in an educated and tolerant manner.

Limitations

Firstly, the sample of this study is small (N = 30) so the results may vary when conducted in different settings. Secondly, the COVID-19 spread and its new variants in Vietnam posed some hindrance to the researcher's process of gathering in-grained data. The lockdown made it impossible to travel and conduct more in-depth and interactive interviews offline. This prevents the researcher from observing their expressions and can cause the respondents to not take

the online survey seriously. More importantly, this research really lacks data input from people whose ethnicity is considered minor. These factors combined led to a data that lacks diversity. Thirdly, the first experiment did not gather an equal distribution of people who share the same or similar culture, so the results may be skewed. Fourthly, adopting a qualitative approach coupled with a lack of data source on TikTok means that this research can only propose theories and patterns. Fifthly, there was not enough diversity in educational backgrounds eg. No participants from the survey had poor literacy, which can dictate users' behavior and levels of awareness on social networking sites. Sixthly, the research has solely been conducted by diving into the lens of browsing users, rather than those who create content. If more TikTok content creators participate in a future research, more diverse information could be gathered.

Potential for future research

This research still has room for improvement. Given more time and resources, more data from a more diverse demographics can be collected, leading to the research results being broader and representative. The researcher's ambition is to apply and extend this research design in other countries or regions so as to gain insights into different cultures. Concerning the research into ethnocentrism, more specific groups of people from different cultures should be involved, and the researcher needs to have a deep insight into those cultures' instances and expressions to minimize chances of bias. Furthermore, face-to-face interviews should be preferably conducted to make room for a comfortable environment in which participants can provide more in-depth and truthful answers to open-ended questions and the researcher can accumulate more qualitative data for thematic analysis.

References

1. NAKAFUJI, REI. "TikTok Overtakes Facebook as World's Most Downloaded App." Nikkei Asia, August 9, 2021. https://asia.nikkei.com/Business/Technology/TikTok-overtakes-Facebook-as-world-s-most-downloaded-app.
2. Doyle, Brandon. "TikTok Statistics - Everything You Need to KNOW [Feb 2021 UPDATE]." Wallaroo Media, June 14, 2021. http://wallaroomedia.com/blog/social-media/tiktok-statistics/.
3. Hamilton, Isobel Asher. "TikTok Hit 1.5 Billion Downloads, and Is Still Outperforming Instagram." Business Insider, November 18, 2019. https://www.businessinsider.com/tiktok-hits-15-billion-downloads-outperforming-instagram-2019-11.
4. Iqbal, Mansoor. "TikTok Revenue and Usage Statistics (2021)." Business of Apps, September 17, 2021. https://www.businessofapps.com/data/tik-tok-statistics/.
5. Graziani, Thomas. "How Douyin Became China's Top Short-Video App in 500 Days." WalktheChat, October 21, 2018. https://walkthechat.com/douyin-became-chinas-top-short-video-app-500-days/.
6. "About: TikTok - Real Short Videos." TikTok. Accessed September 28, 2021. https://www.tiktok.com/about?lang=en.
7. TikTok, Drew Kirchhoff. "More Tok on the Clock: Introducing Longer Videos ON TIKTOK." TikTok Newsroom. TikTok, August 16, 2019. https://newsroom.tiktok.com/en-us/longer-videos.
8. Daniel. "What Is Tik Tok? Why Is It so Popular?" Brandastic, September 1, 2021. https://brandastic.com/blog/what-is-tiktok-and-why-is-it-so-popular/#:~:text=Who%20Invented%20TikTok%3F,users%20(often%20called%20Musers).
9. Graziani, Thomas. "How Douyin Became China's Top Short-Video App in 500 Days." WalktheChat, October 21, 2018. https://walkthechat.com/douyin-became-chinas-top-short-video-app-500-days/.
10. Lin, Liza. "Social-Media App Musical.ly Is Acquired for as Much as $1 Billion." Wall Street Journal, September 11, 2017. https://www.wsj.com/articles/lip-syncing-app-musical-ly-is-acquired-for-as-much-as-1-billion-1510278123.
11. Lee, Dami. "The Popular Musical.ly App Has Been Rebranded as TikTok." The Verge (blog), August 2, 2018. https://www.theverge.com/2018/8/2/17644260/musically-rebrand-tiktok-bytedance-douyin.
12. McFarlane, Greg. "How Facebook, Twitter, Social Media Make Money from You." Investopedia. Investopedia, September 21, 2021. https://www.investopedia.com/stock-analysis/032114/how-facebook-twitter-social-media-make-money-you-twtr-lnkd-fb-goog.aspx#:~:text=The%20primary%20way%20social%20media,before%20social%20media%20companies%20existed.
13. Pariser, Eli. The Filter Bubble: What the Internet Is Hiding from You. Reprinted. London: Viking, 2012.
14. "Filter Bubble." Wikipedia. Wikimedia Foundation, September 15, 2021. https://en.wikipedia.org/wiki/Filter_bubble.
15. "Echo Chamber (Media)." Wikipedia. Wikimedia Foundation, September 12, 2021. https://en.wikipedia.org/wiki/Echo_chamber_(media).
16. Nguyen, C. Thi. "ECHO Chambers and Epistemic BUBBLES." Episteme 17, no. 2 (2018): 141–61. https://doi.org/10.1017/epi.2018.32.
17. Dresser, Sam, ed. "Escape the Echo Chamber." Aeon, April 9, 2018. https://aeon.co/essays/why-its-as-hard-to-escape-an-echo-chamber-as-it-is-to-flee-a-cult.
18. Fletcher, Richard. "The Truth behind Filter Bubbles: Bursting Some Myths." Reuters Institute for the Study of Journalism. Accessed September 15, 2021. https://reutersinstitute.politics.ox.ac.uk/risj-review/truth-behind-filter-bubbles-bursting-some-myths.
19. Knobloch-Westerwick, Silvia, Cornelia Mothes, and Nick Polavin. "Confirmation Bias, Ingroup Bias, and Negativity Bias in Selective Exposure to Political Information." Communication Research 47, no. 1 (February 2020): 104–24. https://doi.org/10.1177/0093650217719596.
20. Hall, M.. "Facebook." Encyclopedia Britannica, September 15, 2021. https://www.britannica.com/topic/Facebook.
21. TikTok. "How TikTok Recommends Videos #Foryou." Newsroom. TikTok, August 16, 2019. https://newsroom.tiktok.com/en-us/how-tiktok-recommends-videos-for-you.
22. Google Developers. "Recommendation Systems Overview." Google Developers. Lecture. Accessed September 15, 2021. https://developers.google.com/machine-learning/recommendation/overview/types.
23. Kharwal, Aman. "TikTok Algorithm with Machine Learning." Data Science | Machine Learning | Python | C++ | Coding | Programming | JavaScript, June 24, 2021. https://thecleverprogrammer.com/2020/09/26/tiktok-algorithm-with-machine-learning/.
24. Schafer, J. Ben, Dan Frankowski, Jon Herlocker, and Shilad Sen. "Collaborative Filtering Recommender Systems." The Adaptive Web, Lecture Notes in Computer Science, 4321 (n.d.): 291–324. https://doi.org/10.1007/978-3-540-72079-9_9.
25. Google Developers. "Collaborative Filtering." Google Developers. Lecture. Accessed September 15, 2021. https://developers.google.com/machine-learning/recommendation/collaborative/basics.
26. "Johari Window." Wikipedia. Wikimedia Foundation, July 15, 2021. https://en.wikipedia.org/wiki/Johari_window.
27. Wang, Catherine. "Why TikTok Made Its User So Obsessive? The Ai Algorithm That Got You Hooked." Medium. Towards Data Science, June 7, 2020. https://towardsdatascience.com/why-tiktok-made-its-user-so-obsessive-the-ai-algorithm-that-got-you-hooked-7895bb1ab423.
28. Roy, Abhijit. "Introduction to Recommender Systems- 1: Content-Based Filtering and Collaborative Filtering." Medium. Towards Data Science, July 31, 2020. https://towardsdatascience.com/introduction-to-recommender-systems-1-971bd274f421.
29. Edpresso Team. "What Is Content-Based Filtering?" Edpresso. Reading. Accessed September 15, 2021. https://www.educative.io/edpresso/what-is-content-based-filtering.
30. Google Developers. "Content-Based Filtering ." Google Developers. Lecture. Accessed September 15, 2021. https://developers.google.com/machine-learning/recommendation/content-based/basics.
31. "What Is the Difference between an Admin and a Moderator in a FACEBOOK Group?: Facebook Help Center." What is the difference between an admin and a moderator in a Facebook group? | Facebook Help Center. Accessed September 15, 2021. https://www.facebook.com/help/901690736606156.
32. 04, May. "What's a Moderator?" Reddit Help. Accessed September 15, 2021. https://reddit.zendesk.com/hc/en-us/articles/204533859-

What-s-a-moderator-.
33. Ling, Rich. "Confirmation Bias in the Era of Mobile News Consumption: The Social and PSYCHOLOGICAL DIMENSIONS." Digital Journalism 8, no. 5 (2020): 596–604. https://doi.org/10.1080/21670811.2020.1766987.
34. Giovanni Luca Ciampaglia Assistant professor, and Filippo Menczer Professor of Computer Science and Informatics; Director of the Center for Complex Networks and Systems Research. "Misinformation and Biases Infect Social Media, Both Intentionally and Accidentally." The Conversation, September 19, 2021. https://theconversation.com/misinformation-and-biases-infect-social-media-both-intentionally-and-accidentally-97148.
35. Mehrabi, Ninareh, Fred Morstatter, Nripsuta Saxena, Kristina Lerman, and Aram Galstyan. "A Survey on Bias and Fairness in Machine Learning". arXiv preprint arXiv: 1908.09635v2 [cs.LG] 17 Sep 2019
36. Rosling, Hans, Ola Rosling, and Rönnlund Anna Rosling. "The Gap Instinct, The Negativity Instinct, The Generalization Instinct." Essay. In Factfulness: Ten Reasons We're Wrong about the World - and Why Things Are Better than You Think. New York: Flatiron Books, 2020.
37. Swayne, Matt. "Video Fake News Believed More, Shared More than Text and Audio Versions." Penn State News, September 8, 2021. https://news.psu.edu/story/668403/2021/09/08/research/video-fake-news-believed-more-shared-more-text-and-audio-versions.
38. McCoy, Erin. "How Our Brains Are Hardwired for Visual Content." Killer Visual Strategies, September 10, 2019. https://killervisualstrategies.com/blog/how-our-brains-are-hardwired-for-visual-content.html.
39. Frost, Peter, Bridgette Casey, Kaydee Griffin, Luis Raymundo, Christopher Farrell, and Ryan Carrigan. "The Influence of Confirmation Bias on Memory and Source Monitoring." The Journal of General Psychology 142, no. 4 (2015): 238–52. https://doi.org/10.1080/00221309.2015.1084987.
40. Mellor, Maria. "Why Is TikTok Creating Filter Bubbles Based on Your Race?" Wired, February 28, 2020. https://www.wired.co.uk/article/tiktok-filter-bubbles.
41. McCluskey, Megan. "These TikTok Creators Say They're Still Being Suppressed for Posting Black Lives Matter Content." Time, July 22, 2020. https://time.com/5863350/tiktok-black-creators/.
42. Mitchell, Taiyler Simone. "Black Creators SAY TikTok's ALGORITHM Fosters a 'CONSISTENT Undertone of Anti-Blackness.' Here's How the App Has Responded." Insider. Insider, August 25, 2021. https://www.insider.com/a-timeline-of-allegations-that-tiktok-censored-black-creators-2021-7.
43. Hao, Karen. "This Is How Ai Bias Really Happens-and Why It's so Hard to Fix." MIT Technology Review. MIT Technology Review, April 2, 2020. https://www.technologyreview.com/2019/02/04/137602/this-is-how-ai-bias-really-happensand-why-its-so-hard-to-fix/.
44. Yale University. "Fear of disloyalty drives bias against bicultural immigrants." ScienceDaily. www.sciencedaily.com/releases/2018/11/181101133858.htm (accessed September 27, 2021).
45. Tajfel, Henri. "Social Identity and Intergroup Behaviour." Social Science Information 13, no. 2 (1974): 65–93. https://doi.org/10.1177/053901847401300204.

Raman Spectroscopy: Basics, Physics and Cancer detection analysis

Vindya Prabhu

Abstract

Raman spectroscopy is a molecular specific technique that can be used to develop a fundamental biochemical understanding of tissue physiology and pathology, which can be used for tissue diagnosis and monitoring. This paper presents a brief history of the development of Raman spectroscopy, a description of the Raman effect, and the current applications of Raman spectroscopy in cancer detection.

Globally, cancer is one of the leading causes of death. 1 in 2 people will develop some form of cancer during their lifetime [1]. Despite the fact that many cancers can be detected, current diagnostic methods can definitely be improved. It is critical to identify and diagnose cancer at an early stage so that the best treatment and intervention options can be offered. Patients diagnosed with early-stage cancers will have better prognosis and a 5-year survival rate [2]. Existing methods are often expensive, invasive, and sometimes inconclusive, or inaccurate. Consequently, there is a crucial need for a reliable, cost-effective, and non-invasive method that can diagnose all forms of cancer in their early stages.

The utilisation of Raman techniques for the detection and diagnosis of prostate cancer, oral cancers, breast cancer, and lung cancer is described. The article is by no means *an exhaustive review of all papers in this research area, but rather highlights selected papers which overall give a representative sample of the current research being undertaken.*

Introduction

Raman spectroscopy technology is optical, and it allows for non-invasive, or at least non-intrusive, data extraction, making it a useful tool in clinical settings. Raman spectroscopy is used for analysing blood serum samples, various tissue samples and oral swabs in research laboratories.

The spectra obtained after analysis, provides a unique chemical molecular 'fingerprint' for each sample. Unwanted signal components can be removed from the results. Raman spectroscopy has the ability to obtain unique information in-vivo, and in real-time which could be useful during operations. For many years, researchers have investigated the use of Raman spectroscopy to distinguish between cancerous and normal tissues, and successful results have been achieved for brain, skin, head & neck, and breast cancers, in adults as well as children [3][4].

The article summarises the history and fundamental physics of Raman Spectroscopy, as well as published literature in detecting cancers in the prostate, breast, lung and oral cavity.

History of Raman Spectroscopy

Chandrasekhara Venkata Raman was an Indian physicist who carried out research on light scattering along with his student, K.S. Krishnan [5]. Raman discovered that deflected light changes its path (wavelength and amplitude) as it passes through transparent materials. This phenomenon was named the Raman effect or Raman scattering [6].

Research in Calcutta, India, was conducted by Raman and his group on the scattering of light in different liquids. Initially, they used sunlight as a light source- where they separated the light using coloured filters, which scattered off the target liquid. As the effect was weak and difficult to see, a telescope was used as a more intense light source. They observed a colour shift in light scattered by various liquids when they used the telescope to concentrate sunlight [7].

Raman's work was greatly influenced by the discovery of the Compton effect in 1922, which discovered that X-rays could lose energy and shift to longer wavelengths when they collided with electrons in an inelastic manner. In Raman's view, visible light scattering inelastically off molecules could demonstrate something similar to the Compton Effect [7].

n February 1928, Raman observed that the scattered light was polarised, which distinguished the new scattering effect from fluorescence. The new scattering effect was observed to some degree in sixty different liquids examined by him and Krishnan, which they submitted to the journal 'Nature' as a short paper named "A New Type of Secondary Radiation" [7].

In general, most of the incident light is scattered elastically (Rayleigh scattering), while very few incident photons undergo inelastic scattering (Raman effect) [4]. Scientists soon realised the value of this phenomenon as an analytical tool and called it the Raman Effect. The current applications of Raman spectroscopy range from the non-destructive identification of minerals to the early detection of life-threatening diseases. For his discovery, Raman was awarded the Nobel Prize in Physics in 1930 [6].

In the 1950s, Raman spectroscopy was also developed in different forms, including Tip Enhanced Raman spectroscopy (TERS), Surface Enhanced Raman spectroscopy (SERS), Coherent Anti-Stokes Raman spectroscopy (CARS), Resonance Raman spectroscopy (RRS), and Stimulated Raman spectroscopy (SRS) which greatly improved the method (e.g., its speed, resolution) compared to spontaneous Raman. It was only in 1953 that the first commercial Raman spectrometer was built [5].

Microanalysis was greatly enhanced by the introduction of Raman spectroscopy with an optical microscope in the late 1970s. The micro-Raman spectrometer became useful in biology, particularly for studying single cells as it is non-destructive and has high resolution imaging [8].

Physics behind Raman effect

The Raman effect is a change in wavelength of light that is scattered by electrons within a material [16]. Raman spectroscopy relies on the inelastic light scattering of a photon after it interacts with a vibrating molecule. The energy required to excite a molecule's specific vibration corresponds to the energy difference between the incident photon and its inelastically scattered counterpart [12]. The Raman effect is used in chemistry for identifying molecules by their structural fingerprint [6] [17].

Most biological molecules are Raman-active, and they each have their own unique structural fingerprint. Raman spectroscopy could be a powerful diagnostic technique as it is extremely sensitive to biochemical and molecular changes- which are vital for differentiation of tissue samples [15] [18].

The interaction of electromagnetic radiation with a molecule, results in three distinct events: absorption, transmission, and scattering. Absorption and transmission are related to middle infrared spectra (IR), whereas scattering is responsible for Raman spectra [19].

There are three types of scattering of radiation: Rayleigh, Stokes, and anti-Stokes. Rayleigh scattering is the most common type of scattering, and no energy is exchanged. Photons will return to the fundamental state after being scattered with very small energy differences, it is hence also called elastic scattering [19].

A polarisation is caused when photons interact with molecules and there is a difference in energy between the incident and scattered photons, and therefore it is called inelastic scattering. In Stokes scattering, energy is transferred from the photon to the molecule, and the scattered photon has a lower energy and a longer wavelength. In anti-Stokes scattering, the scattered photon has a higher energy and shorter wavelength [20]. At room temperature, the molecules are mainly in the fundamental state, so most of the Raman scattering measured is Stokes scattering. Anti-Stokes scattering is only present in a small portion of scattered light.

Very few of the scattered photons are inelastic (i.e., Stokes or anti-Stokes Raman scattering), while most of the scattered photons are elastic (Rayleigh scattering), so leave the molecule with the same energy (and thus, unchanged wavelength) as the incident photon [21].

Chemical and molecular features of biological molecules (lipids, proteins, and DNA) can be identified by their characteristic vibrational frequency, and therefore, any changes in their chemical molecular fingerprints can be analysed, and may provide pathogenic information [12]. Raman spectroscopy creates a molecular fingerprint of a sample, and the Raman spectrum provides quantitative information regarding the chemical composition of the sample [17].

The mutation of DNA in cancer leads to changes in other molecular components as well. Due to this, the cell's chemical composition can be significantly altered. These chemical changes can then appear as new or unique signals related to a disease state. Raman spectroscopy is an excellent tool for detecting these changes in the molecular components that arise from diseases [4].

Advantages	Disadvantages
• Non-invasive • High resolution imaging • Relatively low cost compared to medical imaging techniques (e.g., ultrasound, MRI etc.) • No exposure to ionising radiation • In-vivo fibre optic applications (e.g., for hollow organs, blood vessels) depending on the location of the tumour • Potential early diagnoses of the cancer • Ability to produce a spectral "fingerprint" which represents the biochemical composition of a sample • Accurate identification of organic and inorganic samples • Potential to determine the presence and the stage of disease progression • Minimal/ lack of sample preparation • Ability to use advanced optical technologies in the visible or near-infrared spectral range (lasers, microscopes, fibre-optics) • The possibility of performing analyses in the presence of water since it does not interfere with the spectra (unlike infrared spectra) • No need for labelling, dyes or toxic waste products	• Relatively low efficiency of the inelastic light scattering compared to elastic scattering samples • Sample components with low/weak Raman signals often limit the speed of the technique • Although high levels of chemical specificity- the changes that occur between spectra of different classes can often be minute and difficult to visually observe • Limited in its ability to probe deeper seated tissue at depth beyond near-surface tissue layers • Power and exposure to light must be controlled to prevent phototoxic effects on cells • Autofluorescence (sample dependent) • Low sensitivity • Need advanced data analysis • Access and knowledge of using the equipment in primary care and hospital settings

[22][21][18][17][14][5][15]

Progress in Raman Spectroscopy

Since the emergence of Raman spectroscopy in medical use, significant progress has been made in the field of Raman spectroscopy to aid in its potential transition into clinical environments. Hand-held fibre optics probes, endoscopes, needle probes for deep and subsurface diagnosis have been developed to maximise the diagnostic accuracy and speed of analysis [17].

Advanced statistical analysis, or chemometrics, is used to understand and extract the chemically relevant information found within the data obtained by the Raman spectrometer [18].

The most commonly used analysis strategies for Raman technologies are principal component analysis (PCA), and linear discriminant analysis (LDA). Both LDA and PCA are linear transformation techniques, however, LDA is a supervised technique, whereas PCA is unsupervised. Supervised algorithms use labels for all samples when the model is made. Details of these analysis techniques have not been discussed in this review as it is beyond the intention of this paper [18].

Cancer

Cancer refers to diseases in which abnormal cells divide without control and invade nearby, surrounding tissues. It is also possible for cancer to spread through the lymphatic and blood systems [9]. Cancer is the Latin word for crab, because of the crab-like tenacity in grasping the tissues it invades [10].

In 2020, there were an estimated 19.3 million new cancer cases worldwide and almost 10 million cancer deaths. By 2040, the global cancer burden is projected to reach 28.4 million cases, an increase of 47% from 2020. Global cancer mortality and morbidity control requires early cancer detection measures [11]. The majority of cancer deaths occur in underdeveloped countries due to poor healthcare systems and insufficient medical resources, although even developed countries (i.e., the United States and the United Kingdom) with access to the most advanced technologies and treatments are also projected to see an increase in cancer cases [12].

The goal of early cancer diagnosis is to detect symptomatic patients as soon as possible so they have the best chance of being treated successfully. The cancer survival rate depends on the type and place of the cancer and most importantly on the time of diagnosis [14]. When cancer care is delayed or inaccessible, the chances of survival are reduced, treatment is more problematic, and the cost of care is higher. Cancer diagnosed in early stages improves treatment and survival outcomes, making it an essential public health strategy [13].

Cancer diagnosis is usually made by histopathology, such as by sampling tissue, testing blood, or assessing cytology. Magnetic resonance imaging (MRI), positron emission tomography (PET), ultrasound, and computed tomography (CT) are examples of biomedical imaging techniques that can provide doctors with information about a tumour's tri-dimensional volume, structure, and composition. As a result of these imaging techniques, the ability of surgeons to resect tumours has been greatly improved, and has led to important advancements in cancer management [12].

Diagnostic methods capable of avoiding ionising radiation would have significant benefits. It is necessary to develop a technique that is minimally invasive, accurate, reproducible, fast, non-destructive, and cost-effective [15]. Using Raman technology to differentiate between cancerous and non-cancerous tissues may be useful to evaluate a patient's tissue in real-time during surgery [12].

Samples used for analysis

The ideal biological samples for diagnostic tests are biofluids (i.e., blood, urine, or saliva) as their collection is non-invasive and they can be obtained repeatedly without harming the patient. Also, biofluids are composed of important chemical components, including DNA, hormones and proteins, which make them perfect for analyses via Raman spectroscopy techniques. Therefore, research has been focused on evaluating the use of Raman technologies to analyse the biofluids of cancerous and non-cancerous patients [12].

The use of tissue samples to diagnose various forms of cancer when studied and analysed using Raman spectroscopy and chemometrics, has great potential. While the collection of tissue samples can be invasive and uncomfortable for the patient, histopathological diagnoses- which are usually subjective, experience-based, and not always accurate- can be confirmed using Raman spectroscopy analyses of tissue samples. Raman spectroscopy can therefore increase the accuracy of diagnostic procedures. To understand the full extent and capacity of Raman spectroscopy use in cancer diagnosis, in vivo analysis experiments are being undertaken to see whether the technique has potential intraoperative benefits. Tissue samples are advantageous since they can indicate the presence of cancer in the body, therefore, tissue is frequently biopsied and is readily available for Raman spectroscopic analysis for the purpose of diagnosing cancer [18].

Cytology (cells) samples are easy to collect, cause less discomfort for the patient, cost less money, and are less likely to result in complications in comparison to tissue sample biopsies. Hence, Raman spectroscopic analysis of cells, in combination with advanced analysis methods (chemometrics), have been used for diagnosing cancers [18].

Prostate cancer

There were 1,414,259 recorded prostate cancer cases in 2020 [11]. Prostate cancer is the second most frequent cancer diagnosis in men and the fifth leading cause of death worldwide [23]. Raman spectroscopy has been used as a technique in early, non-invasive detection of prostate cancer since 2003 by numerous researchers. A summary of selected published research has been condensed in the table below.

Author	Cancer site	Samples	Results	Comments
Porter et al. 2003 [12]	Prostate	Serum (blood, and urine) using SERS	96% sensitivity 98% accuracy	Differentiated between prostate cancer patients and healthy volunteers
Li et al. [24]	Prostate	Serum (blood samples) using SERS and silver nanoparticles	Accuracy of 98.1%	Differentiated between malignant condition and healthy volunteers
Medipally et al. [24]	Prostate	Serum (blood plasma) using PCA-LDA and High Throughput-Raman Spectroscopy	Sensitivity 96.5% Specificity 95%	Differentiated between malignant condition and healthy volunteers
Crowe et al. [4]	Prostate	Tissue (biopsy)	Accuracy 89%	Differentiated between benign condition and malignant condition
Corsetti et al. [18]	Prostate	Cells by PCA-LDA	95% sensitivity and 88% specificity	Differentiated between normal and metastatic hormone-resistant prostate cancer

Oral cancers

There have been 377,713 cases of lip and oral cavity cancers in 2020 [11].
Oral cancer incidence has been increasing, and is a large problem worldwide. This cancer can rapidly spread, which emphasises the urgent need for early detection and monitoring [3]. Results from several experiments in selected published research papers have been summarised in the table.

Author	Cancer site	Samples	Results	comments
Murali Krishna et al. 2012 [3]	Oral	Ex-vivo tissues analysed using fibre-optic probe Raman spectrometer	prediction accuracies ranging from 72–96%	Differentiated between normal control, pre-malignant, and malignant sites
Sahu et al. 2013 [12]	Oral	serum (blood and urine) samples by conventional PCA–LDA spectral analysis	diagnostic sensitivity, specificity, and accuracy of 98.6%, 87.1%, and 93.7%, respectively	Differentiated between buccal mucosa and tongue cancer, and healthy volunteers
Daniel et al. 2014 [18]	Oral	tissue using PCA and KCA	98.9% accuracy for discerning the two groups	Differentiated between normal and cancerous oral tissue
Pachaiappan et al. 2015 [18]	Oral	blood plasma and saliva by PCA-LDA	Algorithms separated the normal group from the pre-malignant group with 96.3% sensitivity and 80.0% specificity and the normal group from the malignant group with 91.2% sensitivity and 96.7% specificity	Differentiated between healthy individuals and patients with oral sub mucous fibrosis and oral SCC
Pachaiappan et al. 2015 [18]	Oral	blood plasma and saliva by PCA-LDA with LOO-CV (Leave-One-Out Cross-Validation)	PCA-LDA with LOO-CV algorithms separated normal from pre-malignant samples with 96.4% sensitivity and 70.2% specificity and normal from malignant samples with 93.8% sensitivity and 95.7% specificity	Differentiated between healthy individuals and patients with oral sub mucous fibrosis and oral cancer
Brindha et al. 2015 [16]	Oral	Serum (urine) sample	98.6% sensitivity and 87.1% specificity, with an overall accuracy of 93.7% for identifying the cancer patients	Differentiated between oral cancer and healthy donors
Guze et al. 2014 [16]	Oral	In vivo using Raman fibre-optic systems PCA-LDA with LOO-CV.	100% sensitivity and 77% specificity	Differentiated between pre-malignant/malignant lesions and normal/benign tissue

Breast cancer

Worldwide, there have been 2,261,419 reported cases for female breast cancer in 2020 [11]. It is the most common cancer in women [10]. The management of these cancers not only has significant mortality and morbidity but also has significant emotional and psychological effects on the patients. The use of minimally invasive optical imaging Raman spectroscopy techniques can greatly improve the accuracy of breast cancer diagnosis, by reducing the false positivity rates associated with mammography, as well as differentiating between benign and malignant conditions [10].

Lung cancer

Lung cancer had the highest cancer mortality rate in 2020, with 2,206,771 cases, and 1,796,144 deaths worldwide [11]. Lung cancer is one of the most common causes of cancer related death, impacting millions of people annually [3]. Despite advances in surgical, radiotherapeutic, and chemotherapeutic treatments, the long-term survival rate for lung cancer remains low. Low survival-rates are a reflection of the fact that almost 75% of patients have late-stage disease, when effective treatment is unlikely to succeed [17].

Author	Site	Sample	Results	Comments
Wong et al. [12]	Lung	Tissue by CARS microscopy	accuracies of 91% for normal tissue and 92% for cancerous tissue	Differentiated between normal, benign, and malignant lung tissues
Li et al. [18]	Lung	Serum (blood) by	100% sensitivity and specificity	Differentiated between healthy volunteers and malignant/cancerous

Conclusion

1 in 2 people will develop some form of cancer during their lifetime, and it is a leading cause of death worldwide. Because of its association with mortality and morbidity, it is vitally important to diagnose cancer as early-on in its progression as possible. This review proposes Raman spectroscopy as a potential additional diagnostic tool for this task. In a variety of different ways, the combination of Raman spectroscopy with advanced chemometric analysis has proven its usefulness for diagnosing cancer.

These techniques have considerable potential to reduce large numbers of normal invasive biopsies, reduce the time delay between screening and diagnosis, and therefore diminish patient anxiety, and make treatment more effective and successful to reduce the worldwide cancer mortality rates.

The next important step is to bring this technology into clinical usage by taking Raman technologies and placing them in the hands of a primary care team i.e., general practitioners, nurses, or caregivers.

References

1. www.nhs.uk/conditions/cancer/. 2019. Accessed August 16, 2021
2. https://www.cancerresearchuk.org/health-professional/cancer-statistics/survival/all-cancers-combined#heading-One. Accessed August 10, 2021
3. Pence I, Mahadevan-Jansen A. Clinical instrumentation and applications of Raman spectroscopy. Chemical Society Reviews. 2016;45(7).
4. Devpura S, Barton KN, Brown SL, Palyvoda O, Kalkanis S, Naik VM, et al. Vision 20/20: The role of Raman spectroscopy in early stage cancer detection and feasibility for application in radiation therapy response assessment. Medical Physics. 2014 Apr 18;41(5).
5. Mitsutake H, Poppi R, Breitkreitz M. Raman Imaging Spectroscopy: History, Fundamentals and Current Scenario of the Technique. Journal of the Brazilian Chemical Society. 2019;
6. en.wikipedia.org/wiki/C._V._Raman. Accessed August 14, 2021
7. https://www.aps.org/publications/apsnews/200902/physicshistory.cfm. 2009. Accessed August 12, 2021
8. blog.bccresearch.com/a-history-of-raman-spectroscopy. 2019. Accessed August 16, 2021
9. WHO.int/news-room/fact-sheets/detail/cancer. 2021. Accessed August 20, 2021
10. en.wikipedia.org/wiki/cancer. Accessed August 12, 2021
11. Sung H, Ferlay J, Siegel RL, Laversanne M, Soerjomataram I, Jemal A, et al. Global Cancer Statistics 2020: GLOBOCAN Estimates of Incidence and Mortality Worldwide for 36 Cancers in 185 Countries. CA: A Cancer Journal for Clinicians. 2021 May 4;71(3).
12. Austin LA, Osseiran S, Evans CL. Raman technologies in cancer diagnostics. The Analyst. 2016;14(2).
13. who.int/activities/promoting-cancer-early-diagnosis. 2021. Accessed August 14, 2021
14. Eberhardt K, Stiebing C, Matthäus C, Schmitt M, Popp J. Advantages and limitations of Raman spectroscopy for molecular diagnostics: an update. Expert Review of Molecular Diagnostics. 2015 Jun 3;15(6).
15. Kallaway C, Almond LM, Barr H, Wood J, Hutchings J, Kendall C, et al. Advances in the clinical application of Raman spectroscopy for cancer diagnostics. Photodiagnosis and Photodynamic Therapy. 2013 Sep;10(3).
16. Collinsdictionary.com/dictionary/english/raman-effect. Accessed August 19, 2021
17. Kong K, Kendall C, Stone N, Notingher I. Raman spectroscopy for medical diagnostics — From in-vitro biofluid assays to in-vivo cancer detection. Advanced Drug Delivery Reviews. 2015 Jul;89.
18. Ralbovsky NM, Lednev IK. Raman spectroscopy and chemometrics: A potential universal method for diagnosing cancer. Spectrochimica Acta Part A: Molecular and Biomolecular Spectroscopy. 2019 Aug;219.
19. Mitsutake H, Poppi R, Breitkreitz M. Raman Imaging Spectroscopy: History, Fundamentals and Current Scenario of the Technique. Journal of the Brazilian Chemical Society. 2019;
20. https://www.horiba.com/at/scientific/products/raman-spectroscopy/raman-academy/raman-tutorial/raman-scattering/. 2021. Accessed August 10, 2021
21. Huang C-C, Isidoro C. Raman Spectrometric Detection Methods for Early and Non-Invasive Diagnosis of Alzheimer's Disease. Journal of Alzheimer's Disease. 2017 Apr 19;57(4).
22. Nicolson F, Kircher MF, Stone N, Matousek P. Spatially offset Raman spectroscopy for biomedical applications. Chemical Society Reviews. 2021;50(1).
23. Rawla P. Epidemiology of Prostate Cancer. World Journal of Oncology. 2019;10(2).
24. Auner GW, Koya SK, Huang C, Broadbent B, Trexler M, Auner Z, et al. Applications of Raman spectroscopy in cancer diagnosis. Cancer and Metastasis Reviews. 2018 Dec 19;37(4).

Stem Cells in the Regeneration of Major Visceral Organs: Reduction in Xenotransplantation-associated Medical and Ethical Complications

Hemshankar Laugi

Abstract

Organ failure at a higher pace than organ supply has been a burning issue in today's world with respect to the growing life expectancy and the human population of the world. Although several modern advances have been made in cell regeneration to date, the demand for healthy and well-functioning human organs exceeds the organ supply. This research serves to discuss the current trends of organ demand and organ supply, highlight the breakthroughs in organ transplantation, analyze the prospects and challenges of xenotransplantation, and seek an alternative and reliable method to mitigate the challenges seen in xenotransplantation.

Introduction

Organ transplantation is an effective approach to replacing a malfunctioning organ with a well-functioning one. With the advent of the growing lifespan of people, the number of cases of organ failure is rising at a higher rate than the supply of well-functioning organs. According to the US Government Information on Organ Donation and Transplantation, more than 27000 patients were on the transplant waiting list in the year 1992, but only around 16000 transplants were done [1]. In 2016, around 86000 patients were left without a well-functioning organ. As of January 2019, more than 112000 patients were on the transplant waiting list, but only about 39000 transplants were done and around 73000 patients didn't get organs [1]. The trend of organ demand and supply in other parts of the globe is even worse than this. As shown in figure 1 below, the number of patients on transplant waiting lists has increased around four-fold between 1992 and 2019, while the number of donors has increased only marginally. Hence, it is the need of the time to develop an appropriate method of organ transplantation to reduce deaths related to organ failure.

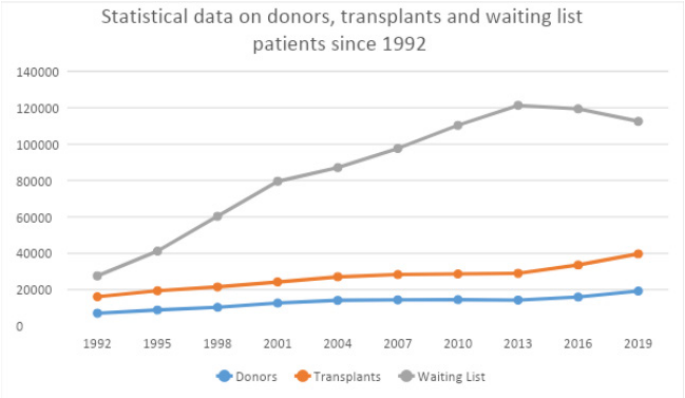


Figure 1: Organ Donation and Transplantation Statistics in the United States (Source: https://www.organdonor.gov/statistics-stories/statistics/data.html)

Methods

Databases like Google Scholar, Pubmed, and SCOPUS were searched to find and study the available research papers in detail. The prospects and challenges of xenotransplantation were analyzed. After researching several potential approaches to mitigate the challenges in organ transplantation, a conclusion of using stem cells for the purpose was generated.

Results

Xenotransplantation has the potential to reduce the gap between organ demand and supply Pigs are commonly used animals, and show cardiac output, glomerular filtration rate, and insulin production mechanisms similar to those of humans. This similarity increases the chances of success of pig organs in humans. These animals can be genetically manipulated and reproduced in a lab to get more organs that perform similar functions to that of human organs without autoimmune rejection. However, ethical implications such as animal mistreatment, and medical complications such as organ rejection and infection with Porcine Endogenous Retrovirus doesn't make xenotransplantation an effective solution to solve organ crisis. The use of pluripotent stem cells is found to be a promising way of generating viscera. Although there are some chances of cellular contamination, ensuring a hygienic environment while growing stem cells could easily reduce the contamination. The current knowledge of using stem cells in organ regeneration is still limited. Hence, adequate research is to be done in order to use reprogrammed pluripotent stem cells in organ regeneration and transplantation in such a way that tumor formation, immunologic stem cell rejection, and other stem cell therapy-associated risks can be limited.

Discussion

Xenotransplantation is a scientific approach to transferring healthy and well-functioning organs from one species to another in a way that the transplanted organ doesn't get rejected by the recipient's immune system [2]. The difference in molecular and biological processes going in immune systems of different species differ posing a risk of rejection. This concept was put forth after the Xenotransfusion of blood from lambs to humans in 1667 [3]. The idea of organ xenotransplantation was hypothesized by Professor Keith Reemtsma in the late 1960s. According to him, chimpanzees bear similar genetic makeup and close evolutionary relationships with humans. Hence, their kidneys can be transplanted into humans to solve organ crises [4]. During the period 1963-1964, 13 kidney transplantations were performed. However, the majority of patients died due to either organ rejection or infection. This highlighted the complexity of the human immune system in using organs from other animals [5]. With the advent of these medical reports, researchers noticed that non-human primates were not suitable source animals for clinical xenotransplantation. Moreover, difficulty in animal breeding for organs further created hindrance in xenotransplantation [6]. After the 1990s, successful experiments on pigs drew the attention of researchers in using porcine organs to solve organ crises.

Date	Organ	Past Record	Result after the Transplant	Surgeon	References
1906	Out of two patients, goat kidney into one and pig kidney into another	Renal Failure	Sudden death	Jaboulay	[39]
1 March, 1963	liver		Death during the surgery due to coagulation disorder & uncontrolled bleeding	Starzl et.al	[40]
11 June, 1963	Left Lung	Left lung bronchial carcinoma	Death after 18 days due to renal failure and infection	James D. Hardy	[41]
1963/64	Chimpanzee kidney	Renal failure	Death after 9 month due to acute electrolytic disturbance	Keith Reemtsma	[4,15]
18 Jan, 1964	Chimpanzee heart	Atheromatous vascular disease	Death after 2 hour due to inadequate blood circulation	James Hardy	[4,15]
1967	liver	Hepatocellular cancer	Death after 1 year due to disease reoccurrence	Thomas Starzl	[40]
2/3 Dec, 1967	Orthotopic heart transplant	Coronary insufficiency	Death after 14 days due to radiographic infiltrates in lungs	Christian Barnard	[42]
1993	Pig islet	Diabetes	Success after reporting porcine C-peptide in some patients only	Carl Groth	[4]

Table1: History of human organ Transplantation.

Pig as a source of organ

Animal breeders have reported possible benefits in using porcine organs as human organs because pigs can be grown quickly to about the desired size. Also, they produce large litters and can be reared in specific pathogen-free conditions [7]. Moreover, pigs can be genetically manipulated to produce porcine organs that are resistant to rejection [8]. These all have drawn attention to the use of porcine organs in reducing organ shortage. Physiologically, pigs show identical cardiac output, stroke volume, heartbeat rate, mean arterial pressure, and myocardial blood flow to that of humans that meet physiological needs of humans [9].

The kidney plays a significant role in maintaining homeostasis, salt and water balance, excretion of metabolites, electrolyte balance, and regulating body fluid and osmolarity. Porcine kidneys exhibit the maximum concentrating ability of 1080 mOsm/L and a glomerular filtration rate of 126–175 ml/h [10]. These are found to be identical to those of human kidneys that have a glomerular filtration rate of 125 ml/min [10]. This shows that pig kidney anatomy is remarkably similar to the human kidney, giving better hope in using porcine kidneys to solve ongoing kidney shortages [10]. Erythropoietin is a hormone synthesised by the kidneys, which is essential for regulating the production and maturation of red blood cells, also known as erythropoiesis. With regard to the transplant of a porcine kidney into the human, it has been reported that human erythropoietin receptors on red blood cell precursors in the bone marrow can't recognize porcine erythropoietin. As a result, this hormone from pigs doesn't function well in humans. However, using recombinant human erythropoietin has shown positive results [7].

Pig lung is reported to be able to provide adequate oxygenation and carbon dioxide exchange in nonhuman primates, although enough experiments haven't been carried out with the xenotransplantation of pig lungs [11]. Pig lungs exhibit survival up to only 5 days in non-human primates and hence no definite conclusions can be made about the survival rates in humans [11]. Researchers have reported a limited survival rate with pulmonary xenotransplantation from pig-to-human as compared to other major solid organs [15]. Lungs are able to release large amounts of von Willebrand Factor that binds GPIb on human platelets thereby triggering hemostasis. This eventually activates blood platelets leading to blood clotting. A possible future strategy to control this includes using transgenic pigs that express tissue factor pathway inhibitors or CD39 [15].

Insulin extracted from pig islet cells after transplantation into humans has been used for the treatment of diabetes for decades. As porcine islets produce insulin which is physiologically similar to that of humans, pigs are found to be a reliable source of obtaining islets which greatly help in the treatment of T1D (Type-1 Diabetes) [12]. Several experiments conducted on genetically engineered pigs have revealed a greater success rate in using pigs for xenotransplantation. For example, the use of alpha 1,3-galactosyltransferase gene knockout (GTKO) pigs greatly helps in reducing cases of immune rejection thereby improving compatibility between the donor and recipient [13]. Valdez-Gonzalez demonstrated a new way to reduce insulin requirement by the human body by grafting neonatal porcine islets into the subcutaneous tissue of 12 children with Insulin-Dependent Diabetes Mellitus (IDDM) without using immunosuppressive drugs [25]. Hence, pigs could be a possible source of major organs required for xenotransplantation in the future which can successfully limit the gap between the organs need and supply.

Organ Rejection

When the immune system of the organ recipient recognizes the donor organ as foreign elements such as microorganisms detrimental to the recipient, it rejects the donor organ and thus donor organ can't function in the recipient's body. Organ transplant rejection is usually of three types namely hyperacute, acute and chronic rejection. Hyperacute rejection is normally caused within minutes or hours after the organ transplant when the recipient's immune system produces specific antibodies against the organ, as it considers the donor organ as a foreign harmful element. Acute rejection occurs when specific lymphocytes in the recipient's body recognize Human Leucocytes Antigens (HLAs) in the donor organ transplanted in the recipient's body. It generally takes place within some days or weeks after the organ transplantation. Finally, chronic rejection usually occurs within months or years after the transplantation. Various mechanisms involving chronic inflammation, humoral, and cellular immune reactions contribute to the immune pathogenesis of chronic rejection [14].

Concerns with Xenotransplantation

Medical concerns deal with the recipients being highly susceptible to xenozoonosis after receiving porcine organs. The recipients of porcine organs are prone to Porcine Endogenous Retrovirus (PERV), a pig virus from the same family as HIV, which gets incorporated into human deoxyribonucleic acid (DNA) after undergoing xenotransplantation. The genome of pigs contains many loci coding for Endogenous Retrovirus (ERV) which aren't found in humans. These viruses are inherited after the transplantation causing infection in the recipient's body [16]. Retroviral transmission after xenotransplantation leads to altered gene expression in the recipient's genome. So far, three closely related C-type porcine endogenous retroviruses that are capable of retroviral transmission have been identified in the pig. They are labeled as PERV A, B, and C. Although PERV-C infects only pig cells, PERV-A and –B can infect both human and pig cells in vitro. Human cellular receptors for PERV-A namely HuPAR-1 and HuPAR-2 have been identified. PERV mRNAs are expressed in all pig tissues and in all breeds of pig tested to date [17].

In addition to serious medical concerns with xenotransplantation, there have been several ethical issues with it. The most important criticism expressed is going against religious norms and values. Consequentialist arguments consider the process of transgenic animals' organ transplantation into the human to be unacceptable due to several immunological barriers seen after the transplant. Animal rights activists strongly oppose the use of pigs in breeding genetically for more organ production required for the welfare of humans by solving the organ crisis [18].

Overcoming Xenotransplantation-associated Risks

With the advent of xenotransplantation-associated risks, scientists are seeking out new reliable techniques to reduce these risks. They are trying to use transgenic pigs (pigs that have foreign genes inserted into their genome) in the transplantation. The human gene responsible for producing a complement regulating protein namely CD55 and CD59 is incorporated into the pig genome. Kidneys taken from these transgenic pigs having gene coding for CD55 in their genome showed increased protection against hyperacute rejection, even in the absence of immunosuppressive drugs [19].

CRISPR/Cas9 genome editing system was first discovered as an RNA-guided defense mechanism and a self-adapted bacterial immunity for the protection from bacteriophages and other foreign elements. It helps to edit or remove any genetic elements responsible for causing the infection. It is used by bacteria as an adapted immunity system to be protected from phages when they infect the bacteria the second time. It is a kind of memory [20]. Scientists have successfully reported high chances of survival with the use of porcine organs by using CRISPR-Cas9 genome editing to modify the genes which cause the immunologic responses to the porcine grafted organs namely HAR, AHXR, immune cell-mediated rejection, and chronic rejection [21]. In 2017, scientists successfully inactivated PERVs in a porcine primary cell line and generated PERV-inactivated pigs using the same technology,using Somatic Cell Nuclear Transfer (SCNT) technology to produce embryos of pigs that are free from PERV [22]. Although the use of CRISPR-Cas technology helps to produce transgenic pigs to increase the rate of successful organ transplantation, the aforementioned ethical issues remain unsolved.

Stem Cells in generating functional organs: Future Perspectives

Stem cells are the specialized cells that possess the ability to perform specialized functions after undergoing genetic reprogramming. For instance, a single stem cell can be specialized to perform functions of nerve cells, blood cells, osteoblasts, etc. after undergoing genetic reprogramming. Hematopoietic Stem Cells (HSCs), found in bone marrow, can be reprogrammed to perform functions of nerve cells and so on. Despite several complications seen in stem cell therapy, these cells have created a new platform for the treatment of a number of dreadful diseases like cancer, diabetes, cardiovascular diseases, sight loss, memory loss, Alzheimer's disease.

They are used in checking the efficiency of new drugs. For example, a drug prepared by a researcher to cure a nerve disease can test on nerve cells created from stem cells in a lab before undergoing human trials. Adult stem cells have self-renewal capacity and, though limited in magnitude, pluripotency. Generally, these primitive cells are stored in a specialized environment called a niche, where they are protected from harmful foreign particles and are provided with a favorable environment required to grow [43]. Within the niche, they remain connected to supporting cells and are kept quiescent until some activating signal is exposed to them. As soon as they are activated, tissue-specific adult stem cells divide, migrate to leave the niche, and differentiate to replace senescent or worn-out cells within the damaged organ. These specialized cells can repair mild

injuries in various organs such as the liver, intestine, skin, kidney,, and thus, make damaged organs well-functional, thereby solving organ crisis [26].

A number of researches on stem cell therapy have revealed the chances of cloning new organs from stem cells. This helps to reduce the demand for organs. Researchers have successfully generated mammary glands and the prostate in vivo from a single adult tissue stem cell [23]. On transplanting single stem cells from adult mouse mammary glands in the fat-pad mice, researchers have reported the successful production of the secretory mammary gland in the recipient mice. It is long believed that mammary glands undergo extensive growth at puberty and further expansion and retraction during pregnancy under the influence of the female sex hormone called estrogen. These natural phenomena have revealed the availability of stem cells in the mammary glands [24].

Heart Regeneration

Cardiac cells get destroyed under the attack of heart diseases, which limits the proper functioning of the heart. Reparative and regenerative stem cells have the capability to restore cardiac function. Stem cell therapy has emerged as a protective and effective strategy to improve heart function disturbed by the attack of heart diseases via repairing the infarcted myocardium and promoting cardiac regeneration. Reprogrammed stem cells are able to replace senescent cells in the damaged heart. Various cell types, including embryonic stem cells (ESC), induced pluripotent stem cells, bone marrow, and other adult tissue-derived mesenchymal stem cells (MSCs), hematopoietic stem cells (HSCs), and endothelial progenitor cells can transform into cardiac myocytes both in vivo and in vitro after undergoing genetic reprogramming. Cardiac damage leads to the loss of cardiomyocytes, which needs to be repaired for the well-functioning of the heart. With the advent of using stem cell therapy in restoring cardiac function, resisting the pro-death environment in the myocardial tissue has been a major obstacle to patient recovery. Stem cell survival signaling pathways such as Phosphatidylinositol 3-Kinase (p13K)/ AKT and Mitogen-Activated Protein Kinase (MAPK) ensure cell survival after stem cell transplant [45]. With regard to several complications seen after stem cell transplantation, pretreatment of stem cells with exosomes, small molecule inhibitors, miRNAs, peptides, etc, has given positive results in reducing these complications. Moreover, undergoing genetic modification further solves the problem [27].

Liver Regeneration

The liver is an organ having maximum regenerative capacity in the body. Direct reprogramming in fibroblasts or human Induced Pluripotent Stem Cells (iPSCs) leads to the production of new hepatocytes that can successfully restore liver function. However, any error in iPSC genetic reprogramming triggers carcinogenesis in vivo. Also, incomplete differentiation of iPSC into hepatocytes results in impaired hepatocyte function. With regard to solving these challenges in using iPSCs, Human Hepatic Stem Cells (HpSCs) from the liver lineage has therefore become a reliable alternative source to generate primary hepatocytes. It has been found that HpSCs obtained from fetal and postnatal livers give rise to differentiated hepatocytes in vitro and more mature hepatocytes in vivo [28]. Migrating inactive or senescent hepatocytes into the cell cycle and undergoing their development beyond the restrictive point in the G1 phase of the cell cycle leads to liver regeneration. Scientists have reported successful hepatic regeneration with the restored liver function via the mitogenic action of three main growth factors namely hepatocyte growth factor (HGF), epidermal growth factor (EGF), and transforming growth factor-alpha (TGF-a) on hepatocytes after stimulating DNA synthesis [29].

Kidney Regeneration

Pluripotent stem cells, iPSCs, and ESCs have been considered as potential means of repairing senescent cells in kidneys damaged due to kidney diseases. In course of using these cells in restoring kidney function, these cells need to be differentiated into mature renal cells. This greatly reduces tumorigenicity intrinsic in pluripotent stem cells. Genetic reprogramming in these stem cells helps to obtain desired target tissue [30]. It has been successfully reported that iPSC can retain both genetic background and distinct epigenetic memory of the cells of origin. This potentiality of iPSC can greatly help in using these stem cells in cell therapy and kidney regeneration [31]. Cinzia Rota et. al have shown that injecting iPSC-derived RPCs into mice with acute kidney injury (AKI) leads to improvement in renal function. Moreover, it has been found that injecting murine bone marrow in cisplatin mice results in improved renal function. This has shown that MSCs can also help in the regeneration of the damaged kidney [32]. Besides these, Togel and co-workers, while working on the case with reperfusion injury, have found a renoprotective effect with bone marrow MSCs through the secretion of several biologically active factors with anti-apoptotic, immunomodulatory, anti-oxidative, and pro-angiogenic properties. Thus, these cells show promising results in repairing damaged kidneys [30].

Lung Regeneration

Stem cell differentiation mediates lung repair and regeneration. Signaling pathways like Wnt and Notch greatly help in stem cell self-renewal and in regulating regenerative responses [44]. So far, several groups have successfully demonstrated activation of canonical Wnt signaling in various compartments in the lung undergoing active regrowth and regeneration via the use of Wnt reporter lines [33]. Different specialized cell types with lungs have created a challenge in generating human airway and alveolar epithelial cells from iPSCs. In course of lung regeneration, human iPSCS should be reprogrammed to obtain definitive endoderm followed by generation of anterior foregut endoderm. Lung endoderm can be derived from this anterior foregut endoderm. It can then subsequently be guided towards bronchial progenitor cells (Sox2+) or alveolar progenitor cells (Sox9+), and finally towards bronchial or alveolar epithelial faith. Several studies have shown the differentiation of ESCs and iPSCs into the most numerous cells of alveoli i.e AT-II cells [44]. Moreover, research on MSCs has revealed the potentiality of these cells derived from human umbilical cord blood in

generating alveolar cells by culturing them in lung-specific differentiation media [34].

Limitations of using Stem cells in Organ Regeneration

Stem cells should undergo genetic reprogramming to be able to regenerate a damaged organ. A myriad of biomolecules such as transcriptional factors, enzymes, signaling molecules, and miRNAs are found to be associated with the genetic reprogramming of stem cells. Recent molecular and cell biology studies have revealed how a limited number of reprogramming factors can initiate specific genomic remodeling that changes the molecular and cellular machinery of cells [35]. Genetic modification leads to epigenetic alterations and a single alteration in any locus of a gene leads to further change in the entire cellular mechanism which can be challenging to be tackled. Retroviruses and lentiviruses possess the capacity to generate human iPSCs. These viruses undergo genetic alteration to encode the genes required for transformation into an iPSC. By using the derived iPSCs, the used viruses can integrate into the cell genome. This leads to the risk of getting infected by these viruses [37]. There has been a major social risk in using ESCs as a source for organ regeneration. In order to use ESCs in repairing diseased or damaged organs, new ESCs lines need to be created. This results in the destruction of the embryo, which raises a major ethical concern in using them as a potential source of organ regeneration. Other risks of stem cell transplantation include tumor formation, inappropriate stem cell migration, and immune rejection of transplanted stem cells [36]. Furthermore, several cases of organ and tissue transplantation have revealed a greater chance of transmission of several infectious agents from donor cells to the recipients [38].

Conclusion

Xenotransplantation has been found to be an effective way to reduce the gap between organ demand and supply thereby saving thousands of lives worldwide. However, with regard to serious medical and ethical complications observed in xenotransplantation, the use of pluripotent stem cells is found to be a promising way in organ regeneration and transplantation. There is a greater chance of cellular contamination while growing stem cells. Therefore, all the steps involved in this process must be conducted in a safe and hygienic environment. The current knowledge of using iPSCs or MSCs in organ regeneration is still limited. Hence, adequate research is to be done in order to use reprogrammed pluripotent stem cells in organ regeneration and transplantation in such a way that tumor formation, immunologic stem cell rejection, and other stem cell therapy-associated risks can be limited.

References

1. U.S. Health Resources and Service Administration. 2020. "Organ Donation Statistics". 2020. Organdonor.gov. https://www.organdonor.gov/statistics-stories/statistics/data.html
2. U.S. Food and Drug Administration 2019. "Xenotransplantation" https://www.fda.gov/vaccines-blood-biologics/xenotransplantation.
3. Aristizabal AM, Caicedo LA, Martinez JM, Moreno MGJE. Clinical xenotransplantation, a closer reality: literature review. Cir Esp. (2017) 95:62– 72. doi: 10.1016/j.cireng.2017.03.007
4. Cooper DKC, Ekser B, Tector AJ. A brief history of clinical xenotransplantation. Int J Surg. 2015 Nov;23(Pt B):205-210. doi: 10.1016/j.ijsu.2015.06.060. Epub 2015 Jun 26. PMID: 26118617; PMCID: PMC4684730.
5. Berki, D. 2018. "A Brief History of Xenotransplantation". Omixon NGS For HLA.
6. https://www.omixon.com/brief-history-xenotransplantation/
7. Cooper DKC, Gaston R, Eckhoff D, Ladowski J, Yamamoto T, Wang L, et al. Xenotransplantation-the current status and prospects. Br Med Bull. (2018) 125:5–14. doi: 10.1093/bmb/ldx043
8. Weiss, R A. "Xenotransplantation." BMJ (Clinical research ed.) vol. 317,7163 (1998): 931-4. doi:10.1136/bmj.317.7163.931
9. Cooper DK, Gollackner B, Sachs DH. Will the pig solve the transplantation backlog? Annu Rev Med. (2002) 53:133–47. doi: 10.1146/annurev.med.53.082901.103900
10. Ibrahim Z, Busch J, Awwad M, Wagner R, Wells K, Cooper DKC. Selected physiological compatibilities and incompatibilities between human and porcine systems. Xenotransplantation. 2006; 13:488–499. [PubMed: 17059572]
11. Ekser B, Cooper DKC, Tector AJ. The need for xenotransplantation as a source of organs and cells for clinical transplantation. Int J Surg. 2015 Nov;23(Pt B):199-204. doi: 10.1016/j.ijsu.2015.06.066. Epub 2015 Jul 16. PMID: 26188183; PMCID: PMC4684733.
12. Navarro-Alvarez, N et al. "The Effects of Exogenous Administration of Human Coagulation Factors Following Pig-to-Baboon Liver Xenotransplantation." American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons vol. 16,6 (2016): 1715-1725. doi:10.1111/ajt.13647
13. Cheng, M. Islet Xeno/transplantation and the risk of contagion: local responses from Canada and Australia to an emerging global technoscience. Life Sci Soc Policy 11, 12 (2015). https://doi.org/10.1186/s40504-015-0030-2
14. Liu, Zhengzhao et al. "Pig-to-Primate Islet Xenotransplantation: Past, Present, and Future." Cell transplantation vol. 26,6 (2017): 925-947. doi:10.3727/096368917X694859
15. Justiz Vaillant AA, Mohseni M. Chronic Transplantation Rejection. [Updated 2020 Jul 5]. In: StatPearls [Internet]. Treasure Island (FL): StatPearls Publishing; 2020 Jan-. Available from: https://www.ncbi.nlm.nih.gov/books/NBK535435/
16. Michel SG, Madariaga MLL, Villani V, Shanmugarajah K. Current progress in xenotransplantation and organ bioengineering. International Journal of Surgery (London, England). 2015 Jan;13:239-244. DOI: 10.1016/j.ijsu.2014.12.011.
17. Schmidt, P., Andersson, G., Blomberg, J. et al. Possible Transmission of Zoonoses in Xenotransplantation: Porcine Endogenous Retroviruses (PERVs) from an Immunological Point of View. Acta Vet Scand 45, S27 (2004). https://doi.org/10.1186/1751-0147-45-S1-S27
18. Fishman, Jay A. "Infectious disease risks in xenotransplantation." American journal of transplantation : official journal of the American Society of Transplantation and the American Society of Transplant Surgeons vol. 18,8 (2018): 1857-1864. doi:10.1111/ajt.14725
19. Behnam Manesh, Shima et al. "Ethical issues of transplanting organs from transgenic animals into human beings." Cell journal vol. 16,3 (2014): 353-60.
20. Ménoret, Séverine et al. "Characterization of human CD55 and CD59 transgenic pigs and kidney xenotransplantation in the pig-to-baboon combination." Transplantation vol. 77,9 (2004): 1468-71. doi:10.1097/01.tp.0000111758.35048.ea
21. Barrangou, Rodolphe. "The roles of CRISPR-Cas systems in adaptive immunity and beyond." Current opinion in immunology vol. 32 (2015): 36-41. doi:10.1016/j.coi.2014.12.008
22. Naeimi Kararoudi, Meisam et al. "Clustered Regularly Interspaced Short Palindromic Repeats/Cas9 Gene Editing Technique in Xenotransplantation." Frontiers in immunology vol. 9 1711. 5 Sep. 2018, doi:10.3389/fimmu.2018.01711
23. Niu, Dong et al. "Inactivation of porcine endogenous retrovirus in pigs using CRISPR-Cas9." Science (New York, N.Y.) vol. 357,6357 (2017): 1303-1307. doi:10.1126/science.aan4187
24. Shackleton, M., Vaillant, F., Simpson, K. J., Stingl, J., Smyth, G. K., Asselin-Labat, M. L., Wu, L., Lindeman, G. J., & Visvader, J. E. (2006). Generation of a functional mammary gland from a single stem cell.

Nature, 439(7072), 84-88. https://doi.org/10.1038/nature04372
25. Liu, Yunying et al. "Generation of functional organs from stem cells." Cell regeneration (London, England) vol. 2,1 1. 22 Jan. 2013, doi:10.1186/2045-9769-2-1
26. Valdés-González, Rafael A et al. "Xenotransplantation of porcine neonatal islets of Langerhans and Sertoli cells: a 4-year study." European journal of endocrinology vol. 153,3 (2005): 419-27. doi:10.1530/eje.1.01982
27. Montagnani, Stefania, et al. "Adult Stem Cells in Tissue Maintenance and Regeneration." Stem Cells International, 2016. Gale OneFile: Health and Medicine, . Accessed 4 Oct. 2020.
28. Abdelwahid, Eltyeb et al. "Stem cell death and survival in heart regeneration and repair." Apoptosis : an international journal on programmed cell death vol. 21,3 (2016): 252-68. doi:10.1007/s10495-015-1203-4
29. Zhang, Ran-Ran et al. "Hepatic stem cells with self-renewal and liver repopulation potential are harbored in CDCP1-positive subpopulations of human fetal liver cells." Stem cell research & therapy vol. 9,1 29. 5 Feb. 2018, doi:10.1186/s13287-017-0747-3
30. N.N. Than, P.N. Newsome, Stem cells for liver regeneration, QJM: An International Journal of Medicine, Volume 107, Issue 6, June 2014, Pages 417–421, https://doi.org/10.1093/qjmed/hcu013
31. Rota, Cinzia et al. "Stem Cell Therapies in Kidney Diseases: Progress and Challenges." International journal of molecular sciences vol. 20,11 2790. 7 Jun. 2019, doi:10.3390/ijms20112790
32. Liu, D., Cheng, F., Pan, S. et al. Stem cells: a potential treatment option for kidney diseases. Stem Cell Res Ther 11, 249 (2020). https://doi.org/10.1186/s13287-020-01751-2
33. Morigi, Marina et al. "Mesenchymal stem cells are renotropic, helping to repair the kidney and improve function in acute renal failure." Journal of the American Society of Nephrology : JASN vol. 15,7 (2004): 1794-804. doi:10.1097/01.asn.0000128974.07460.34
34. El-Badrawy, Mohammad K et al. "Stem Cells and Lung Regeneration." International journal of stem cells vol. 9,1 (2016): 31-5. doi:10.15283/ijsc.2016.9.1.31
35. Schilders, K.A.A., Eenjes, E., van Riet, S. et al. Regeneration of the lung: Lung stem cells and the development of lung mimicking devices. Respir Res 17, 44 (2016). https://doi.org/10.1186/s12931-016-0358-z
36. Hirschi, Karen K et al. "Induced pluripotent stem cells for regenerative medicine." Annual review of biomedical engineering vol. 16 (2014): 277-94. doi:10.1146/annurev-bioeng-071813-105108
37. Master, Zubin et al. "Benefits, risks and ethical considerations in translation of stem cell research to clinical applications in Parkinson's disease." Journal of medical ethics vol. 33,3 (2007): 169-73. doi:10.1136/jme.2005.013169
38. Herberts, Carla A et al. "Risk factors in the development of stem cell therapy." Journal of translational medicine vol. 9 29. 22 Mar. 2011, doi:10.1186/1479-5876-9-29
39. Wei-Hua Wang, Xiao-Fang Sun, Human embryonic stem cell lines are contaminated: what should we do?, Human Reproduction, Volume 20, Issue 11, November 2005, Pages 2987–2989, https://doi.org/10.1093/humrep/dei197
40. C. J. E. Watson, J. H. Dark, Organ transplantation: historical perspective and current practice, BJA: British Journal of Anaesthesia, Volume 108, Issue suppl_1, January 2012, Pages i29–i42, https://doi.org/10.1093/bja/aer384
41. Meirelles Júnior RF, Salvalaggio P, Rezende MB, et al. Liver transplantation: history, outcomes and perspectives. Einstein (Sao Paulo). 2015;13(1):149-152. doi:10.1590/S1679-45082015RW3164
42. Venuta F, Van Raemdonck D. History of lung transplantation. J Thorac Dis. 2017 Dec;9(12):5458-5471. doi: 10.21037/jtd.2017.11.84. PMID: 29312756; PMCID: PMC5757055.
43. Brink, J. G., & Hassoulas, J. (2009). The first human heart transplant and further advances in cardiac transplantation at Groote Schuur Hospital and the University of Cape Town - with reference to : the operation. A human cardiac transplant : an interim report of a successful operation performed at Groote Schuur Hospital, Cape Town. Cardiovascular journal of Africa, 20(1), 31–35.
44. Morrison SJ, Spradling AC. Stem cells and niches: mechanisms that promote stem cell maintenance throughout life. Cell. 2008 Feb 22;132(4):598-611. doi: 10.1016/j.cell.2008.01.038. PMID: 18295578; PMCID: PMC4505728.
45. Katoh M, Katoh M. WNT signaling pathway and stem cell signaling network. Clin Cancer Res. 2007 Jul 15;13(14):4042-5. doi: 10.1158/1078-0432.CCR-06-2316. PMID: 17634527.
46. Rheault-Henry M, White I, Grover D, Atoui R. Stem cell therapy for heart failure: Medical breakthrough, or dead end? World J Stem Cells. 2021 Apr 26;13(4):236-259. doi: 10.4252/wjsc.v13.i4.236. PMID: 33959217; PMCID: PMC8080540.

Automated system to detect and solve water parameters issues

Alhassan Ahmed

Abstract

The study aims to establish a comprehensive, efficient, and sustainable automated system in order to solve the issue of water pollution. The main focus of the study is to resolve the consequences of manual and sporadic water analysis by creating a system that efficiently treats deviations in different water parameters. Water parameters are the physical, chemical and biological properties of water that defines its usage and example of such parameters includes total dissolved solids (TDS), pH, heavy metals, temperature, dissolved oxygen (DO), and turbidity. Through analyzing the defined water parameters frequently, any deviation from the safe range can be solved immediately. Another main concern was determining the modifiers that will be used to fix flaws in the defined parameters. The final system was decided to consist of Arduino board, TDS sensor, pH sensor, temperature sensor, GSM module, heavy metals sensor, WI-FI module, and a receiver. In fact, determining the best types of sensors was a concerning demand for the study. The results showed that the system will decrease the time required and the effort exerted in order to check the water parameters regularly. In addition, the system displayed high efficiency in fixing any flaw in water parameters, consequently lowering the repercussions of water pollution.

Introduction

Recently, water pollution has increased drastically resulting in many devastating consequences such as the destruction of ecosystems, poisoning cases, crops corruption, as well as the spread of fatal diseases (cholera, diarrhoea, dysentery, hepatitis A, typhoid, and polio). Many solutions were found to treat only one type of water pollution. However, the weaknesses of these solutions are vast. First, the solutions are incomprehensive as they fix one flaw and neglect the others. For instance, using sodium carbonate (Na2Co3) to solve the high pH problem and neglecting the issue of high TDS. Second, the irregular checking of water parameters can cause dramatic consequences so finding a method that measures the water parameters instantly is important. Consequently, the study tried to create an integrated system that will automate the process of checking water parameters. The study focused on two aspects:

1- The types of sensors used in measuring the parameters.
2- The modifiers used to fix the flaw of different water parameters.

For example, the heavy metals pollution has been a serious pollution type due to the increase of anthropogenic activity, which has increased lately in metal industries. Fortunately, orange peel after undergoing pyrolysis reaction was discovered to be an eco-friendly, sustainable, and efficient solution in detoxifying heavy metals in both the soil and the water. Due to the cellulosic nature and the presence of carboxyl, sulfur and nitrogen acids of orange peel, it will perform as an efficient heavy metals adsorbent after going through the pyrolysis process. The pyrolysis process will ionize the carboxyl, sulfur and nitrogen acids that will acquire negative charge in orange peel making. As the heavy metals ions are positively charged, they will be attracted to the ionized groups making an ion exchange process between the heavy metals and the orange peel that will eliminate heavy metals pollution. In fact, orange peel has proved to be reliable in decreasing the TDS and stabilizing neutral pH between 6.9-7.1 making the water available for most potable uses.

Results and Discussion

A. Sensors

1. Temperature sensor

There are various types of temperature sensors: thermocouples, RTD (resistance temperature detectors), thermistors and infrared sensors. Each of these sensors have unique features and limits as well that will be discussed in detail.

I- Thermocouples

The thermocouples sensor is a very common type of temperature sensors that relies on the voltage difference between two used metal rods. When heat is applied to the two rods (of different material), a voltage difference starts to occur due to the excitation of the atoms making electrons travel along the rods. As each material's

resistivity is unique, a voltage difference between the two rods will occur. To determine the measured temperature, the sensor is calibrated at different temperatures. Different combinations of metals guarantee different ranges of temperature measurement. The downsides of these thermometers are the nonlinear relationship between temperature and voltage, the high probability of zero errors and the large time consumption to reset and measure the temperature.
[1]

$$R = R_0(1 + \alpha \Delta T)$$

R_0: Initial resistance.

R: Resistance after the temperature change.

α: Temperature coefficient of resistance "constant for each material".

ΔT: Change in temperature.

$$R = \frac{V}{I}$$

R: Resistance.

V: Voltage.

I: Electric Current

II- RTD (Resistance Temperature Detectors)

Similar to the thermocouples sensor, the RTD sensor relies on the effects of temperature on electrical characteristics namely, electrical resistance in the case of the RTD. What makes the RTD better than the thermocouple is the linear relationship between resistance and temperature: As resistance increases, temperature increases. [2]

III - Infrared Sensors

Like any light wave, infrared waves can be absorbed or reflected. The infrared sensor absorbs the emitted infrared from the target. Afterwards, a device called thermopile converts the thermal effect of the infrared waves into an electrical signal that indicates the temperature measurement. To increase efficiency, infrared sensor uses thermopile to determine the intensity of infrared waves emitted from the object "water in that case". Consequently, the temperature will be measured using The Stefan-Boltzmann Equation:

$$I = \varepsilon \sigma T^4$$

I: Intensity of infrared emission.

ε: Emissivity constant "between 0.95-1.00 for water depending on its purity".

σ: Stefan Boltzmann's constant = 5.67 × 10−8 watt. meter^{-2}.kelvin^{-4}.

T: Temperature in kelvin.

In order to measure the water temperature accurately, the water emissivity must be determined first by taking a sample and heat it until it reaches a known temperature using infrared source of a known intensity. Eventually, the emissivity could be determined from the Stefan Boltzmann equation. Infrared sensors have many advantages like being efficient and fast in measuring the "surface temperature" of any object. Not to mention, it does not react with the water causing any potential pollution due to unintended chemical reactions. On the other hand, infrared sensors can cause the greenhouse effect, so consistent use can be detrimental to the environment. [3]

IV-Thermistors

The thermistors sensor relies on resistance to measure the temperature. The main difference between thermistors sensors and RTD sensors is the response time and temperature measurement range. Thermistors sensor has short response and narrow temperature measurement range of thermistors. On the other side, the RTD takes longer time, but guarantees higher measurement accuracy and wider temperature measurement range. Moreover, the relationship between temperature and resistance is exponential in thermistors sensors which reduces the accuracy. There are two types of thermistors sensors: NTC (Negative Temperature Coefficient) and PTC (Positive Temperature Coefficient). NTC thermistors have an inverse relationship between resistance and temperature, while PTC has a direct relationship. [4]

2. pH Sensors

The pH sensors are divided into two main types: one consists of glass electrode and the other consists of non-glass electrode. Both types depend on the Nernst equation in determining the value of pH.

Nernst Equation

Nernst Equation:

$$v = \frac{RT}{zF} \ln(Q)$$

V: Voltage

Q: Quotient = $\frac{\text{concentration of products}}{\text{concentration of reactants}}$

R: The gas constant ($8.314\ J\ mol^{-1}\ k^{-1}$)

F: Faraday's constant ($9.649 \times 10^4\ C\ .mol^{-1}$)

Z: Number of electrons.

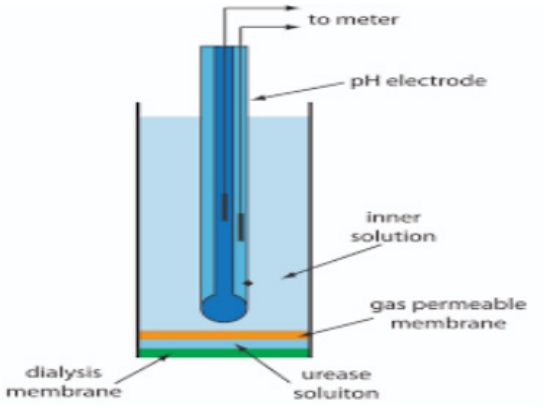
T: Temperature in kelvin.

I-The Glass Electrode pH Potentiometer

The glass electrode pH potentiometer consists of two electrodes: a reference electrode of known potential, usually silver, and a glass electrode (working electrode) of unknown potential, which is used to measure the potential of the measured solution. The glass electrode

has a buffered neutral KCL solution inside which works as an electrolyte to transmit the current. Also, there is a junction with known flow rate which leaks out some of the electrolyte into the measured solution to transmit electricity between the working electrode and the outer solution due to the difference in H+ ions' amount between the interior buffered KCL and the outer solution which generates voltage. If the amount of H+ ions outside the glass electrode is greater than the amount of H+ ions inside the glass electrode, a positive voltage is generated indicating pH < 7 (acid solution). If the amount of H+ ions outside the glass electrode is less than amount of H+ ions inside the glass electrode, negative voltage is generated indicating pH > 7 (basic solution). If the amount of H+ ions inside the glass electrode is equal to the amount of H+ ions outside the electrode, no voltage is generated indicating pH = 7 (neutral solution). The leaked KCl outside the sensor delivers the current generated, due to the voltage difference between the inner and the outer solution, to the wire. Then, the sensor measures the generated voltage as the sensor is connected to a voltameter. After the voltage is determined, the pH could be measured using the Nernst equation. [5]

Figure.1



The glass electrode pH potentiometer

III- Non glass electrode pH Potentiometer or Ion Sensitive Field Effect Transistors (ISFET)

The non-glass electrode potentiometer's principle also relies on the Nernst equation except that the reason of voltage generation is not the concentration difference between H+ ions. Rather, the non-glass electrode pH potentiometer consists of a permeable layer that allows only H+ ions to pass through. Then, the H+ ions are attracted to a metallic conducting surface "working electrode". Inside the working electrode, negatively charged electrons are presented. The electrons are attracted to the H+ ions due to the attraction between the negative charge of the electrons and the positive charge of H+ ions. As quantity of the charge of the H+ ions and electrons are equal but with opposite charges, the amount of electrons attracted is equal to the amount of H+ ions. The attraction of electrons causes the current to run through the wire. Thereupon, the voltage of the current is measured and hence the pH is measured as well using the Nernst equation. [7]

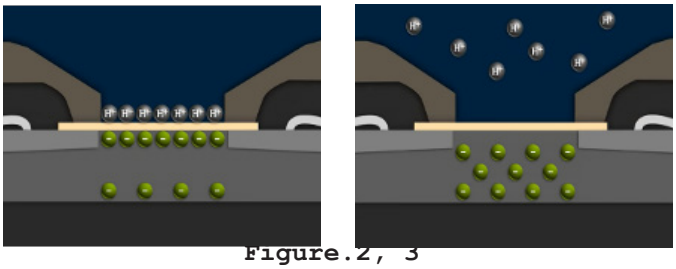


Figure.2, 3

The measurement of pH using non glass electrodes potentiometer

DO (Dissolved Oxygen) Measurements

The most common sensors used to measure dissolved oxygen are galvanic dissolved oxygen sensor (Clark-type sensor) and optical DO sensor.

I- Galvanic Dissolved Oxygen Sensor (Clark- Type Sensor)

The galvanic dissolved oxygen sensor consists of two electrodes: working electrode and counter electrode, usually gold and silver respectively. Also, a Teflon membrane is used to allow only O2 to reach the electrodes. Furthermore, an electrolyte, usually KOH, is used to connect electricity between the two electrodes. Eventually, a power source is used to

generate voltage between the two electrodes causing a current flow in order to complete the reduction reaction. When the dissolved oxygen goes through the Teflon membrane by diffusion due to the pressure difference, it is reduced using the cathode or the gold electrode into OH- ions. This reduction reaction generates a current flow as it requires electrons to occur. The current generated is proportional to the amount of dissolved oxygen presented in the solution. The chemical equation of this reduction reaction is as follows:

$$O_2 + 2H_2O + 4e^- \rightarrow 4OH^-$$

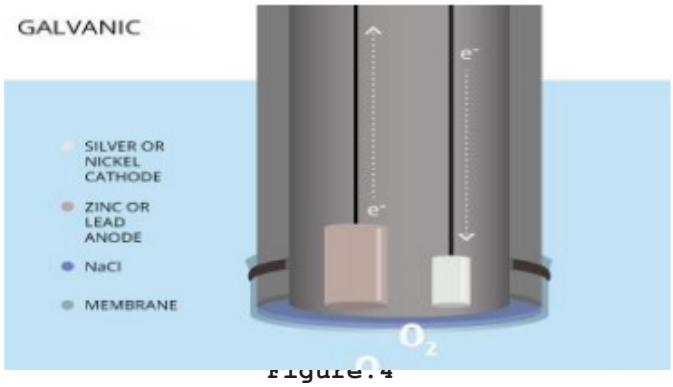


Figure.4

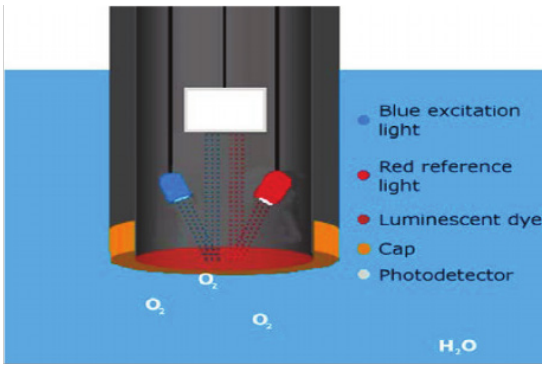
The galvanic DO meter [8]

II- Optical Dissolved Oxygen

Sensors

The optical DO oxygen sensor consists mainly of an oxygen sensitive marker, LED, a photodiode and an oxygen permeable membrane. The oxygen sensitive marker is a substance that binds to the oxygen atoms presented in the solution. the measurement of dissolved oxygen is done by emitting an orange light at specific wavelength using the LED. Then, the oxygen sensitive markers absorb this light and gets excited. Consequently, the markers try to restore their normal state through emitting this excessive energy in the form of NIR-emission (usually dark red light). When the oxygen sensitive marker is bonded to oxygen, the emitted wavelength is attenuated. As the number of markers inside the sensor is known, any reduction in the number of waves emitted by the markers, due to being bonded to oxygen, indicates the presence of oxygen. The light emitted from the marker is absorbed and

converted into electrical signal using the photodiode. The electrical signal is then analyzed to indicate the amount of dissolved oxygen in the solution. [9]



The optical DO meter structure [10]

4. TDS (Total Dissolved Solids) Measurement

The TDS is measured depending on the principle of electric conductivity. A current of certain voltage and intensity is applied across two electrodes in the solution. By measuring the voltage drop of the solution, the resistance of the solution could be determined and thereby the conductance, as it is the reciprocal of the resistance. Consequently, The TDS could be calculated using the following formula:

TDS equation:

$TDS = k_e EC$

EC: Conductance

K_e: Correlation factor (between 0.55 and 0.8)

TDS: Total dissolved solids

5- Heav

The most common sensors of measuring heavy metals in water are ICP or inductively coupled plasma and AAS or atomic absorption spectrometry.

I-ICP-OES (Inductively Coupled Plasma- Optical Emission Spectrometry)

ICP-OES depends on the principle of wavelengths emission of excited particles. After the ions of different metals are excited using the high energy of plasma, each metal uniquely emits specific wave lengths. These waves are then converted into electric signals using photodiodes to be analyzed in order to determine the type and the amount of the metals in the water. [11]

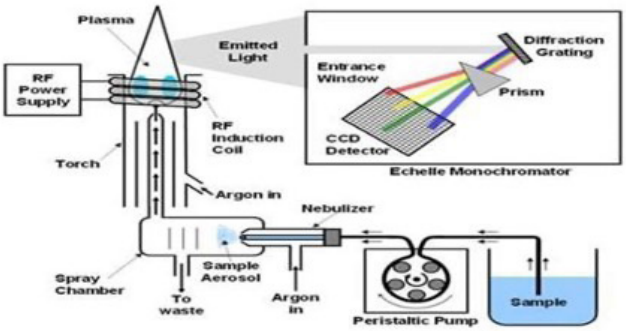


Figure.6
The ICP-OES structure [12]

II-AAS (Atomic Absorption Spectrometry)

In AAS, The Flame is used to dissociate the sample into pure atoms, evaporate the solute, as well as atomizing the atoms, and turning them into aerosol. Then, a beam of light with a certain wavelength is directed into the aerosol produced. The wavelength of the light beam is defined based on the wavelength that the element of interest absorbs. Depending on the intensity loss of the beam after passing through the aerosol, the amount of the element of interest is defined.[13]

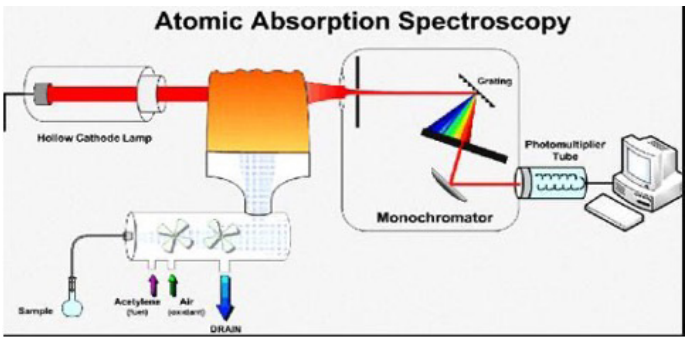


Figure.7
The AAS structure [14]

6-Turbidity Measurement

Turbidity is the measure of water's cloudiness and

transparency. Turbidity is measured using two methods: scattered light measurement (nephelometric measurement) and attenuation measurement.

I-Scattered Light Measurement (Nephelometric Measurement)

When a beam of light goes through a solution, the suspended particles in the solution cause deflection in the light. The deflection angle differs based on the particles size, shape and quantity. To measure the turbidity, usually infrared light of 860 nm wavelength or white light is directed into the solution. Based on the diffraction angle of light, the turbidity is determined. NTU is the unit of turbidity measured using white light, while FTU is the unit of turbidity measured using infrared light. They are approximately equal under ideal conditions. [15] The most accurate scattered turbidity measurement system consists of four sensors:

1- Transmitted light detectors. (Very low turbidity near zero, angle ≈ 0°)
2- Forward light detectors. (Intermediate turbidity, angle < 90°)
3- 90° detector. (Relatively high turbidity, angle = 90°)
4- Back scattered light detector. (High turbidity, 135° > angle > 90°)

Scattered light turbidity equation:

$$T = \frac{I_{90}}{(d_0 \times I_t + d_1 \times I_{fs} + d_2 \times I_{bs} + d_3 \times I_{90})}$$

T: Turbidity in NTU.
d_0, d_1, d_2, d_3: Calibration Coefficients.
I_{90}: 90 Degree Detector Current.
I_t: Transmitted Detector Current.
I_{fs}: Forward Scatter Detector Current.
I_{bs}: Back Scatter Detector Current.

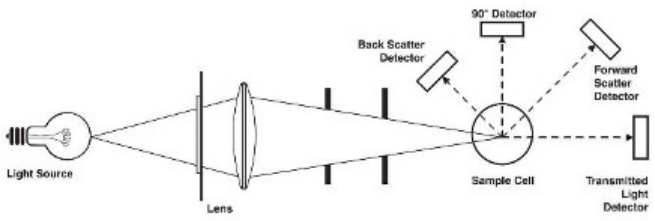


Figure.8
The nephelometer structure [16]

Attenuation Method

Attenuation method measures the loss of light intensity at a wide angle of 180° after the light passes through the solution. Due to the diffraction of suspended particles in the solution, the beam of the light loses some of its intensity. Instead of measuring the light intensity at different angles as in the nephelometric method, the attenuation method measures the light intensity lose at 180°. [17]

B. The Final Used Sensors:

1-Temperature Sensor

Infrared sensor was found to be the best choice due to its high accuracy, ease of use, short response time and non-direct reaction with the water. in addition, its downsides of measuring the surface heat rather than the internal heat will not be impactful on the results. The difference between water's surface heat and internal heat appears in large water depths which is not presented in agricultural circumstances.

2-pH Sensor

ISFET potentiometer sensor was found to be better than glass electrode sensor due to many factors:

1- The need to use a special type of glass in very acidic or basic mediums due to the fact that glass can make chemical reactions in such mediums.
2- The high accuracy and fast response of ISFET.
3- The low zero error percentage of ISEFT due to the ease of cleaning unlike glass electrodes sensor which requires the usage of special chemicals in cleaning the sensor after each use to guarantee accurate measurements.

3-DO Sensor

The optical sensor was found to be more accurate and durable than the galvanic sensor as the optical sensor lasts for about a year which is two times the durability duration of the galvanic sensor.

Furthermore, the optical sensor can measure the DO in different mediums and circumstances unlike the galvanic sensor which is inefficient in high alkaline or acidic mediums. However, the galvanic sensor is faster, but its vulnerability in high and low pH makes the optical sensor better for agricultural circumstances.

4-TDS Sensor

TDS will be measured using electrical conductivity sensor. In fact, it is cheap, reliable and accurate in determining the amount of total dissolved solids in water.

5- Heavy Metals Sensor:

ICP is a better choice due to its fast response and high accuracy in addition to its ability to determine as much as 250 elements during the same measurement trial unlike AAS which measures the amount of one element during the measurement trial.

6- Turbidity sensors:

Attenuation method was found to be a better choice in the determination of the turbidity. Although it is less accurate than the nephelometric method, its measurement range is acceptable for agricultural circumstances as the high accuracy of the nephelometric method is not required. Furthermore, the nephelometric method is more sophisticated and costly than the attenuation method.

C. The Used modifiers:

In case any change occurs in the following safe ranges of water parameters, a modifier should be used whether to increase or decrease the unstable water parameter.[18] [19] [20] [21]

The used parameter	The safe range
TDS	525-1400 PPM
Temperature	17.4-23.7 C°
DO	5-30 mg/L
pH	5.8-8.5
Turbidity	Food crops ≤ 2 Processed food crops ≤ 5

Table.1
The safe ranges of irrigation water's parameters

The metal	As	Cd	Cu	Cr	Mn	Ni	Pb	Zn	Fe
Safe value in ppm	0.01	0.01	0.2	0.02	5	0.2	5	2	5

The safe ranges of metals in irrigation water

1. Orange peel:

Orange peel is a novel solution for flaws in more than one water parameter. Orange peel has high efficiency in reducing the TDS and the heavy metals percentages by about 36% in relatively short time (1-2 weeks). Also, it has a slight effect in stabilizing the pH, but is not good in very high or low pH levels. Eventually, it increases soil fertility as well.

2- Aluminum sulfate or alum ($Al_2(SO_4)_3$):

Aluminum sulfate will be used as a coagulator to neutralize the charge of the suspended particles accumulate them as a result, so they are easily removed. Consequently, turbidity is decreased. Also, the TDS is slightly decreased but by a small percentage.

3- Sulfuric Acid or Phosphorous Acid:

In order to stabilize any increase in the pH, strong acid was used to neutralize the water. However, the used acids anions should be suitable for agricultural uses so as not to cause toxicity. As the soil needs sulfur and phosphorus to increase the nutrition source for the plants, sulfuric acid and phosphorus acids are suitable for agricultural circumstances.[4]

4- Calcium Hydroxide ($Ca(OH)_2$):

Like sulfuric acid, Calcium Hydroxide is a strong base that will stabilize high acidity. Moreover, it will increase soil fertility.[22]

5- Thermostat:

Thermostat will control the temperature of the water. Consequently, the DO levels in the water will be maintained as cold water holds more dissolved oxygen unlike hot water which releases oxygen.

6- Purified O_2:

If there is a drastic decrease in DO levels, purified O_2 will be a quick solution to increase DO levels.

7- N_2 Gas:

Purging N_2 in the water reduces the amount of DO.

4. Conclusion:

The study could successfully achieve the purpose of finding the best combination of sensors and modifiers to create an integrated, comprehensive, and accurate system that could fix any flaw in water parameters to prevent any potential corruption in the crops. The designed system will be able to solve the issues of main water parameters resembled in TDS, pH, heavy metals, temperature, turbidity and DO. As a future recommendation, working on measuring some water parameters like water chlorination and the amount of bacteria like E-coli and coliform in water will be beneficial as it will provide more data about the crops and soil status. Furthermore, making a timetable to measure water parameters when a certain bacteria or insects are active, or a specific chemical reaction occurs due to plant growth will increase the system's efficiency.

5. References:

1. Pollock, Daniel D. "Thermocouples: Taylor & Francis Group." Taylor & Francis. Taylor & Francis, Access August 1, 2021. https://doi.org/10.1201/9780203735824.
2. Bonnie C. Baker. "Precision Temperature-Sensing with RTD Circuits." Microchip. Access date August 1,2021. https://ww1.microchip.com/downloads/en/appnotes/00687c.pdf
3. Jing Zhang. "Development of a Non-contact Infrared Thermometer." Atlantis press. Access date 25 July 2021. https://www.atlantis-press.com/article/25892853.pdf
4. A. Becker, G. L. Pearson, and C. B. Green. "Properties and Uses of Thermistors - Thermally Sensitive Resistors." IEEE Xplore. Access July 30, 2021. https://doi.org/10.1109/EE.1946.6439939
5. G. Haugaard. "The Mechanism of the Glass Electrode." ACS publications. Access date July 21 ,2021 https://doi.org/10.1021/j150406a013
6. Unknown artist. Schematic diagram of a gas-sensing membrane electrode. Photograph. chem.libretexts June 5, 2019. https://chem.libretexts.org/Courses/Northeastern_University/11% 3A_Electrochemical_Methods/11.2%3A_Potentiometric_Methods
7. Duroux, P, C Emde, P Bauerfeind, C Francis, A Grisel, L Thybaud, D Arstrong, C Depeursinge, and A L Blum. "The Ion Sensitive Field Effect Transistor (ISFET) PH ELECTRODE: A New Sensor for Long TERM Ambulatory PH Monitoring." Gut. BMJ Publishing Group, Access August 1, 2021. http://dx.doi.org/10.1136/gut.32.3.240
8. Unknown artist. Cross-section of a polarographic dissolved oxygen sensor. Fondriest. Unknown publication date. https://www.fondriest.com/environmental- measurements/measurements/measuring-

water-quality/dissolved- oxygen-sensors-and-methods/
9. Glazer, Brian T., Adam G. Marsh, Kevin Stierhoff, and George
10. W. Luther. "The Dynamic Response of Optical Oxygen Sensors and Voltammetric Electrodes to Temporal Changes in Dissolved Oxygen Concentrations." Analytica Chimica Acta. Elsevier. Access July 30, 2021. https://doi.org/10.1016/j.aca.2004.05.040
11. unknown artist. Cross-section of an optical dissolved oxygen sensor.. Fondriest. Unknown publication date. https://www.fondriest.com/environmental- measurements/measurements/measuring-water-quality/dissolved- oxygen-sensors-and-methods/
12. Fathabad, Ayub Ebadi, Nabi Shariatifar, Mojtaba Moazzen, Shahrokh Nazmara, Yadolah Fakhri, Mahmood Alimohammadi, Ali Azari, and Amin Mousavi Khaneghah. "Determination of Heavy Metal Content of Processed Fruit Products from Tehran's Market Using ICP- Oes: A Risk ASSESSMENT STUDY." Food and Chemical Toxicology. Pergamon. Accessed July 28, 2021. https://doi.org/10.1016/j.fct.2018.03.044
13. unknown artist. ICP-OES. Photograph. slidetodoc. Unknown publication date. https://slidetodoc.com/einfluss-des-bodens-auf-den-mineralstoffgehalt-in-authentischen/
14. Kenawy, I. M. M., M. A. H. Hafez, M. A. Akl, and R. R. Lashein. "Determination by AAS of Some Trace Heavy Metal Ions in Some Natural and Biological Samples after Their Preconcentration Using Newly Chemically MODIFIED Chloromethylated POLYSTYRENE-PAN Ion-Exchanger." Analytical Sciences. The Japan Society for Analytical Chemistry. Accessed July 25, 2021.
15. https://doi.org/10.2116/analsci.16.493
16. unknown artist. Atomic Absorption Spectroscopy (AAS). Photograph. assignment point. Unknown publication date. https://www.assignmentpoint.com/science/chemistry/atomic-absorption-spectroscopy-aas.html
17. unknown artist. A Ratio Turbidimeter Design Measuring Transmitted Light at 180° and Reflected Light at 90°, at 70° (Forward Scatter), and at 135° (Backward Scatter) Compares the Various Readings to Account for Various Types of Particle Interference in Turbid and Colored Samples. n.d. Photograph.
18. Hach. Unknown publication date. https://www.hach.com/turbidity-article-turbidity101
19. Trevathan, Jarrod, Wayne Read, and Simon Schmidtke. "Towards the Development of an Affordable and Practical Light Attenuation Turbidity Sensor for Remote near Real-Time Aquatic Monitoring." MDPI. Multidisciplinary Digital Publishing Institute. Access July 31, 2021.
20. https://doi.org/10.3390/s20071993
21. Tessier A, Campbell PGC, Bisson M. sequential extraction procedure for the speciation of particulate trace metals. Anal Chem. 1979.
22. Ayers RS, Westcot DW. Water quality for agriculture. Italy: FAO, irrigation and drainage. Paper 29, Rome, 1985.
23. 20- Alloway BJ. Heavy metals in soils. 2nd ed. USA : John Wiley & Sons press, Inc. New York; 1995.
24. Jeong, Hanseok, Hakkwan Kim, and Taeil Jang. "Irrigation Water Quality Standards for Indirect Wastewater Reuse in AGRICULTURE: A Contribution toward Sustainable Wastewater Reuse in South Korea." MDPI. Multidisciplinary Digital Publishing Institute, Access July 22, 2021. https://doi.org/10.3390/w8040169
25. 22- Soros, Ampai, James E. Amburgey, Christine E. Stauber, Mark D. Sobsey, and Lisa M. Casanova. "Turbidity Reduction in Drinking Water by Coagulation-Flocculation With Chitosan Polymers." Journal of Water and Health. IWA Publishing, Access July 20, 2021.
26. https://iwaponline.com/jwh/article/17/2/204/65912/Turbidity-reduction-in-drinking-water-by

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