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EPIGENETIC APPROACHES EXPRESS POTENTIAL IN GENERATING EMBRYONIC STEM CELL REPLACEMENTS: A LITERATURE REVIEW

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ABSTRACT

Embryonic stem cells (ESCs) have significant potential for curing serious diseases such as Parkinson's and Alzheimer's due to their ability to differentiate into most cell types in the body. However, the extraction of ESCs involves the destruction of embryos, raising moral and ethical concerns. Consequently, many studies have explored the possibility of creating ESC replacements. This literature review examines multiple publications to investigate the potential of epigenetic approaches in inducing stem cells that could replace human embryonic stem cells (hESCs) in biomedical research. Findings indicate that a combination of the demethylation enzyme TET dioxygenase, the CRISPR-dCas9 binding system, and directional guide single-guide RNA (sgRNA) can theoretically revert somatic stem cells to an hESC-like state through DNA demethylation, provided that all key regulators of genetic expression in hESCs are identified.

KEYWORDS: ESC, Epigenetics, TET Dioxygenase, CRISPR-dCas9, Gene Expression, Pluripotent Stem Cells

INTRODUCTION

Stem cells are unspecialized cells in the body that can transform into specific cells when needed (Brazier, 2018). Due to their ability to differentiate into specialized cells, they are used to treat many chronic conditions caused by tissue or cell damage, such as Parkinson's, Alzheimer's, heart diseases, type 1 diabetes, leukemia, sickle cell anemia, and other immunodeficiency problems (Brazier, 2018). While stem cells can be found in various locations in the adult human body, only pluripotent stem cells in the human embryo (hESCs) have the potential to differentiate into all cell types in the body, except those used for embryo support. In contrast, non-embryonic stem cells (somatic stem cells, SSCs) have limited differentiation capabilities (Zakrzewski et al., 2019). For this reason, hESCs are widely used in biomedical research and therapy. However, the use of hESCs raises significant ethical concerns since they are obtained from human blastocysts, which are embryos (Zakrzewski et al., 2019). This controversy centers around the morality of destroying embryos, with some advocating for the cessation of hESC use. In contrast, others argue that embryos should not be moralized and should continue to be used in research.

To address these ethical concerns, induced pluripotent stem cells (iPSCs) were developed as potential replacements for hESCs. iPSCs behave similarly to hESCs and can be generated in laboratories using extracts from skin or blood cells (Brazier, 2018). However, iPSCs are produced through retroviral vector-mediated gene insertion into somatic stem cells' genome, making them susceptible to oncogenic issues, as the altered genes are associated with tumor development (Medvedev, Shevchenko, & Zakian, 2010). Additionally, the efficiency of iPSC production decreases over time (Medvedev et al., 2010). These health and efficiency concerns drive researchers to seek alternative hESC replacements.

Methods other than gene insertion should be considered for inducing hESC replacements. Since all cell types within a multicellular organism contain the same DNA, SSCs possess the same genetic information as hESCs, differing only in their gene expression levels (Ralston & Shaw, 2008). TET dioxygenases, an enzyme family capable of altering gene expression, may potentially adjust SSC gene expression to match that of hESCs (Wu & Zhang, 2014). Moreover, CRISPR-dCas9, a modified CRISPR system with directional guide single-guide RNA (sgRNA), allows for precise targeting of DNA sites where gene expression needs to be altered (Yang et al., 2018). The potential integration of TET dioxygenases with CRISPR-dCas9 and sgRNA for inducing pluripotent stem cells offers a significant reduction in ethical concerns, highlighting a novel approach in stem cell research.

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Background

Epigenetics: Different cell types arise through gene expression, where genes irrelevant to the cell type are unexpressed to prevent accidental DNA transcription and unnecessary protein formation. Gene expression is regulated by DNA methylation, a process in which methyl groups are added to genes, inhibiting transcription factors from producing mRNAs (Moore, Le, & Fan, 2012). Since DNA sequences are not altered during methylation, traits formed from this process are considered epigenetic changes (Hamilton, 2011). Using epigenetically modified SSCs as hESC replacements mitigates concerns like tumor formation associated with gene insertion used to create iPSCs.

DNA demethylation: Methyl groups in DNA can be removed to express inhibited traits. This is done through oxidative demethylation, which removes methyl groups from molecules through oxidizing atoms in the substrate ("Oxidative Demethylation"). Though DNA demethylation can occur on other nucleotides, it generally refers to the demethylation in the fifth carbon on cytosine (5mC); this is because Cytosine epigenetics have a significant impact on gene expression and development (Neidhart 2016, Wu and Zhang 2014). The genome of pre-implantation embryos is demethylated (specifically 5mC) in preparation for hESC pluripotency. Thus, demethylation is essential to induce hESC replacements (Wu and Zhang 2014). Specifically, DNA demethylation is done through oxidation catalyzed by the enzyme family TET dioxygenases (Wu and Zhang 2014).

CRISPR-Cas9, found in bacteria and archaea, serves as an immune defense against pathogens (Xu & Li, 2020). Scientists leverage CRISPR's ability to recognize and cut target DNA sequences, enabling manipulation by removing or replacing the sequence with a new one (Xu & Li, 2020). The modified variant, CRISPR-dCas9, does not cut DNA after binding to its target site, making it ideal for tagging target DNA sequences (Yang et al., 2018).

METHODOLOGY

The literature review was conducted by researching articles in credible and accessible academic journals online. Sources in this review were found using a combination of the following keywords: epigenetic modifications, hESC, cell differentiation, pluripotency maintenance, CRISPR-dCas9, and demethylation. The research process began with discovering sources through keywords and then the qualitative content analysis of the sources. All content analysis was conducted through website annotations with a Chrome extension, and similar highlighted concepts were later compared and contrasted against each other to ensure validity. Moreover, quantitative approaches could not be achieved because no research directly related to the theory proposed in this literature review was found.

Sources used in the literature review section included primary (Xu et al., 2016; Choudhury et al., 2016) and secondary (Lunyak & Rosenfeld, 2008). While primary sources provided detailed information regarding experiment methodology and findings, the secondary sources highlighted and condensed a much wider range of primary sources than the ones discussed in this literature review, which builds validity to the topic. In this instance, the secondary source (Lunyak & Rosenfeld, 2008) was used for analyzing the broader topic of epigenetic regulation of cell fate. It summarized primary sources of various related subtopics, such as the role of epigenetics in hESC pluripotency maintenance, and conversion between different specialized stem cells through epigenetic means. Contrarily, primary sources (Xu et al., 2016; Choudhury et al., 2016) were used to discuss the niche topic of the CRISPRdCas9-TET system since it requires a detailed explanation rather than a summary.

In the process of evaluating the methodology, it can be concluded that the sources are valid, as content analysis suggests that the highlighted findings in the secondary and primary sources are generalizable; see Lunyak & Rosenfeld (2008) below, where various epigenetic modifications were discussed and worked on the differentiation change on various stem cell types; additionally, see Xu et al. (2016) and Choudhury et al. (2016), as both groups of researchers concluded similar results through the same mechanism. This further justifies the keywords used to discover the sources. Additionally, the journals from which the sources are retrieved hold high academic integrity, which strengthens the results and discussions stemming from the topic. However, the lack of quantitative data in this methodology is a key limitation since it failed to justify the topic of this review.

LITERATURE REVIEW

Epigenetic regulation of stem cell fate

Lunyak & Rosenfeld (2008) compiled insightful primary resources regarding epigenetics in stem cell potential and the reversibility of its differentiation process. Their review highlighted that hESCs utilize epigenetic mechanisms to maintain pluripotency after cell division and that epigenetics plays a significant role in pluripotent stem cells' differentiation (Lunyak & Rosenfeld, 2008). Additionally, they discussed evidence showing the use of DNA methylation inhibitors to alter stem cell gene expression; for instance, bone marrow stromal cells were converted into neuronal stem cells through epigenetic changes. Similarly, they found that DNA demethylation combined with histone acetylation could convert neuronal stem cells into hematopoietic cells, a type of stem cell that differentiates into all blood cell types. Altogether, their review suggested that epigenetic alterations can reset genetic marks made during cell development, indicating that non-hESCs could be epigenetically reverted to resemble hESCs if key regulators are identified (Lunyak & Rosenfeld, 2008). Moreover, the researchers concluded that there are numerous epigenetic modifications, such as histone modification and chromatin remodeling, that can alter gene expression (Lunyak & Rosenfeld, 2008).

The article reviewed a significant number of studies and presented substantial evidence. The results of the reviewed studies did not contradict each other but rather built a strong argument that epigenetics is crucial in maintaining and reversing stem cell gene expressions. The article, published in "Human Molecular Genetics" by Oxford University Press, a highly respected source, provides reliable information on

molecular genetic disease mechanisms (Lunyak & Rosenfeld, 2008). While the publication date of 2008 is relatively recent, current research reviews would more accurately represent the latest findings in the field.

Effectiveness of the CRISPR Cas9-TET system

A study by Xu et al. (2016) combined Tet1-CD, a catalytic domain of TET dioxygenase, with CRISPR-dCas9 to create a DNA-targeted demethylation system named dCas9-Tet1-CD. This system was developed by modifying CRISPR-Cas9 into dCas9 and then fusing Tet1-CD to CRISPR-dCas9 (Xu et al., 2016). The complex was further integrated with a guiding system using a modified single-guide RNA (sgRNA) that directs it to the target DNA sequence, along with an MS2 coat protein complex that enhances demethylation efficiency (Xu et al., 2016).

The results exhibited the dCas9-Tet1-CD system as an efficient method for targeted DNA demethylation, demonstrating activation of gene expression independent of gene context (Xu et al., 2016). Moreover, the study assessed the system's accuracy, concluding that it had low off-target effects. The researchers suggested applying the system to potentially cure diseases caused by gene hypermethylation (Xu et al., 2016).

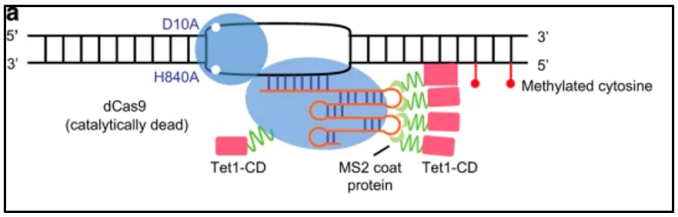

Figure 1: Visual representation of dCas9-Tet1-CD system by Xu et al. (Xu et al. 2016)

The study by Xu et al. was well-designed, successfully applying the system to three hypermethylated genes, all of which demonstrated upregulation and demethylation (Xu et al., 2016). This increases the system's validity. Moreover, the study was published in 2016 in "Cell Discovery," a credible international journal that publishes significant papers on molecular and cell biology (Nature.com, 2024).

A study by Choudhury et al. (2016) created a system similar to that of Xu et al., combining CRISPR-dCas9 with Tet1-CD and using a regular sgRNA as a directional guide. The researchers utilized the system to demethylate 38 sites at target hypermethylated regions in the tumor suppressor gene BRCA1 (Choudhury et al., 2016). The results showed efficient demethylation at target sequences and insignificant off-target effects, similar to the findings of Xu et al., supporting the viability of combining CRISPR-dCas9 with TET dioxygenases.

The study produced substantial evidence as it was tested at a large number of methylated sites. It remains relevant today, having been published in 2016 in "Oncotarget," a peer-reviewed, reliable source focusing on oncology and cancer research (Choudhury et al., 2016).

DISCUSSIONS

The studies reviewed indicate that a DNA demethylation system composed of Tet1-CD, CRISPR-dCas9, and single-guide RNAs can efficiently activate gene expression at target regions with minimal errors. Moreover, the application of epigenetic mechanisms in stem cells can potentially revert them to a state similar to hESCs (Choudhury et al., 2016; Lunyak & Rosenfeld, 2008; Xu et al., 2016). Consequently, it is theoretically feasible to target and demethylate ESC-related gene sequences in SSCs using a system similar to dCas9-Tet1-CD, thereby altering gene expressions to produce hESC-like cells. This approach could address ethical concerns in pluripotent stem cell research and therapy by providing an alternative to hESCs. However, it is important to note that this remains a theoretical proposal due to the current lack of empirical evidence supporting this claim. To date, studies have employed various epigenetic mechanisms, but the resultant stem cells do not exhibit the same behavior as hESCs (Lunyak & Rosenfeld, 2008).

Assuming the proposed method is experimentally viable, several limitations must be considered. One significant limitation is the need for additional epigenetic measures to maintain the pluripotency of hESC replacements. hESCs have intrinsic epigenetic mechanisms that sustain their pluripotency after cell division (Lunyak & Rosenfeld, 2008). Therefore, additional methylation inhibitors may be necessary to ensure the stability and functionality of the hESC replacements upon conversion. Moreover, while some regulatory genes of hESCs have been identified, the complete collection remains incomplete, which poses a challenge for accurate and comprehensive gene targeting (Zhong et al., 2007). Additionally, methylation is not the only factor that affects gene expression. Other mechanisms, such as transcription factors, should also be considered.

Furthermore, the validity of this proposal is constrained by the fact that it is a theoretical hypothesis, and the author does not possess advanced expertise in this specific field of science. Future research should aim to empirically test the feasibility of this approach and address the identified limitations to establish a more solid foundation for the potential application of epigenetic mechanisms in generating hESC replacements.

In conclusion, while the integration of Tet1-CD, CRISPR-dCas9, and single-guide RNAs presents a promising avenue for creating hESC-like cells through DNA demethylation, further research and evidence are required to substantiate this theoretical framework. This approach holds potential for advancing pluripotent stem cell research and addressing ethical concerns, but practical application necessitates overcoming significant scientific and technical challenges.

CONCLUSION

In conclusion, the integration of TET dioxygenases with CRISPR-dCas9 and sgRNA to induce pluripotent stem cells presents a theoretically promising approach in stem cell research. The literature review supports the potential validity of this system, as demonstrated by multiple studies. Specifically, research by Lunyak et al. highlights the feasibility of reverting stem cells through epigenetic mechanisms (Choudhury et al.,

2016; Lunyak & Rosenfeld, 2008; Xu et al., 2016). Producing hESC replacements could significantly reduce ethical concerns and increase the availability of samples, addressing the limited number of embryos donated for hESC research (Salari et al., 2023).

To address the limitations discussed, it is essential to map the hESC genome and identify all key regulators involved in pluripotency expression and maintenance. Investigating the effects of dCas9-Tet1-CD-mediated demethylation on currently identified key regulators will provide crucial evidence for the proposed method's validity. Additionally, exploring other epigenetic methods beyond demethylation is vital, as modifying gene expression in stem cells is not limited to this approach (Xu et al., 2016). For example, a study by Liang and Zhang found that enriched histone acetylation is significant in recreating an ESC-like state in chromatin (Liang & Zhang, 2012). Considering the integration of alternative epigenetic modifiers with CRISPR-dCas9 could further enhance the proposed approach.

Future research should focus on experimentally validating the theoretical framework presented in this review. By addressing the identified challenges and exploring complementary epigenetic modifications, the potential for generating ethical and effective hESC replacements could be realized, advancing both stem cell research and its applications in medicine.

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