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How has societal demand influenced the application of induced pluripotent stem cell and CRISPR-Cas9 technology as an autologous, combined cell and gene therapeutic treatment method for sickle cell disease?

SHE focuses: Influence & Application and Limitation

Influence:

- *The acceptance and use of scientific knowledge can be influenced by social and ethical considerations.*

Application and Limitation:

- *Scientific knowledge and understanding can enable scientists to develop solutions.*
- *The use of scientific knowledge may unexpected consequences; this requires monitoring, assessment, and evaluation of risk, and provides opportunities for innovation.*

Word count: 1498

INTRODUCTION

Sickle cell disease (SCD) is an inherited blood disorder that results from a substitution of glutamic acid for valine at the 6th codon on the β-globin gene and causes the production of abnormal hemoglobin protein (refer to Figure 1; Huang et al., 2015). Haemoglobin polymerisation, leading to erythrocyte rigidity and vaso-occlusion, results in the manifestation of multiple complications such as anemia, progressive organ damage, pain crisis and early mortality (Sargent et al., 2016). Since the only clinical curative treatment of an allogenic donor bone marrow transplant is highly limited by accessibility for the majority of patients, a great societal demand exists for developing new genetic engineering technologies and procedures, to improve treatment methodologies using autologous cell and gene therapy. This treatment uses patient-derived induced pluripotent stem cells (iPSCs: adult reprogrammed cells that have the potential to differentiate into any cell type) and CRISPR-Cas9 designer nuclease system to correct the Glu6Val mutation in order to use their differentiated products for therapeutic transplantation (Papapetrou, 2017). The **Influence** aspect of Science as a Human Endeavour will be explored on the use and acceptance of the autologous cell and gene replacement therapy since it is directly influenced by social and ethical considerations. The **Application and Limitation** aspect will also be examined regarding the application of scientific knowledge into the development and utilisation of the therapy, which requires considerable preclinical monitoring due to limitations in this treatment method.

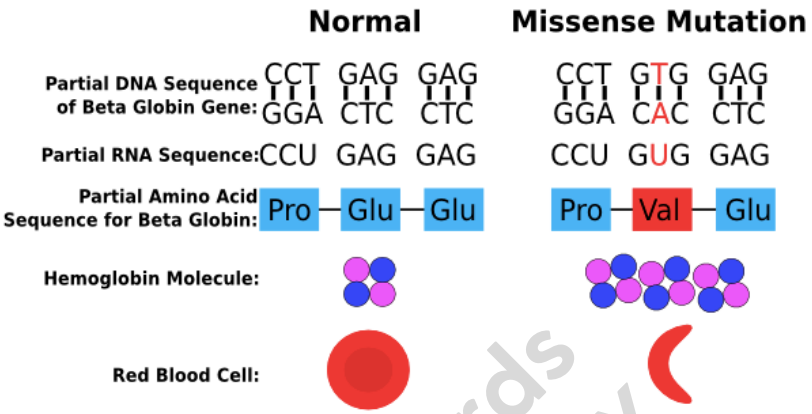

Figure 1: The inherited single base pair Glu6Val missense point mutation, in which adenine is replaced by thymine, leads to sickle cell disease. Source: Buratov, 2019

BIOLOGICAL BACKGROUND

Various preclinical studies provide proof-of-principle that the β-globin gene locus can be targeted in hiPSCs to perform genome editing of the sickle point mutation and differentiated to produce gene-corrected hematopoietic stem cells to infuse into the patient (Reis et al., 2017; Wang et al., 2015; Chou et al., 2016). The principal steps of this process are as follows:

Isolation of somatic cells and cell preparation

Once scientific protocol gains approval from the ethics committee and following acceptance and signature of the informed patient consent, a blood sample is collected to acquire DNA (Reis et al., 2017). The peripheral blood mononuclear cells (MNCs) are separated to culture *in vitro* and supplemented with cytokines for 12 days to allow selective culture expansion to occur (Wang et al., 2015).

Reprogramming of iPSCs using plasmid vectors

The expanded MNCs are transfected with plasmid vectors carrying the transcription factors OCT4, SOX2, KLF4, and c-MYC to begin the reprogramming process through forced gene expression (Chou et al., 2016). After 3-4 weeks, colonies of pluripotent cells with the properties of human embryonic stem cells (hESCs) emerge (Reis et al., 2017).

Glu6Val mutation correction using CRISPR-Cas9 and DNA donor template vectors

CRISPR-Cas9 nucleases, in combination with single-stranded oligodeoxynucleotides (ssODNs) containing the correct, wild-type hemoglobin subunit beta (HBB) sequence, are engineered to repair disease-associated point mutations in patient-specific iPSCs (Park et al., 2017). Accordingly, two β-globin-gene-specific guide RNAs (gRNAs) are manufactured to direct the Cas9 complex to locate the mutated target sequence; the Cas9 protein cuts at the highly specific recognition site and removes the point mutation to integrate the ssODNs, correcting the genotype (refer to Figure 2; Huang et al., 2015).

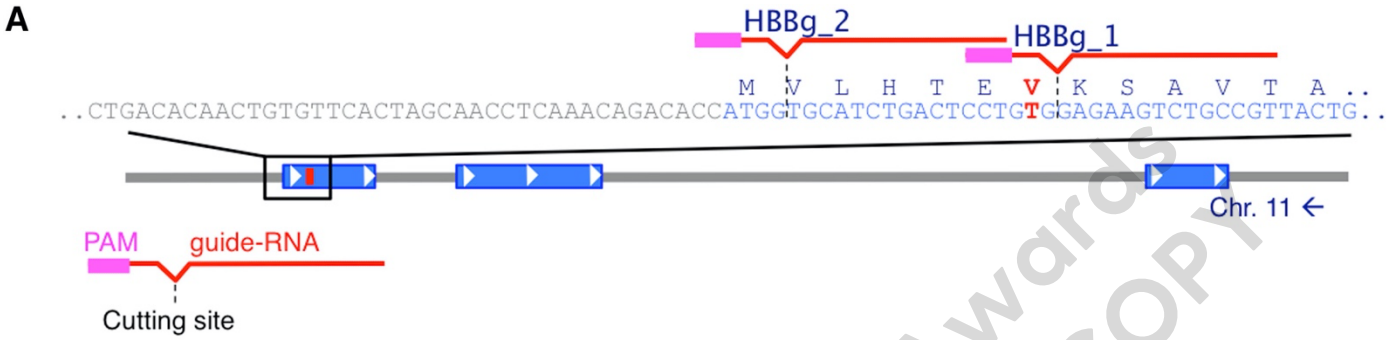

Figure 2: (A) The guide RNAs HBBg_1 and HBBg_2 are engineered to target the mutated sequence and direct the Cas 9 protein to remove the genomic Glu6Val mutated HBB gene
(B) Integration of donor ssODNs templates allow for the restoration of normal HBB sequence. Source: Park et al., 2017

Picking corrected clones

Using polymerase chain reaction (PCR), allele-specific primers anneal to the corrected gene sequences, distinguishing the mutated and altered DNA sequences to screen for the gene-corrected clones (Niu et al., 2016). Scientists must meticulously monitor through using PCR to quantify Cas9 off-target activity and ensure that these events do not have a carcinogenic effect.

Differentiation into hematopoietic stem cells and infusion into the patient

The corrected iPSC clones are differentiated into hematopoietic stem cells (which later develop into all types of blood cells) and get infused into the patient following chemotherapy. This treatment scheme is illustrated in Figure 3.

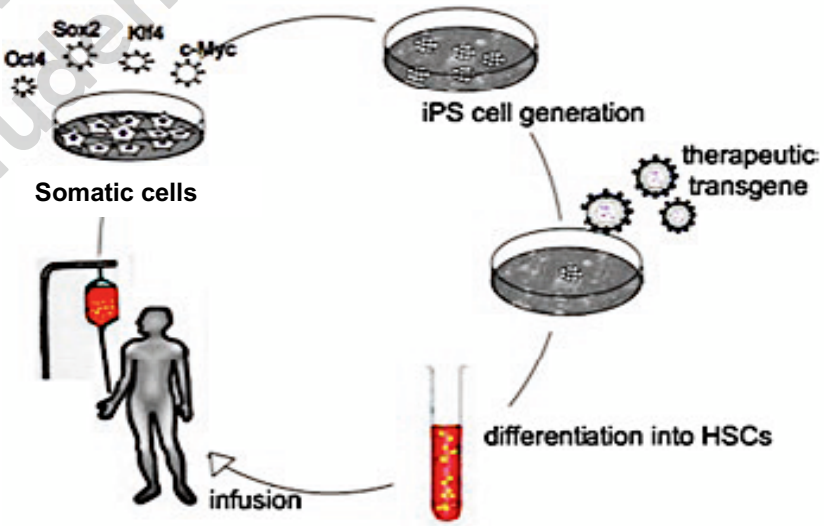

Figure 3: Treatment scheme of autologous cell and gene therapy for SCD utilising iPSCs and CRISPR technology. Source: Papapetrou, 2017

SCIENCE AS A HUMAN ENDEAVOUR

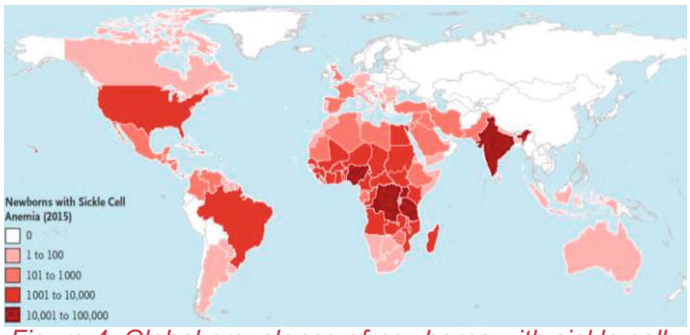

Figure 4: Global prevalence of newborns with sickle cell disease. Source: The New England Journal of Medicine, 2015

The use and acceptance of SCD cell and gene therapy has been significantly influenced by social and ethical considerations. A large societal demand exists for practical treatment methods as SCD is the most common hemoglobinopathy with a worldwide prevalence (refer to Figure 4); although the World Health Organisation estimates that approximately greater than 300,000 infants are born with severe haemoglobin disorders annually, mainly palliative SCD treatment options are available (Brandow et al., 2020; WHO, 2020). In response to this demand, scientists have investigated a range of technologies as procedures that potentially provide a cure, including iPSCs and CRISPR; subsequently, various proof-of-concept studies demonstrate that iPSCs and CRISPR-Cas9 can correct the SCD mutation in *ex vivo* cell culture conditions and mouse models (Reis et al., 2017; Wang et al., 2015; Chou et al., 2016). Moreover, the acceptance of this cell and gene therapy is influenced by ethical considerations because iPSCs circumvent ethical concerns of using and correcting human embryonic stem cells (hESCs). Prior to the discovery of iPSCs, the method of hESCs questioned the ethics of the destruction of human embryos; this raised the ongoing debate of whether the destruction of an embryo is justified for research to discover a possibly new SCD treatment (Kyba et al., 2018). However, the somatic cells that iPSCs are derived from do not face this ethical concern as the adult provides full consent and gene editing of somatic cells affects only the patient being treated and will not influence the gene pool of future generations. Hence, the level of acceptance in society for use of iPSC-corrected therapy is higher than alternative hESCs (Kyba et al., 2018). This increases the likelihood of widespread use of regenerative transfusion therapy for SCD, which improves its efficiency as a future treatment method.

The use of scientific knowledge regarding the application of iPSCs and CRISPR as a treatment method is allowing scientists to develop solutions for patients that do not qualify for the only available cure of bone marrow transplants involving donor hematopoietic stem cells (HSPCs). Currently, the curative therapy of an allogeneic bone marrow transplant is exclusively recommended for the most severe SCD cases, requiring a closely-matched related donor, typically a sibling, which a significant proportion of the candidates (>80%) do not suitably possess (Chou et al., 2016; Papapetrou, 2017). In addition, the transplantation replaces the patient's bone marrow with donor HSPCs without the mutation, posing significant risks such as immune rejection of the cell graft, graft-versus-host disease and transplant-related mortality (Papapetrou, 2017). In response to this issue, scientists are investigating the possibility of utilising various technologies for SCD treatment involving iPSCs, which are autologous and thus circumvent immunological problems. Furthermore, by discovering and understanding that donor HSPCs cannot remain at an undifferentiated state *ex vivo* for more than 72 hours (Papapetrou, 2017), scientists were able to determine that HSPCs impose severe time constraints to genetic modification strategies. Hence, this advance in understanding provided researchers with the opportunity to conduct experimental trials on iPSCs, leading to the discovery that iPSCs have a greater capacity for survival and growth *in vitro* and their pluripotency provides greater control over experiments to pass quality control testing (Huang et al., 2015), which is crucial to ensure patient safety in methods of genetic engineering involving CRISPR by increasing its reliability as a treatment method. Hence, the advances in scientific understanding of the properties of iPSCs and CRISPR technology and their potential uses, along with the application of this knowledge in a biomedical context, is enabling scientists to ongoingly develop a potentially improved, more efficient sickle cell disease treatment method.

Further application of scientific knowledge about iPSCs and CRISPR into the utilisation of regenerative transfusion therapy as a method of SCD treatment has resulted in the discovery of limitations of this strategy. The production of new iPSC lines is limited due to the time-consuming, laborious nature of cell reprogramming and CRISPR editing. Hence, by existing standards, the prospect of this treatment method is prohibitively costly. This, combined with the time period required to carry out safety screening of edited cells, is a disadvantage, especially in the case of patients with severe sickle cell disease. In addition, this method of genetic manipulation necessitates extended time in cell culture which increases acquisition of genetic abnormalities; likewise, existing literature, such as those by Young et al. (2012), Abyzov et al. (2018) and Taapken et al. (2011) demonstrate that iPSC reprogrammed cells harbour genetic aberrations, in the form of subtle single-nucleotide variants, that pre-exist in the starting cell and this may impact on reprogramming and editing, which drives or predisposes to cancer. Therefore, while this transfusion technology improves upon previous treatment methods in terms of using the patient's somatic cells to correct the mutations without ethical concerns, it is still limited by various aspects and further scientific inquiry and preclinical monitoring must be conducted for continuing improvements. Further comprehensive studies can be undertaken to test iPSCs cultured for cell therapies with high-resolution techniques, such as whole-genome sequencing to enhance patient safety. To keep editing efficiency sufficiently high for clinical application, acceptable off-target cleavage thresholds must be determined and revised as a greater amount of genomic data becomes available for assessment from future investigations. Through the discovery of solutions to these biotechnological limitations across preclinical monitoring, the reliability of iPSC reprogramming and the effectiveness of CRISPR as a method to perform SCD cell and gene replacement therapy would be dramatically enhanced.

POTENTIAL APPLICATION: iPSC-BASED DRUG SCREENING

A potential future application using tissue culture hiPSC technology in the pharmaceutical sector provides an opportunity to improve drug discovery methodologies. The utilisation of human, patient-specific somatic cells offers an alternative humanised platform that avoids interspecies differences, resulting in higher predictive value (Huang et al., 2015), and simultaneously enables scientists to carry out drug and toxicology testing which are unique to the patient's genetic and epigenetic makeup. Additionally, since the majority of individuals with SCD live in developing nations that face economic hardships, and take combinations of drugs in the absence of targeted therapies, scientists could potentially use iPSC-based drug screening to increase the effectiveness of drugs that improve haemoglobin polymerisation for more affordable palliative treatment.

CONCLUSION

In response to the social demand for improved sickle cell disease treatments, scientists are investigating the prospect of CRISPR-mediated gene editing of patient-specific iPSCs for autologous transplantation, to develop a treatment solution which will particularly benefit patients that are not eligible for standard bone marrow transplants. This influence of societal demands has resulted in the preclinical development of a more ethically sound method of treatment through the application of scientific knowledge. Furthermore, the use of iPSCs has great potential in drug screening by providing opportunities to enhance drug discovery methodologies. However, the application of iPSCs and CRISPR in cell reprogramming and genetic manipulation is subject to limitations and there are significant aspects of this technology that require further monitoring and assessment in order to facilitate its movement from proof-of-concept to the clinic.

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