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Editorial

Genetic Modulation of Pluripotent Stem Cells

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Human pluripotent stem cells (hPSC) have a major potential in advanced medical applications, and have become the center of current cell and molecular biology studies. The fantasy of both scientists and lay people has been captured by culturing immortal human cell lines with the full potential of generating any kind of tissues or organs, including non-regenerating neuronal or heart muscle tissues. The ethical concerns regarding research on human embryonic stem cells [1] disappeared when it was discovered that human induced pluripotent stem cells (iPSCs) can be generated from a range of differentiated cell types, and these iPSCs fully mimic the regenerative and differentiation properties of embryonic stem cells [2].

The major current challenges of working with hPSCs are to establish proper methods for targeted genetic modulation together with directed tissue differentiation, to devise human cell-based disease models for applications in drug screening, and to develop safe and efficient medical use of pluripotent cell-derived tissues or organs. To achieve these aims, the specific use and further development of the tools of genetic engineering are essential and remain a challenge.

The aim of this Special Issue was to publish original research as well as comprehensive reviews on the recent advances in the field of targeted genetic modifications in hPSCs and their derivatives. A review of these issues was published by Xu *et al*. [3], describing the challenges of the medical interventions related to the use of human iPS cells in the treatment of diabetic nephropathy. This review describes the use of kidney organoids derived from human iPSCs, which replicate key features of diabetic nephropathy pathology, making them useful models for validating therapeutic targets and assessing drug efficacy. While genetic engineering of iPSCs using the most recent methodologies may further increase their use as important model systems, the therapeutic application of iPSCs still faces major challenges, including tumorigenicity and genomic instability. Mesenchymal stem cells (MSCs) have already shown promising results in improving renal function in preclinical models. Specific differentiation protocols for iPSC-based tissue engineering should successfully address these problems and have the potential to provide a major breakthrough in the treatment of diabetic nephropathy.

The other three papers published within the frame of this special issue are more related to the new techniques helping to understand the stages of development and specificities of potential genetic engineering of stem cells. The paper by Miao *et al*. [4] details the application of single-cell RNA sequencing in the recognition of the structural features of human tooth and periodontium under normal and pathological conditions. This approach helps to understand the intricate cellular composition of teeth and periodontium, leading to the identification of new cell types and tracing their lineage profiles. These data may provide insights into dental disease mechanisms at the cellular level and promote therapeutic interventions.

A paper by Chung [5] gives a detailed guideline for the use of Bacterial Artificial Chromosomes (BAC) for homologous recombination, and the identification of this recombination by detecting single-nucleotide polymorphisms (SNPs). The use of more than 150 kb BAC constructs can substantially enhance genetic recombination and SNPs that are useful markers for accurately docking BAC constructs at target sites. The advanced method of analyzing SNP patterns in this paper for large-scale genetic engineering should promote the use of therapeutic resources and disease models based on BAC-mediated homologous recombination.

The contribution of Xing *et al*. [6] deals with a derivative of hPSCs, the umbilical cord-derived mesenchymal stem cells (MSCs). This review summarizes the role of the Notch signaling pathway in the stability and further differentiation of these MSCs, providing an overview of the fate of these medically important stem cell preparations. MSCs have potent self-renewal and differentiation features, and due to their immunomodulatory, anti-inflammatory, and wound healing potential, have potential regenerative medicine applications. The Notch signaling pathway has a pivotal function in MSC proliferation and differentiation, and this paper summarizes the recent progress concerning the role of this pathway in maintaining homeostasis and determining the cell fate of human umbilical cord-derived MSCs with an outlook for the therapeutic use of these MSCs.

Of course, these papers, with a limited scope, could not deliver a full message of the increasing importance of using genetically engineered human stem cells in research, biotechnology, and medical treatment. According to the recent guidelines of the FDA and EMA, the new approach methodologies (NAMs) are important alternatives to animal

testing in the preclinical stages of the development of new medicines. These NAMs, which are compliant with the so-called 3Rs principles, that is, the replacement, reduction, and refinement of animal use in medicine testing, require the use of complex human tissues both for toxicity and efficacy investigations [7]. hPSC-derived differentiated tissue models, 3D spheroids, and organoids are the best candidates for these new test systems. Proper human disease models can now be developed by using genetically engineered iPSCs or various progenitor cells.

There are several goals and methods of genetically modifying hPSCs, and it is worth adapting these methods to the specific goals. The creation of induced pluripotency began with a retroviral stable genetic modification method [8], but it soon became clear that to produce iPSCs, a stable gene integration is not necessary, and transient expression of the factors can also result in permanent pluripotency. Stable, transient, or inducible gene expression methods helped to generate different pluripotent cell types, and trans-differentiation methods have evolved from these studies.

The first major goal of these studies was to track the iPSCs and their differentiation using a variety of markers. Special genes coding for fluorescent proteins were introduced, as these markers can be followed by using flow cytometry or imaging systems, and the cell cycle—e.g., by using the fluorescence ubiquitin cell cycle indicator (FUCCI)-based system, can also be monitored. Knock-out cell lines, marked by fluorescent marker expression, can also be generated and, when linked to specific promoters, selected differentiation pathways can be followed [9].

In order to follow specific cellular activities, such as calcium transients, voltage changes, neurotransmitter release, sensing pH, ion concentration, and metabolites, the introduction of genetically engineered indicators (GEIs), which are mostly fluorescent proteins, has now been widely used. There is a great advantage to using these systems in iPSCs, as the modified cells can be selected, cloned, and fully characterized, and their further differentiated offspring are well-suited for reporter-based studies in specific tissues. These methods have been used to monitor calcium signaling in PSC-derived cardiac and nervous tissues [10].

Genetic modification of iPSCs has major importance in the generation of human-specific disease models. Up-to-date gene knockout or gene editing methods (e.g., CRISPR) are regularly used to examine the function or the disease-related variants of specific proteins. An important point is that in normal iPSCs, a disease-causing mutation can be generated by targeted gene editing, thus isogenic normal and disease model cell lines and differentiated tissues can be established. Moreover, for the exploration of the genetic background of complex diseases, in patient-derived iPSCs, one or more genes can be selectively edited, again providing isogenic cell lines and tissue models for these complex studies [11].

Genetic engineering of human iPSCs may also result in major breakthroughs in cell therapy applications. Most recently, such genetic modifications have been devised with the aim of producing iPSCs and their derivatives that are generally usable, safe, and effective. These gene modifications aim to allow universal human use by avoiding recognition by the immune system [12]. The built-in suicide genes ensure safety [13], and the cells can reliably produce biologicals with specific therapeutic targets [14].

In summary, we suggest that further exploration of the tools of genetic engineering of iPS cells should provide the basis of directed 3D differentiation and drug screening applications in complex human tissues, as requested by the regulatory agencies. In addition to helping targeted drug development, genetically engineered iPSCs may significantly promote the clinical treatment of human diseases.

Author Contributions

BS and AA have substantial contributions to the conception and design of the work. BS wrote the initial draft and both authors contributed to the final form and the editorial changes in the manuscript. Both authors read and approved the final manuscript. Both authors have participated sufficiently in writing the paper and agreed to be accountable for all aspects of the work.

Ethics Approval and Consent to Participate

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Conflict of Interest

Balázs Sarkadi is an employee of Salus Ltd., however, Salus Ltd. had no involvement in the actual planning, or interpretation of the article. Given his role as the Guest Editor, Balázs Sarkadi had no involvement in the peer-review of this article and has no access to information regarding its peer review. Full responsibility for the editorial process for this article was delegated to Graham Pawelec.

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