Cell Research (2012) 22:283-284.

www.nature.com/cr

RESEARCH HIGHLIGHT

Cut and Paste: restoring cellular function by gene correction

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Cell Research (2012) **22**:283-284. doi:10.1038/cr.2011.192; published online 6 December 2011

Gene-editing technologies and patient-specific induced pluripotent stem cells (iPSCs) may represent an unprecedented opportunity for merging the stem cell and traditional gene therapy fields to fulfill the promises of regenerative medicine.

Gene correction technologies may allow for the precise excision of mutant genes responsible for monogenic disease and further replacement with its wild-type version. When combined with the use of pluripotent cells, gene correction approaches could ultimately lead to the cure or alleviation of human disease. Jaenisch and colleagues published the first demonstration of phenotypic improvement upon gene correction in 2007 [1]. By using a mouse model of sickle cell anemia, the authors were able to "edit" the genome of murine iPSCs, thereby allowing for the re-introduction of a wild-type version of the defective hemoglobin gene. Upon transplantation into the mouse model, hematopoietic progenitor cells derived from corrected iPSCs led to normal erythrocyte function. Subsequently, two different reports demonstrated the phenotypic correction of Hemophilia A by either allogeneic transplantation of endothelial cells derived from wild-type iPSCs or by *in vivo* correction of the mutant factor VIII [2, 3]. The studies by Xu *et al*. did not involve genetic correction of the generated iPSCs, but were rather a proof-of-principle application of wild-type iPSC differentiated cells as a "dominant positive" graft alleviating the disease phenotype [2]. On the other hand, the approach by Li *et al*. involved Zinc Finger Nuclease (ZFN)-mediated *in vivo* correction and alleviation of the hemophilic phenotype without iPSC generation or cell transplantation [3]. The ZFN-mediated correction of Hemophilia, although a more "canonical" gene therapy approach, actually showed efficient targeting, splicing and replacement with the wild-type version of the mutated DNA fragment *in vivo*.

More recently, different research groups have demonstrated the successful generation and correction of patient-specific iPSCs. They include the progeria syndrome [4, 5], Parkinson's disease [6], gyrate atrophy [7] as well as hematological diseases such as Thalassemias [8], Fanconi Anemia [9], and sickle cell anemia [14]. Even though these studies lacked *in vivo* confirmation of the phenotypic correction, some of them demonstrated functional correction of the phenotype upon differentiation *in vitro*. Thus, *in vitro* differentiation of pluripotent cells seems to mimic physiological processes, accelerate the time required for symptomatic manifestation of the disease *in vitro* and accordingly represent a suitable platform for gene correction studies. Of note, methods based on the application of Helper-dependent Adenoviral Vectors (HDAdVs) have proven successful with editing large genomic regions, potentially allowing for the single-step correction by homologous recombination of genes bearing multiple point mutations or even certain deletions and duplications [5, 14]. Most importantly, even though different methods were used in the various reports, gene targeting itself did not seem to affect epi/genomic integrity [5-7, 14]. Thus, the broad range of applications for gene editing technologies may not only be circumscribed to gene correction and regenerative medicine, but it could also allow for concise analysis of the molecular mechanisms leading to disease manifestation and progression.

Taking into account previously described epigenetic as well as genetic abnormalities found in iPSCs, the use of ESCs could prove a suitable complement not only for safer gene correction strategies, but also for cell's modification in the opposite direction, the generation of disease-specific ESCs [10, 11]. Such an approach, based on the use of site-specific nucleases, has been recently demonstrated. Hockemeyer *et al*. have described for the first time the use of Transcription Activator-like Effector Nucleases (TALE) for gene editing purposes in humans [12]. TALEN are site-specific nucleases that

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can be engineered similarly to ZFNs for the targeting of site-specific locations. The report describes the use of these novel nucleases for the efficient targeting of up to five different loci in both ESCs and iPSCs [12]. Importantly, the efficiencies described as well as the specificity shown for these nucleases were comparable to those achieved by employing more traditional methodologies such as ZFNs. Along the same line, Soldner *et al*. have reported the editing of the α-synuclein gene, whose different variants have been linked to Parkinson susceptibility [6]. In this work the authors do not only described the efficient engineering and correction of this gene by ZFNs in Parkinson patient-derived iPSCs, but also demonstrated the generation of Parkinson-specific ESCs lines [6].

More recently, Yusa *et al*. described the development of a novel gene targeting approach by combining the use of ZFN and piggyBAC transposon technology [13]. In their approach the authors were able to excise back the targeting construct from the host genome in the absence of the typical genomic scars induced by other commonly employed excisable methods such as the Cre-LoxP system. Of note are the facts that correction of iPSCs generated by non-integrative approaches was equally efficient as compared to the correction of iPSCs generated by integrative viruses and that the frequency for biallelic correction reaches up to 11% in some of the corrected clones. Moreover, even though the generated iPSCs and their derivatives bear certain mutations acquired during reprogramming, gene targeting and/or culture conditions, the mutations observed did not hamper the phenotypic correction *in vivo*, neither led to tumor formation. This work as well as several other reports published earlier this year highlight the need for exhaustive testing of iPSCs and their derivatives in the context of specific applications rather than absolute number of mutations acquired, i.e., iPSCs bearing mutations in genes compromising the function of differentiated cells should be discarded in favor of lines whose alterations do not hamper normal cell function upon differentiation and transplantation [13]. With these criteria in mind, one would rather prime functionality in a particular context instead of the absolute number of mutations observed.

The genetic correction of monogenic mutations responsible for the development of disease and generation of isogenic iPSC/ESC lines may not only contribute towards more reliable and experimentally matched control sets in future drug discovery studies, but also brings the opportunity for combining gene therapy and regenerative medicine in novel therapeutical treatments. Although these two fields of study are relatively recent, the astonishingly rapid pace of new development leads us to assume that our long-lasting bench dreams might become real, allowing us to witness one of the most revolutionary ages in personalized medicine.

Acknowledgments

Work in the laboratory of JCIB was supported by Sanofi-Aventis, The Helmsley Charitable Trust and The Ellison Medical Foundation.

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