NEWS AND VIEWS / NOTICIAS Y OPINIONES

A review of cellular reprogramming: limitations and recent advances.

Revisión sobre la reprogramación celular: límites y avances recientes.

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Abstract: Cellular reprogramming has been around for many years offering opportunities in areas such as regenerative medicine. New technologies and methods have merged since its origin. However, no method has yet been totally successful. A wide range of possible applications, from healing small wounds to curing complex illnesses like Alzheimer, is the reason to continue the exhaustive research in this area. In this review paper, we make a compilation of the most relevant reprogramming technologies. We go over the initial techniques to the most recent advances, especially highlighting each of their benefits and limits. Finally, we make a comparison of the current reprogramming technologies in regenerative medicine and remark the importance of continuing the present investigations.

Keywords: reprogramming, transfection, nanotechnology, regenerative medicine, iPSCs.

Resumen: La reprogramación celular existe desde hace muchos años ofreciendo oportunidades en áreas como la medicina regenerativa. Nuevas tecnologías y métodos se han fusionado desde su origen. Sin embargo, ningún método ha sido totalmente exitoso hasta la actualidad. Una amplia gama de posibles aplicaciones, desde curar pequeñas heridas hasta enfermedades complejas como el Alzheimer, es la razón para continuar la investigación exhaustiva en esta área. En este documento de revisión, hacemos una compilación de las tecnologías de reprogramación más relevantes. Repasaremos desde las técnicas iniciales hasta los avances más recientes, destacando especialmente cada uno de sus beneficios y límites. Finalmente, hacemos una comparación de las tecnologías actuales de reprogramación en medicina regenerativa y destacamos la importancia de continuar las investigaciones actuales.

Palabras clave: reprogramación, transfección, nanotecnología, medicina regenerativa, iPSCs.

Introduction

Differentiation was frequently thought as a one-way traffic in which cells pass from an undifferentiated or progenitor state to a mature one, without the ability to switch function[1]. However, since the discovery of stem cells (SC), scientists started to look for strategies that could allow them to mimic SCs' behavior and reverse that dogma. The technique that has allowed investigators to achieve that change is reprogramming, an event based on giving plasticity to terminally differentiated cells. This befalls through transfection, which is the introduction of foreign nucleic acids into cells to induce genetic modification[2]. Somatic cells are transformed into induced pluripotent stem cells (iPSCs) which, in fact, shown to be functionally equivalent to stem cells[3]. Reprogramming somatic cells directly to iPSCs eliminates the necessity of using embryonic material and additionally contributes to the production of patient-specific cells of any type[4].

Background

The first approach to iPSCs technology was somatic cell nuclear transfer (SCNT). The basis of this method consists in the incorporation of the nucleus of a somatic cell to an enucleated oocyte, generating cloning[5]. This demonstrated that even stable differentiated cells can be inverted to their original state because of the genetic information contained and that some factors present at oocytes can help reprogramming somatic cell nuclei[6]. Nuclear cloning generated doubt about the epigenetic mechanisms that were transforming somatic to embryonic cells, giving the first clues that had to be solved for the explanation of cellular reprogramming[7]. However, nuclear cloning triggered insertional mutations and abnormal pattern of expression under study due to unsatisfactory reprogramming[8].

Later, in 2001, Takashi Tada's group integrated the presence of reprogramming factors of somatic cells in embryonic stem cells such as integrating vectors, non-integrating vectors[9]. This generated another reprogramming technique based on the combination of somatic cells and embryonic stem cells[5]. Epigenetic reprogramming of somatic nuclei was accomplished and proved in murine hybrids. The development and results of some experiments were accurate but none gave full confidence[7]. With this two first approaches, researchers got convinced with the idea that a combination of factors is what drives reprogramming of somatic cells.

Cellular reprogramming research has now focused on overcoming obstacles, developing and improving new direct reprogramming techniques. Various methods are being implemented, each comprising better characteristics but based on the same principle of working. We consider the most important and recent are micro RNA, messenger RNA, and transcription factors (Figure 1).

Micro RNA (miRNA)

miRNAs are part of the trending factors that researchers have seen as influencers in converting somatic to embryonic cells. Micro RNA comprises approximately 22 nucleotides of non-coding RNA that commonly promotes the degradation or

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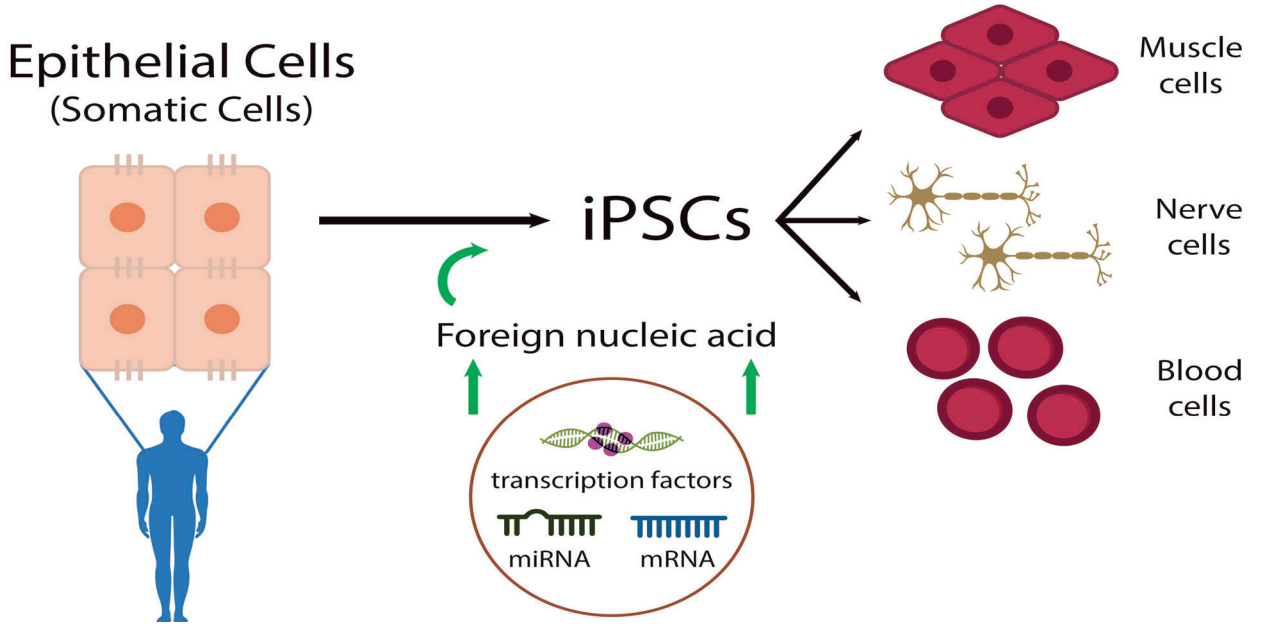

Figura 1. Principle of working of the most recent methods of cellular reprogramming. We show epithelial cells as example of starting point, which are then transformed to iPSCs by the insertion of any foreign nucleic acid (miRNA, mRNA or transcription factors). From there, we can obtain any type of somatic cell be it muscle, nerve, blood or other cell.

inhibit the translation of messenger RNA by binding within it. Essentially some clusters, specifically miR-209-295 and miR-302-367 seem to present some evidence of promoting cellular trans-differentiation and reprogramming into IPSCs and even replace some transcription factors. These processes were found to occur by inhibiting enzymes and signing paths[10]. Similarly, some other studies have shown that miRNAs play a crucial role in the regulation of self-regeneration of stem cells and differentiation.

Transcriptional and post-transcriptional gene regulation of miR-302-367 with embryonic stem cells (ESCs) tend to maintain "stemless" over differentiating leading a delay for early differentiating ESCs. miR-9 and miR-124 mediate cell trans-differentiation while inducing the conversion of fibroblasts into neurons through mesenchymal to epithelial transition (MET). And most essential, reprogramming somatic cells into IPSCs and human ESCs, uses these bundles of miRNA. Its tendency to undergo reversible MET sustains the expression of pluripotency among these cells by activating OCT4 gene expression cooperating with Hdac2 suppression showing some powerful pathways in reprogramming somatic cells into pluripotency[11, 12].

Messenger RNA (mRNA)

An additional reprogramming tool that many groups of scientists have been using is mRNA, having various degrees of success[13]. Messenger RNA is a subtype of RNA that takes a portion of the DNA code to other parts of the cell for processing[14]. The mRNA-based reprogramming technology is a non-integrating, non-viral, highly clinically applied. Their potential is due to the reduction of the risk of integration and mutagenesis in the genome[15, 16]. One study shows the efficiency of repeated administration of synthetic messenger RNAs. The modifications made include the incorporation of additional factors to overcome innate antiviral responses. In addition, the mRNA reprogramming suggests a titratable dose of expression of different mRNAs, which provides stoichiometric control of essential factors during reprogramming[16]. This simple, non-mutagenic, and controllable technology can be applied to directed differentiation of RNA-iPSCs (RiPSCs) to terminally differentiated myogenic cells[17].

The application of mRNA gives advantages in comparison to orthodox drugs because mRNA does not use biological structures, avoiding biodegradation and environmental issues with a high efficacy and fast kinetics[18]. However, there are some limitations such as some transfections needed to induce iPSCs due to the short half-life of mRNAs[15, 18]. The most recent report in this field, non-modified mRNA, showed no toxicity and immune response in the generation of iPSCs[13]. The original paper details the principal steps that have to be followed to develop this method successfully[13].

Transcription factors

Scientists have found that differentiated somatic cells can be directly transformed into embryonic stem cells by ectopic co-expression of specific transcription factors[18, 19]. This epigenetically resets somatic cells into an early development stage which then develop to other cell types[3]. Since this technique showed up, some transcription factors have been used; cells from different somatic lineages of a varied group of species. The potential that has been generated by forced expression in transcription factors is limited since the majority has not been able to support the development of animals completely derived from iPSC[18].

Hence, scientists started to use new resources to develop new methods for reprogramming, replacing transcription factors. The main reason for the replacement was because the majority of animals with iPSCs and their progeny increased potentially the incidence of tumors. However, according to new studies, this constitutes the most promising field for iPSCs' technology and actually, is the one that has more investigation made[18]. Tremendous innovation has occurred principally in the method of factor delivery and the type of somatic cells being reprogrammed[3].

Delivery techniques

The initial delivery method were viral vectors. Their efficiency for cell reprogramming is variable. However, in some cases, the genome of the viral vector can integrate into the host genome and influence differentiation. This additionally activates an oncogene that can cause inflammation and even become into a cancerous cell[20–23]. The principal reason of the development of non-integrating approaches is to make iPSCs more therapeutically applicable[4]. A recent investigation showed the potential of electroporation-based transfection for delivery of transcription factors. Bulk electroporation (BEP) elaborates pores under the influence of an electric field, allowing the entry of transcription factors into the cell and posterior reprogramming[24–26]. There are no much chances for safe electroporation because the plasticity of the cell is usually affected[27].

After BEP failures, scientists started to develop a better method for the transfer of transcription factors. Researchers at Ohio State University Wexner Medical Center created the most recent technology, one that can reprogram cells with no damage, known as Tissue Nano transfection (TNT)[28]. Its process is based on the direct cytosolic delivery of reprogramming factors into cells' outer membranes through temporary channels[27]. They use a chip which is loaded with the required reprogramming factors and placed on the skin. A highly intense and focused electric field is applied, and the canals become opened[29]. Then, they inject the desired genes, those reach the chosen somatic cells by vesicle transport and transform them[29]. The technique has been successfully proved in two in vivo experiments. The first transformed adult skin cells into vascular cells and the other reprogrammed fibroblasts into induced neurons[27, 29].

Conclusions

From the beginning of the discovery of reprogramming, there has been a vast advance which reflects the magnitude and the importance of the possible future results. There is still a lot of work to be done to understand the principles by which reprogramming happens in somatic cells but definitely, this technique is worth to continue with investigations. Right now, the technique that is being investigated the most is the one that uses transcription factors for reprogramming, however, there is also a recent (2016) research with non-synthetic mRNA that shows potential for its capacity of transforming somatic cells to an embryonic state and miRNA also showed to be powerful.

About the models of delivery, the more accurate process until now is tissue nanotransfection (TNT). It is the safer, inexpensive, more flexible, fast an antiviral. Possess a simple way for reprogramming, avoiding large laboratory processes and allowing the generation of any cell type and recovery of injured tissue, using the patients' own cells. Combination of this procedure with any of the factors used for reprogramming provides a promising future for this field.

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