

Concise Review: ES vs. iPS Cells; the Game is on

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ABSTRACT

Extraordinary advances in pluripotent stem cell research have initiated an era of hope for regenerative strategies to treat human disease. Alongside embryonic stem (ES) cells, the discovery of induced pluripotent stem (iPS) cells widened the possibility of patient specific cell therapy, drug discovery and disease modelling. Although similar, it has become clear that these two pluripotent cell types display significant differences. In this review, we explore current

knowledge of the molecular and functional similarities and differences between these two cell types to emphasize the necessity for thorough characterization of their properties both in the pluripotent state as well as their differentiation capabilities. Such comparative studies will be crucial for determining the more suitable cell type for future stem cell-based therapies of human degenerative diseases.

INTRODUCTION

Since the advent of human embryonic stem (ES) cells in 1998[1], stem cell research has been developing at a breathtaking pace. The pluripotent nature of these cells renders them able to differentiate into any cell type - including into those with therapeutic potential - after practically unlimited self-renewal in the stem cell state. ES cells hold enormous promise as tools for understanding normal development and disease, and just as importantly, for cell therapy applications to treat devastating and currently incurable disorders, such as spinal cord injury, neurological disease, blindness and Type 1 diabetes. On the other hand, the use of human embryos to derive these cells has ignited a diverse ethical debate rooted in the complex

background of human historical, cultural and religious differences. In this review, we are not going to pursue a discussion of ethical issues, but rather focus on the potential of pluripotent cells in general to cure disease and eliminate human suffering.

Following the characterization of the first human ES cells cell lines in the late 1990's, standard protocols have steadily been developed to accommodate future clinical applications, including maintenance of these cells in the absence of animal-derived culture components. Furthermore, guided by insights gained from decades of research on the molecular genetic basis of mammalian development, detailed protocols have emerged for the reproducible generation of enriched populations of various differentiated cell lineages in mouse and human,

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including neurons, cardiomyocytes, and hematopoietic cells[2]. Numerous preclinical animal studies have demonstrated that the differentiated derivatives of ES cells can provide functional replacements for diseased tissues, such as for Parkinson's disease[3], and clinical trials are currently underway for human ES cell based cellular therapy for spinal cord injury and macular degeneration in the US and UK [4].

Six years ago, Takahashi and Yamanaka astonished the world by showing that enforced expression of four key transcription factors: Oct4, Sox2, Klf4 and c-Myc can reprogram mouse somatic cells such as fibroblasts to pluripotency, and achieve similar developmental potential as ES cells, without the requirement for an embryo[5]. They named these new cells "induced Pluripotent Stem cells" or iPS cells. A year later several groups, including Yamanaka's, reported the successful generation of iPS cells from human somatic cells[6,7]. With this step forward, a race was initiated. The expectation that iPS cells will offer the same therapeutic potential as human ES cells, and the robust, reproducible method of deriving iPS cells, have spawned hundreds of studies addressing *in vitro* disease modeling and cell therapy strategies in pre-clinical animal models. Indeed, iPS cell lines have now been generated from patients of several monoallelic and complex genetic disorders (reviewed in [8]). These developments have brought the field a hopeful step closer to the promises of *in vitro* disease modeling, disease specific pharmacological treatment testing, and in some cases individualized cell replacement therapy. Several examples of the differentiation of disease specific iPS cells into the cell types that are implicated in the disorder's pathogenesis have been reported, and therefore this technology is particularly attractive for the diseases for which animal models are either not available, or do not accurately represent the human disease etiology.

The following question has thus subsequently arisen: can iPS cells replace ES cells in clinical application and disease modeling? Although all

of us would likely welcome a "yes", it is clear that we are not yet in the position to answer this question, despite the unprecedented speed of development in the iPS cell research area. Our understanding of the full characteristics of iPS cells and the mechanistic details of reprogramming to pluripotency is far from complete[9]. Although several analyses indicate that iPS cells share many key properties with ES cells including morphology, pluripotency, self-renewal and similar gene expression profiles, there are just as many published examples that point out their differences. There is an urgent need to shed more light on the complex landscapes of safety, efficacy, economy and disease coverage associated with clinical use of these new and exciting pluripotent cell types. At the current state of knowledge, we promote the view that parallel, direct studies on iPS and ES cells, including detailed characterization of mechanisms of pluripotency and differentiation are required to make the promise of stem cell based therapeutics for human disease a reality.

Genome integrity

During expansion and prolonged passage, human ES cell lines frequently acquire abnormal karyotypes such as trisomy 12 and 17[10,11] as well as genetic amplification at 20q11.21, which has been associated with oncogenic transformation[12,13]. iPS cell lines are also subjected to similar selective forces, resulting in cell culture adaptation frequently manifested in karyotypic abnormalities[14]. So in this respect ES and iPS cells are likely equivalent.

The distinct cellular origin, however, could lead to significant differences between these two pluripotent stem cell types. ES cells are derived from the inner cell mass of a blastocyst stage embryo before the soma and the germ cell lineages separate. iPS cells, however, are derived from somatic cells.

It was August Weismann who in 1889 first recognized that in most organisms, the somatic and germ cell lineages separate very early in development and pointed out the evolutionary

consequences of this separation[15]. Weismann postulated that hereditary information moves only from germ cells to somatic cells. The reverse direction, soma to germ line, would be impossible. Moreover, the genome of the germ cell lineage is passed to the new generation and in this respect it is immortal. On the other hand, the genome of the somatic cells is mortal, since it is discontinued with the death of the organism. Therefore, mutations generated in the soma are not subject to evolutionary selective forces such as natural selection or genetic drift. Instead, the immortal germ cell genome remains mostly locked into the germ cell lineage with a brief passing through the very early developmental stages (pre- and early postimplantation in the mouse and human), prior to the germ-soma separation (Figure 1).

Since evolutionary selective forces act only on mutations in the germ line genome, the expectation is that the strength of genome integrity protection might therefore be different between germ line and soma. A putative differential genome protection could have significant consequence regarding the genome integrity of ES vs. iPS cells. ES cells derived from the inner cell mass of the blastocyst have never had a journey through a stage in the soma.

John Gurdon's somatic cell nuclear transfer (SCNT) in frogs was the first example that with experimental manipulation it is possible to return the genome of a somatic cell to the germ line [16]. This discovery was later followed by success in sheep[17], mice[18], and numerous agricultural species[19]. SCNT reprograms the somatic cell genome into a totipotent cell state (Figure 1). Since SCNT has become a routine procedure in many mammalian species, it has become evident that cloned animals suffer increased risk of abnormalities ranging from prenatal death to altered development[20]. It is still not completely clear what proportion of these abnormalities are due to incomplete epigenetic reprogramming or to permanent genetic changes occurring during somatic cell

development or during the reprogramming process (see below).

The generation of iPS cells by reprogramming using enforced expression of a finite number of transcription factors is similar in this respect. The genome of a fully differentiated somatic cell is returned to pluripotency, which theoretically includes germ line competence (Figure 1). Therefore, iPS cells can acquire genetic alterations at two additional phases; during somatic differentiation and during reprogramming. It is likely that none of these phases have developed genome-protecting mechanisms responding to evolutionary pressure.

Several recent studies have demonstrated that the reprogramming process leads to genomic instability and genomic abnormalities, with a notable proportion of lesions mapping to known cancer causative loci[14,21,22]. Reprogramming causes genomic copy number variations (CNV) to occur early in iPS cell passage leading to mutations and a mosaic iPS cell population [21,22]. During passage, iPS cells undergo strong selection pressure against most of the mutations and reach a CNV load similar to that of ES cells. Nevertheless, human iPS cells contain *de novo* mutations that are not detected in human ES cells, suggesting that certain mutations are selected for and are advantageous to reprogramming[14,21,22]. Taken together the available data suggest that reprogrammed cells indeed likely pose a greater risk for accumulation of deleterious genomic mutations. Furthermore, when the reprogramming factors are not silenced, iPS cells are predisposed to further genomic instability[23]. These findings underscore the critical requirement for detailed characterization of the genome integrity of iPS cells in comparison to that of ES cells and the human genome for correct interpretation of experimental results using these cell lines, and also for safe future therapeutic applications.

Genetic and epigenetic regulation of the pluripotent state

Comparisons of iPS and ES cells have indicated that major features of the ES epigenome are reproduced in iPS cells, including genome wide methylation patterns and the establishment of bivalent histone marks at specific loci[24-26]. However, some analyses of reprogramming in mouse cells have shown that differences in gene expression and differentiation potential are observed specifically in early passage iPS cells, and have led to the concept that an “epigenetic memory” of previous fate persists in these cells[27-31]. Epigenetic memory has been attributed to the incomplete removal of somatic cell specific DNA methylation at regions in proximity to CpG islands known as “shores”[28,32]. The residual DNA methylation pattern and resulting gene expression of the somatic cell of origin are lost upon continued serial passage of derived iPS cells, and after treatment with molecular inhibitors of DNA methyltransferase activity[28,29] suggesting that “epigenetic memory” also identifies cells that are incompletely reprogrammed. On the other hand, these findings suggest that cell type of origin could affect results in disease modeling since iPS cells show distinct cellular and molecular characteristics based on the cell type of origin. However it has been noted that this property may improve the prospects of generating some cell types for cell replacement therapy, in particular for those that are difficult to generate by differentiation from ES cells, including insulin producing pancreatic beta cells[27].

Gene expression

In agreement with the epigenetic similarity of the two pluripotent cell types, comparative transcriptome analyses using microarray also indicate that human ES and human iPS cells are highly alike on a global scale, with gene expression patterns clustering together, and separate from the originating somatic cells[9]. iPS cells may retain, however, a unique gene expression signature, including that of miRNAs and long non-coding RNAs[33-37]. In addition, a few studies have noted that some

transcriptional differences can also be attributed to latent expression of the four reprogramming factors, to genetic background, and to differences in *in vitro* microenvironment and handling conditions in different laboratories[24,38]. These findings collectively suggest that detailed analyses, and standardization of reprogramming and cell culture protocols will be required to validate whether small variations in gene expression seen between iPS and ES cells have biological significance.

Developmental potential vs. disease risk

Since mouse ES cells have the capacity to generate an entire normal adult mouse, they are considered the gold standard against which all other cell types are compared with respect to pluripotency. The ability to significantly contribute to chimeras is considered the most stringent test of pluripotency for mouse iPS cells. Interestingly, available data suggest that compared to ES cells, only a small percentage of mouse iPS cell lines can contribute to strong chimeras or quite infrequently form completely iPS cell-derived animals in tetraploid embryo complementation[39]. Furthermore, the earliest studies on iPS derived chimeric mice demonstrated that they were prone to cancer and attributed this property to the re-expression of the c-myc reprogramming factor[40]. C-myc is a well-studied oncogene, and the expression of the other three reprogramming factors has been associated with several forms of human cancer[41]. For this reason, substantial efforts have been made to find reprogramming methods that do not require permanent transgene integrations. During the last three years, several such factor delivery methods have been developed using adenovirus, the piggyBac transposon, as well as direct protein transduction among others[42].

Pluripotency of ES and iPS cells, as defined by the ability to differentiate into tissues of all three germ layers is also assessed using the *in vivo* teratoma assay, the only pluripotency test available for the study of human pluripotent cells.

Detailed pathological characterization of teratomas in immunocompromised mice has recently revealed surprising differences between human ES and iPS cells. iPS cell induced teratomas were more aggressive, with a shorter latency than ES cells and frequently contained areas more aggressive teratocarcinoma characteristics[43]. It remains to be determined whether such pathological features can be directly attributed to alterations at the genome level during reprogramming and prolonged passage *in vitro*. Recent analyses suggest that the pluripotent and tumorigenic capacity of ES cells may be governed by different cell signaling pathways[44], a property that most likely also applies to iPS cells. This necessitates a thorough molecular understanding of the differences between ES and iPS cells with respect to their developmental potential and risk of ill behaving if their derivatives were grafted into an individual.

Ironically, the vast proliferation and tissue differentiation potential of iPS and ES cells *in vivo*, is considered to be one of their main obstacles for clinical use. For example, formation of teratoma-like tumours was observed in one test for the efficacy of hES cells in a mouse model of Parkinson's disease, and interfered with the grafted cells ability to restore dopaminergic neural function[3]. Furthermore, a survey of teratoma formation by grafted neural tissue obtained from iPS cells that were derived from different cellular sources and with different methods has identified another important aspect of the safety of cellular therapy. Tumour formation was positively correlated only with the residual presence of undifferentiated cells, but, interestingly, not with the presence of c-myc, or with other variables in the iPS cell derivation process[45]. These reports demonstrate that the elimination of residual pluripotent cells is a major challenge and an issue that is equally potent for ES cells as is for iPS cells. With current protocols, it is very difficult to produce completely pure populations of differentiated derivatives from ES cell or iPS cell cultures for transplantation. In the future, stringent cell

surface marker based cell separations or depletion of undifferentiated cells, or modifications of the starting iPS cell or ES cell populations that permit deletion of undifferentiated cells *in vivo* will have to be considered.

Differentiation and disease modeling

For clinical applications, reprogramming is the first step with the ultimate goal being reproducible differentiation and maximum enrichment to specific cell lineages. While this property is established for ES cells, albeit still with technical barriers, very recent studies have begun to address the differentiation capacity of human iPS cells and the functionality of their differentiated derivatives. Although multiple protocols have been developed to derive specific cell types *in vitro*, there is considerable variability in the efficiency of generating differentiated lineages among independent human ES and iPS cell lines[46]. The production of hemangioblast cells and other derivatives occurred at a much lower efficiency from human iPS cells than from human ES cells[47]. Similarly, human iPS cells differentiate to neural lineages at a much lower frequency than ES cells regardless of the means of derivation[48]. The molecular signature of iPS cells can be influenced by the cell type of origin, and in one case, can explain this biased differentiation potential[27]. Premature senescence of differentiated endothelial cells and retinal pigment epithelium from iPS have also been observed[49,50] suggesting that the differentiated progeny of iPS cells may also display significant functional differences that could undermine their therapeutic utility. Thus, it is important to consider that genetic or epigenetic features that affect iPS cells during differentiation could also do so after transplantation, generating cells with gene expression patterns or phenotypic characteristics that are different from ES cell-derived transplants.

CONCLUSION

Permanent cell lines of pluripotent ES and iPS cells, and our increasing ability to direct them into any cell type for therapeutic potential holds enormous promise for future regenerative medicine. ES cells are considered to be the gold standard of pluripotency, while iPS cells offer the development of cells from any adult individual, which advances the possibility of curing devastating degenerative diseases using cell or tissue grafts with perfect histocompatibility match. This potential calls for efforts to characterize and compare the nature of these pluripotent cell types in great detail. Only

such deep studies can give us sufficient insight into the potential, efficacy and safety to reach a decision; which one will be more favorable for future clinical applications. At the current state of knowledge, we are not in a position to make such a decision. The game between ES and iPS cells is still on with no obvious indication of the winner.

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REFERENCES

1. Thomson JA, Itskovitz-Eldor J, Shapiro SS et al. Embryonic stem cell lines derived from human blastocysts. *Science*. 1998; 282:1145-1147.
2. Murry CE and Keller G. Differentiation of embryonic stem cells to clinically relevant populations: lessons from embryonic development. *Cell*. 2008; 132:661-680.
3. Bjorklund LM, Sánchez-Pernaute R, Chung S et al. Embryonic stem cells develop into functional dopaminergic neurons after transplantation in a Parkinson rat model. *Proc Natl Acad Sci U S A*. 2002; 99:2344-2349.
4. Aboody K, Capela A, Niazi N et al. Translating Stem Cell Studies to the Clinic for CNS Repair: Current State of the Art and the Need for a Rosetta Stone. *Neuron*. 2011; 70:597-613.
5. Takahashi K and Yamanaka S. Induction of Pluripotent Stem Cells from Mouse Embryonic and Adult Fibroblast Cultures by Defined Factors. *Cell*. 2006; 126:663-676.
6. Park I-H, Zhao R, West JA et al. Reprogramming of human somatic cells to pluripotency with defined factors. *Nature*. 2007; 451:141-146.
7. Takahashi K, Tanabe K, Ohnuki M et al. Induction of Pluripotent Stem Cells from Adult Human Fibroblasts by Defined Factors. *Cell*. 2007; 131:861-872.
8. Wu SM and Hochedlinger K. Harnessing the potential of induced pluripotent stem cells for regenerative medicine. *Nat Cell Biol*. 2011; 13:497-505.
9. Plath K and Lowry WE. Progress in understanding reprogramming to the induced pluripotent state. *Nat Rev Genet*. 2011; 12:253-265.
10. Baker DEC, Harrison NJ, Maltby E et al. Adaptation to culture of human embryonic stem cells and oncogenesis in vivo. *Nat Biotechnol*. 2007; 25:207-215.
11. Draper JS, Smith K, Gokhale P et al. Recurrent gain of chromosomes 17q and 12 in cultured human embryonic stem cells. *Nat Biotechnol*. 2003; 22:53-54.
12. Lefort N, Feyeux M, Bas C et al. Human embryonic stem cells reveal recurrent genomic instability at 20q11.21. *Nat Biotechnol*. 2008; 26:1364-1366.
13. Spits C, Mateizel I, Geens M et al. Recurrent chromosomal abnormalities in human embryonic stem cells. *Nat Biotechnol*. 2008; 26:1361-1363.
14. Mayshar Y, Ben-David U, Lavon N et al. Identification and Classification of Chromosomal Aberrations in Human Induced Pluripotent Stem Cells. *Cell Stem Cell*. 2010; 7:521-531.
15. Weissman A. (1889). *Essays Upon Heredity and Kindred Biological Problems*. Clarendon Press, Oxford.
16. Gurdon JB, Elsdale T and Fishberg M. Sexually mature individuals of *Xenopus Laevis* from the transplantation of single somatic nuclei *Nature*. 1958; 182:64-65.
17. Wilmut I, Schnieke AE, McWhir J et al. Viable offspring derived from fetal and adult mammalian cells. *Nature*. 1997; 385:810-813.
18. Wakayama T, Perry AC, Zuccotti Met al. Full-term development of mice from enucleated oocytes injected with cumulus cell nuclei. *Nature*. 1998; 394:369-374.
19. Vajta G and Gjerris M. Science and technology of farm animal cloning: State of the art. *Anim Reprod Sci*. 2006; 92:211-230.
20. Wilmut I, Beaujean N, de Sousa PA et al. Somatic cell nuclear transfer. *Nature*. 2002; 419:583-586.
21. Hussein SM, Batada NN, Vuoristo S et al. Copy number variation and selection during reprogramming to pluripotency. *Nature*. 2011; 470:58-62.
22. Laurent LC, Ulitsky I, Slavin I et al. Dynamic Changes in the Copy Number of Pluripotency and Cell Proliferation Genes in Human ESCs and iPSCs during

- Reprogramming and Time in Culture. *Cell Stem Cell*. 2011; 8:106-118.
23. Ramos-Mejia V, Munoz-Lopez M, Garcia-Perez JL et al. iPSC lines that do not silence the expression of the ectopic reprogramming factors may display enhanced propensity to genomic instability. *Cell Res*. 2010; 20:1092-1095.
 24. Guenther MG, Frampton GM, Soldner F et al. Chromatin Structure and Gene Expression Programs of Human Embryonic and Induced Pluripotent Stem Cells. *Cell Stem Cell*. 2010; 7:249-257.
 25. Lister R, Pelizzola M, Kida YS et al. Hotspots of aberrant epigenomic reprogramming in human induced pluripotent stem cells. *Nature*. 2011; 470:68-73.
 26. Meissner A. Epigenetic modifications in pluripotent and differentiated cells. *Nat Biotechnol*. 2010; 28:1079-1088.
 27. Bar-Nur O, Russ HA, Efrat S et al. Epigenetic Memory and Preferential Lineage-Specific Differentiation in Induced Pluripotent Stem Cells Derived from Human Pancreatic Islet Beta Cells. *Cell Stem Cell*. 2011; 9:17-23.
 28. Kim K, Doi A, Wen B et al. Epigenetic memory in induced pluripotent stem cells. *Nature*. 2010; 467:285-290.
 29. Polo JM, Liu S, Figueroa ME et al. Cell type of origin influences the molecular and functional properties of mouse induced pluripotent stem cells. *Nat Biotechnol*. 2010; 28:848-855.
 30. Ghosh Z, Wilson KD, Wu Y et al. Persistent Donor Cell Gene Expression among Human Induced Pluripotent Stem Cells Contributes to Differences with Human Embryonic Stem Cells. *PLoS One*. 2010; 5:e8975.
 31. Marchetto MCN, Yeo GW, Kainohana O et al. Transcriptional Signature and Memory Retention of Human-Induced Pluripotent Stem Cells. *PLoS One*. 2009; 4:e7076.
 32. Doi A, Park I-H, Wen B et al. Differential methylation of tissue- and cancer-specific CpG island shores distinguishes human induced pluripotent stem cells, embryonic stem cells and fibroblasts. *Nat Genet*. 2009; 41:1350-1353.
 33. Mikkelsen TS, Hanna J, Zhang X et al. Dissecting direct reprogramming through integrative genomic analysis. *Nature*. 2008; 454:49-55.
 34. Chin MH, Mason MJ, Xie W et al. Induced Pluripotent Stem Cells and Embryonic Stem Cells Are Distinguished by Gene Expression Signatures. *Cell Stem Cell*. 2009; 5:111-123.
 35. Loewer S, Cabili MN, Guttman M et al. Large intergenic non-coding RNA-RoR modulates reprogramming of human induced pluripotent stem cells. *Nat Genet*. 2010; 1-7.
 36. Stadtfeld M, Apostolou E, Akutsu H et al. Aberrant silencing of imprinted genes on chromosome 12qF1 in mouse induced pluripotent stem cells. *Nature*. 2010; 465:175-181.
 37. Wilson KD, Venkatasubrahmanyam S, Jia F et al. MicroRNA Profiling of Human-Induced Pluripotent Stem Cells. *Stem Cells Dev*. 2009; 18:749-757.
 38. Newman AM and Cooper JB. Lab-Specific Gene Expression Signatures in Pluripotent Stem Cells. *Cell Stem Cell*. 2010; 7:258-262.
 39. Zhao XY, Li W, Lv Z et al. iPSC cells produce viable mice through tetraploid complementation. *Nature*. 2009; 461:86-90.
 40. Okita K, Ichisaka T and Yamanaka S. Generation of germline-competent induced pluripotent stem cells. *Nature*. 2007; 448:313-317.
 41. Ben-David U and Benvenisty N. The tumorigenicity of human embryonic and induced pluripotent stem cells. *Nat Rev Cancer*. 2011.
 42. González F, Boue S and Belmonte JCI. Methods for making induced pluripotent stem cells: reprogramming à la carte. *Nat Rev Genet*. 2011; 12:231-242.
 43. Gutierrez-Aranda I, Ramos-Mejia V, Bueno C et al. Human Induced Pluripotent Stem Cells Develop Teratoma More Efficiently and Faster Than Human Embryonic Stem Cells Regardless the Site of Injection. *Stem Cells*. 2010; 28:1568-1570.
 44. Li Y, Yokohama-Tamaki T and Tanaka TS. Short-Term Serum-Free Culture Reveals that Inhibition of Gsk3 β Induces the Tumor-Like Growth of Mouse Embryonic Stem Cells. *PLoS One*. 2011; 6:e21355.
 45. Miura K, Okada Y, Aoi T et al. Variation in the safety of induced pluripotent stem cell lines. *Nature Biotechnol*. 2009; 27:743-745.
 46. Osafune K, Caron L, Borowiak M et al. Marked differences in differentiation propensity among human embryonic stem cell lines. *Nature Biotechnol*. 2008; 26:313-315.
 47. Feng Q, Lu S-J, Klimanskaya I et al. Hemangioblastic Derivatives from Human Induced Pluripotent Stem Cells Exhibit Limited Expansion and Early Senescence. *Stem Cells*. 2010; 28:704-712.
 48. Hu BY, Weick JP, Yu J et al. Neural differentiation of human induced pluripotent stem cells follows developmental principles but with variable potency. *Proc Natl Acad Sci U S A*. 2010; 107:4335-4340.
 49. Narsinh KH, Sun N, Sanchez-Freire V et al. Single cell transcriptional profiling reveals heterogeneity of human induced pluripotent stem cells. *J Clin Invest*. 2011; 121:1217-1221.
 50. Kokkinaki M, Sahibzada N and Golestaneh N. Human Induced Pluripotent Stem-Derived Retinal Pigment Epithelium (RPE) Cells Exhibit Ion Transport, Membrane Potential, Polarized Vascular Endothelial Growth Factor Secretion, and Gene Expression Pattern Similar to Native RPE. *Stem Cells*. 2011; 29:825-835.

