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Will Undifferentiated Induced Pluripotent Stem Cells Ever have Clinical Utility?

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Abstract

The emergence of induced pluripotent stem cell (iPSC) technology, with the capability of iPSCs to differentiate into any type of cell, has advanced the field of stem cell therapies. As the field has progressed towards pre-clinical transplantation of iPSCs, polarizing views of the tumorigenic potential of undifferentiated iPSCs has left many researchers believing that there is no future in the clinical utility of transplanting undifferentiated iPSCs. The potential for insertional mutagenesis and the integration of oncogenes in iPSCs, as well as the teratoma assay in nude mice, has fueled the rationale for one side of the argument, while some iPSC transplantation studies into healthy, immunocompetent and animals have provided evidence that clinical utility is possible. This brief review highlights the perspectives of both sides of the debate while providing representative examples of iPSCs studies, as well as possible safeguards against iPSC-induced tumor formation.

Keywords: Induced pluripotent stem cells; Teratoma; Undifferentiated

Inducing Pluripotency

Induced pluripotent stem cells (iPSC) have gained enormous notoriety and are pushing the scientific and medical frontiers to new levels of recognition, as indicated by the 2012 Nobel Prize for Physiology or Medicine. However, a critical question concerning the clinical utility of transplanting these cells to treat a variety of medical conditions remains unresolved. The advantages of using iPSCs for treating such disorders is that these cells have the capability of differentiating into any type of cell, with the distinct advantage over using embryonic cells in that iPSCs can be generated from the patient's own cells, thus reducing the chances of rejection. Human somatic cells can be reprogrammed into iPSCs by ectopic expression of four transcription factors (OCT4, KLF4, SOX2, and c-MYC; "OKSM"). Originally the method of reprogramming these cells was by utilizing retrovirus-mediated genomic integration [1,2]. However, there now exists several reprogramming gene combinations and a multitude of methods for generating iPSCs [3]. Recently, two publications in Nature described the reprogramming of somatic cells, without the introduction of reprogramming genes, by stressing the cells through transient pH changes in the cell culture media [4,5]. However these findings have proven difficult to replicate. Genome activation (or re-activation) can also be obtained by somatic nuclear cell transfer at least in mouse, demonstrated by Egli and colleagues [6].

Tumorigenesis

Although the potential application of iPSCs for human transplantation is the focus of several ongoing research projects, there exists a plethora of safety concerns surrounding the clinical application of iPSCs technology, especially considering that virally integrated DNA possesses a high risk of insertional mutagenesis. Furthermore, two of the reprogramming genes, KLF4 and c-MYC, are, themselves, potent oncogenes. Hence, the possible development of tumors after transplantation of iPSCs clearly exists. Teratocarcinoma and teratoma (defined as benign germ cell tumors) are the most common type of tumors which are derived from various populations of iPSCs. In fact, the gold standard for describing the pluripotency of an iPSC line is the demonstration of their ability to form tissues of all three germ layers, via the formation of teratomas in severe combined immunodeficiency (SCID) mice.

Clinical Safety

Concerns surrounding the safety of iPSC transplantation have created two distinct camps of researchers with polarizing views on the ultimate safety of using undifferentiated iPSCs for human transplantation. This controversy has persisted with the advent of pre-clinical trials of iPSC transplants for treating a variety of diseases, even when such studies did not show evidence of tumor formations in the transplanted animals. An example of this came in 2012 with a rather strong rebuke of the potential clinical application of iPSCs, following the publication by Yang and colleagues, which indicated that intravenous injections of iPSCs reduced endotoxin-induced acute lung injury in mice, without evidence of teratoma formation [7]. The claim that these transplants did not form tumors was quickly challenged [8], and it was soon apparent that several researchers seriously doubted that transplantation of undifferentiated iPSCs was even possible without observing the formation of tumors following transplantation. This response was heightened by statements from Okita and colleagues who questioned the rationale of Zhao and colleagues who compared immunogenicity of undifferentiated iPSCs against embryonic stem cells [9,10]. In their commentary on this latter study, Okita and colleagues

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Authors	iPSC Source	Vector	Genes	Host	Transplantation Organ	Transplantation Timeline	Teratomas
Nelson et al., [11]	mice	lentivirus	OKSM	mice	intramyocardial	8 weeks	NO
Kawai et al., [12]	mice	retrovirus	OKSM	mice	striatum and cortex	2,4 weeks	YES
Yamashita et al., [13]	mice	retrovirus	OKSM	mice	striatum and cortex	2 weeks	YES
			OKSM			4 weeks	YES
	mice	adenovirus	OKSM	mice	striatum and cortex	4 weeks	YES
Yang et al., [7]	mice	retrovirus	OKSM	mice	IV	13 weeks	NO
Zhang et al., [14]	rat	lentivirus	OKSM	rat	intramyocardial	2, 4, 6 weeks	NO in 80.6%
Fink et al., [15]	rat	adenovirus	OKSM	rat	striatum	13 weeks	NO
Durruthy Durruthy et al., [16]	human	lentivirus	OKSM	mice	seminiferous tubules	8 weeks	YES
	human	lentivirus mRNA	OKSM-V	mice	seminiferous tubules	8 weeks	NO

Table 1: Outcomes on the tumor formation after transplantation of undifferentiated iPSc.

indicated that "…undifferentiated iPSCs for transplantation…would never be used for medical applications".

Current Status of Preclinical Research with iPSCs

The position that iPSCs have limited clinical utility is held by many researchers and is supported by several findings that show tumors are readily formed following transplantation of undifferentiated iPSCs in a variety of preclinical studies (Table 1). Nonetheless, it is interesting to note that nearly half of the studies listed in Table 1 did not show obvious signs of tumor formation when undifferentiated iPSCs were transplanted into similar animal models of human diseases.

It is important to note that all the studies listed in Table 1 were conducted on immune-competent animals, rather than SCID or nude mice, which have compromised immune systems. It is difficult to make direct comparisons between the various studies in which the authors reported the occurrence of tumors following transplantation of undifferentiated iPSCs in comparison to those which did not, given the wide variety in the methodologies, including differences in the use of species from which the iPSCs were derived (e.g. human, rat, mice), vectors used (e.g. retrovirus, adenovirus, lentivirus), types of transplants (e.g. allo- or xeno-transplantation), targeted organs, and timelines for transplantation and histological verification. There appears to be no consistent patterns to explain why the presences of tumors following transplantation were observed in some of studies and not in others. Although it is possible that differences in sensitivity of screening for tumors may explain some of the discrepancy, it is also possible that there exists protocols, for which their use might lead to minimizing or circumventing the formation of tumors. However, until these can be identified, it appears that the clinical utility of using undifferentiated iPSCs remains an unresolved question, and clearly it would be premature to advocate for their use in clinical trials.

Potential Safeguards

Although use of undifferentiated iPSCs in clinical trials carries to great of a risk, there may be acceptable alternatives for using different forms of iPSCs that may have the potential for clinical utility. One of these alternatives has been described by Miura and colleagues [17,18], and involves the use of what are called 'safe iPSC clones'. Most of these "safe-cell lines"appear to provide some degree of efficacy in pre-clinical trials of spinal cord injury are semi-differentiated (i.e., neither undifferentiated nor fully differentiated) iPSC-derived cells [19,20]. It may also be possible to genetically manipulate cells in 'unsafe' cell lines to make them safer or to remove tumorigenic cells in a population of iPSCs prior to transplantation, in a manner similar to what has already been done with embryonic cells [21]. Possible options that could be used to decrease the chance of iPSC-induced teratoma formations include the use of suicide genes to safeguard the iPSCs [22], which could be activated to control the cell-fate of the stem cells and their derivatives. The tumorigenic tendency of iPSCs could also be inhibited during the cell cycle. Lin and colleagues have demonstrated that microRNA (miR-302) may silence cyclin-dependent kinases, which play a major role into the cell proliferation [23]. The use of pharmacological agents, such metformin, could provide a second option for safeguarding iPSC-induced teratoma formation. Metformin is an antidiabetic drug that appears to be able to activate the metabolic tumor suppressor AMP-activated protein kinase (AMPK), and, thereby, block the metabolic pathways involved in tumor formation, but without interfering with the pluripotency properties of the iPSCs [24]. A critical test for using semi-differentiated stem cells, which were derived from iPSCs, for clinical use is underway in Kobe, Japan [25], where Masayo Takahashi and colleagues at the RIKEN Center for Developmental Biology will be testing the efficacy of autologous iPSC-derived epithelial cells that will be used to repair the damaged retinal pigment epithelium of patients with macular degeneration.

Conclusion

In conclusion, a new era of clinical use of iPSCs has started. Clearly, it is premature to test undifferentiated iPSCs in the clinic at this point in time, but semi-differentiated cells derived from iPSCs may prove to be efficacious, while reducing the chances of iPSC-induced tumor formation. However, the question of how efficacious these semi-differentiated cells prove to be is of particular interest. Finding the delicate balance of semi-differentiated cells, derived from iPSCs, without losing their pluripotent properties (and presumably their full therapeutic efficacy), while mitigating the possibility of tumor formation may remain a focus of research in the near future. With the proliferation of new safeguarding tools and the seemingly constant evolution of exciting new applications in regenerative medicine, it is highly likely that a safe and effective means of using iPSCs, in some form, will eventually be developed, and more effective cellular therapies for a variety of diseases will emerge.

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