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Emerging innovation towards safety in the clinical application of ESCs and iPSCs

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The Review by Behfar and colleagues (Cell therapy for cardiac repair—lessons from clinical trials. *Nat. Rev. Cardiol.* **11**, 232–246; 2014)[1] summarized that 'first-generation' cell therapies for heart failure[2] using autologous cells are safe for use in humans. Conversely, 'next-generation' cell therapies, which include pluripotent stem cells such as embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs), have major safety concerns, because contamination of undifferentiated cells might lead to teratoma formation.[3] However, novel and efficient protocols for selective shutdown of tumour formation in these cells have been reported in several studies, which merit discussion (Table 1).

Firstly, chemical inhibitors of survivin potently induce selective and complete cell death of undifferentiated human ESCs or iPSCs.[4,5] A single pretreatment exposure to survivin inhibitors is sufficient to completely inhibit teratoma formation after transplantation.[4] Importantly, differentiated cells derived from human ESCs or iPSCs maintain their functionality after treatment with survivin inhibitors.[4] The survivin inhibitor QC has been widely used as nutritional supplement and no adverse effects have been reported.[4] Secondly, chemical inhibitors of oleate synthesis have been identified as compounds for selective elimination of human ESCs or iPSCs.[6,7] Oleate synthesis inhibitors lead to apoptosis in human ESCs or iPSCs through lipid metabolism, revealing a dependence of these cells on oleate. At present, application of oleate synthesis inhibitors is limited to *in vitro* culture before transplantation; whether these inhibitors might be applied *in vivo* remains to be determined. Thirdly, the diabetes mellitus drug metformin[8] can reduce tumour forming potential of iPSCs without affecting pluripotency;[9] however, in this study only mouse iPSCs were investigated. Metformin, an agonist of AMP-activated protein kinase, suppresses the expression of Oct4 and survivin thereby showing previously unrecognized stem-cell toxicity.[10] Finally, an antibody against stage-specific embryonic antigen-5 (a newly identified PSC-specific surface antigen) can be used to remove undifferentiated cells by fluorescence-activated cell sorting.[11] However, because this method depends on cell sorting, which includes *ex vivo* manipulation (such as single-cell dissociation and cell-staining techniques), cells might lose viability. New synthesized small molecules (such as JC011), which selectively induce a cytotoxic endoplasmic reticulum stress response in ESCs and iPSCs, have also been reported, but further studies should reveal the precise mechanisms of this pathway.[12]

We believe that two issues relating to the use of ESC or iPSC therapies need to be addressed. After treating cells with chemical inhibitors to prevent teratoma, these cells should be tested to ensure that they have maintained functional properties, including differentiation capacity[13] and engraftment potential. Efficiency, as well as safety, is required for ideal cell transplantation. A second problem is that malignant cell transformation, other than teratoma formation, after transplantation of PSC-derived cells might also exist. Pluripotent tumour forming potential can be divided into two categories: malignant transformation of differentiated PSCs, and benign teratoma formation from residual undifferentiated PSCs.[14,15] The former should be also investigated for safety. For example, CD30, which is a biomarker for transformed human ESCs, is correlated with karyotype abnormalities such as partial duplication of chromosome.[16] Further elucidation of this issue is needed before a judgement on iPSC clinical safety can be made.

Table 1 | Novel strategies for selective elimination of teratoma

Reference	Chemical or antibody	Mode of action	Drug
Lee *et al.*[4]	Chemical inhibitor	Survivin inhibition	QC; YM155
Ben-David *et al.*[6]	Chemical inhibitor	Oleate synthesis inhibition	PluriSIn #1
Vazquez-Martin *et al.*[9]	Chemical inhibitor	AMP-activated protein kinase activation	Metformin
Tang *et al.*[11]	Antibody	SSEA-5 purging	Anti-SSEA-5 monoclonal antibody
Richards *et al.*[12]	Chemical	Endoplasmic reticulum stress	JC011

Abbreviations: PluriSIn, pluripotent cell-specific inhibitor; SSEA-5, stage-specific embryonic antigen-5.

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Regulating ES or Induced Pluripotent Stem Cells by Innate Lymphoid Cells

To The Editor:

Natural killer (NK) cells are innate lymphocytes that are able to be cytotoxic via perforin and granzyme B to cells with low expression of major histocompatibility complex class I molecules. ILC1s lack perforin and granzyme B. It would be meaningful if some ILCs might have a novel role in immunity during allogeneic transplantation, such as rejecting ES cells. Interestingly, it has been reported that RORγt-NKR-LTi cells express perforin and granzyme B, leading to cytotoxicity (*5*). Regarding specificity of antibodies, for example, anti–asialo-GM-1, which is well known to be capable of depleting NK cell subsets, has been revealed to also deplete basophils as off-target effect (*6*). It would be essential to understand expression patterns exactly.

In a study by Perez-Cunningham et al. (*1*), the authors demonstrate that a subset of NK cells is responsible for embryonic stem cell (ESC) rejection; they show that, after injecting anti–asialo-GM-1 or anti-Ly49G2 into mice, ESC rejection rate was remarkably decreased, suggesting that a subset of Ly49G2$^+$ NK cells would play a crucial role in killing ESCs (*1*).

Recently, innate lymphoid cells (ILCs) are emerging as novel modulators of innate immunity, enabling early immune responses (*2*). Actually, conventional NK cell is a member of ILCs. It has been proposed that ILCs should be classified into three distinct groups based on functional characteristics and cytokines that they can produce. Group 1 ILCs are defined by production of Th1 cell-associated cytokine interferon-γ, and include NK cells and ILC1s. Group 2 ILCs produce Th2 cell-associated cytokines (interleukin [IL]-5 and IL-13), and include ILC2s. Group 3 ILCs secrete IL-17 and/or IL-22, and include lymphoid tissue inducer (LTi) cells and natural cytotoxicity receptor (NCR)$^+$ ILC3s (*2*).

Are there any cells that express NK cell receptors other than conventional NK cells? Recent evidence suggests that an NK cell receptor-expressing innate lymphocyte subset has been identified as intraepithelial ILC1-like cells (*3*). Moreover, NCR+ ILC3s could be converted to ILC1s under the influence of IL-12 (ref. *4*). These cells are expressing NK cell receptors, but their functions remain poorly understood.

In the present study by Perez-Cunningham et al. (*1*), it would be critical to explore whether not only conventional NK cells but also ILC1s (if expressing NK cell receptors) can be depleted by treatment with anti-Ly49G2; whether a subset of mouse ILC1s express Ly49G2 NK receptor would be interesting. One hypothesis is that interferon-γ–secreting ILC1s would also have a pivotal role in regulating immune responses in transplantation, although

When we transplant human ES-derived or induced pluripotent stem (iPS)-derived cells into patients in allogeneic settings in clinical trials, we will, under treatment with immunosuppressants, use differentiated cells (expressing major histocompatibility complex class I molecules) but not undifferentiated cells. Indeed, recent studies have shown that terminally differentiated cells derived from ES or iPS cells elicit negligible immune rejection in their host, although recipients are syngeneic (*7–9*). Therefore, condition in the present study (*1*), where undifferentiated cells were used, would be quite different from that of clinical settings. However, in view of removal of undifferentiated cells, we can apply a strategy of regulating immune responses as shown in the present study (*1*). Collectively, it could be rational hypothesis to modulate ILC function in transplantation immunity, thereby providing principle of concept.

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