New trends in cellular therapy

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ABSTRACT

Regenerative therapies, including both gene and cellular therapies, aim to induce regeneration of cells, tissues and organs and restore their functions. In this short Spotlight, we summarize the latest advances in cellular therapies using pluripotent stem cells (PSCs), highlighting the current status of clinical trials using induced (i)PSC-derived cells. We also discuss the different issues and other challenges in iPSC-based cell therapy.

KEY WORDS: Cardiomyopathy, Diabetes, Parkinson’s disease, Pluripotent stem cells, Regenerative therapy

Introduction

Cellular regenerative therapies seek to use cells transplanted into a patient to restore the impaired functions of living tissues and organs. There are high hopes that cellular therapies, particularly stem cell-based therapies, may provide a fundamental treatment option for conditions that cannot yet be treated with conventional drugs. The sources for cellular therapy include tissue stem cells (of adult or fetal origin) and pluripotent stem cells (PSCs), including both embryonic stem cells (ESCs) and induced pluripotent stem cells (iPSCs). We are now at a point where therapies using PSCs are becoming a reality: evidenced by the increasing number of applications for human clinical trials submitted to the Food and Drug Administration (FDA) each year. In fact, a total of 636 clinical trials (Phase I: 242; Phase II: 348; Phase III: 46) were under way worldwide as of the end of the third quarter of 2019: these can be classified as gene-modified trials of stem cell-based cellular therapy. In this article, we aim to describe the current status and future directions of planned/ongoing clinical trials using induced (i)PSC-based cell therapy.

Principles of PSC-based cellular therapy

PSC-based cellular therapy is increasingly being applied for various disorders, such as diabetes, age-related macular degeneration (AMD), Parkinson’s disease (PD), myocardial infarction, corneal epithelial dysfunction and spinal cord injuries (SCI) (summarized in Table 1). Before discussing specific cases, we first outline key principles to consider when developing cellular therapies from PSCs, and discuss the issues involved in choosing an appropriate cell source from which to derive these products.

ESCs versus iPSCs

Unlike ESCs that are established from the blastocyst inner cell mass, iPSCs are artificially generated from somatic cells through the transduction of reprogramming factors. Reprogramming can be incomplete, and partially reprogrammed iPSCs tend to exhibit high teratoma-forming propensities due to incomplete suppression of the reprogramming transgenes (Miura et al., 2009; Okano et al., 2013) and biased differentiation propensities due to the epigenetic memory of the somatic cells of the origin (Kim et al., 2010, 2011). These are potential disadvantages of iPSCs compared with ESCs in terms of clinical application. However, significant progress in reprogramming methods means that the quality of iPSCs is improving dramatically (Jacobson and Tzankakis, 2018; Umekage et al., 2019; Doi et al., 2020) and there are several advantages of iPSCs for clinical use, including the potential to avoid an immune response (discussed later).

Differentiating PSCs

For effective use of human PSCs in cell replacement therapy, drug development and disease modeling, it is essential to develop reproducible and robust protocols for differentiation into desired cell types (Okano and Yamanaka, 2014; Fujimori et al., 2017). One approach to achieve this is stepwise directed differentiation of PSCs following developmental principles. Although this has been very successful, a limitation of this method is that the cells obtained can be immature compared with their in vivo counterparts, making it difficult to model late-onset aging-related diseases. An alternative approach is direct reprogramming of somatic cells to other cell-types, avoiding the iPSC-stage (Vicente et al., 2018). Alternatively, introduction of particular transcriptional factors and microRNAs can be used to induce PSCs into specific cell types (Zhang et al., 2013; Ishikawa et al., 2020). Furthermore, organoid-based approaches can be used to induce PSC differentiation into more mature cells in a three-dimensional tissue environment (McCauley and Wells, 2017).

Which kind of cell products (single cells versus aggregates versus organized tissue) should be used for cell replacement therapy would differ depending on the context of the disease. For example, suspensions of single cells of purified dopamine neurons would be most appropriate for Parkinson disease (Kikuchi et al., 2017). On the other hand, aggregates of neural stem/progenitor cells (neurospheres) might be more suitable for transplant into a site of spinal cord injury (Tsuji et al., 2019). Of note, trials in non-human primates have suggested that iPSC-derived highly organized retinal tissue could be transplanted to treat, for example, patients with retinitis pigmentosa (Tu et al., 2019).

Escaping the immune system

One key issue with using PSCs for cellular therapies is the patient immune response: unless the therapy is derived from the patient’s own cells, there is a risk that the transplanted cells will be rejected because of incompatibility between human leukocyte antigen (HLA) types. iPSCs used for clinical trials can be classified into
autologous iPSCs (derived from patients’ own somatic cells), universal iPSCs (generated by genetic manipulation of the HLA genes) (Suzuki et al., 2020), HLA-matched allogeneic iPSCs [established from donors that are homozygous at three HLA loci (HLA-A, HLA-B, and HLA-DR)] (Umekage et al., 2019) and HLA-matched allogeneic universal iPSCs (generated by genetic manipulation of the HLA genes) (Suzuki et al., 2020), HLA-matched allogeneic iPSCs

**Autologous iPSCs**

Autologous iPSCs are likely to be the safest option, as they should not trigger an immune response. For example, a patient’s own iPSC-derived retinal pigment epithelial (RPE) cells were transplanted into the retina of a patient with AMD, with a successful outcome in the first trial (Mandai et al., 2017a). However, this in-human trial cost nearly ¥100m (~US$1m) for a single patient. On the other hand, the cost and effort needed for autologous transplantation might decrease in the future with further technological developments. Recently, midbrain dopaminergic progenitor cells derived from autologous iPSCs were transplanted into a patient with idiopathic Parkinson’s disease (Schweitzer et al., 2020).

**Universal iPSCs with HLA engineering**

To avoid an allogeneic immune response from the host, universal PSCs can be generated from both iPSCs and ESCs by genetic manipulation of the HLA genes (Suzuki et al., 2020). For example, it has been shown that HLA class I-negative cells, in which expression of all HLA-A, B, C and E antigens is depleted, can be obtained by targeted disruption of both alleles of β2-microglobulin (β2M – part of the HLA-I complex) (Riolobos et al., 2013). These HLA-I-negative cells can still be attacked by human natural killer (NK) cells after transplantation (Lanier, 2008; Vivier et al., 2011; Long et al., 2013). Various strategies are available to avoid this, including overexpression of single-chain HLA-E fused with B2M (Gornalusse et al., 2017), but it is too early to conclude which method will prove best to eliminate NK cell attack on these HLA-I-negative cells.

However, grafting cells that are not recognized by the host immune cells carries a risk in terms of potential tumor development. Development of a fail-safe-system, which aims to kill or suppress the proliferation of the tumorigenic cells, could be effective in overcoming this pitfall (Itakura et al., 2017; Kojima et al., 2019; Liang et al., 2018).

HLA-matched allogeneic iPSCs

It has been expected that grafting of HLA-matched allogeneic iPSCs established from donors homozygous at three HLA loci (HLA-A, HLA-B and HLA-DR) could reduce the immune response (http://hla.or.jp/med/frequency_search/en/haplo/; Okita et al., 2011; Gourraud et al., 2012; Umekage et al., 2019). The Center for iPSC Cell Research and Application (CiRA), Kyoto University, Japan, has released clinical-grade iPSCs from its stock, including three lines from peripheral blood mononuclear cells (PBMCs) with first-, second- and third-ranked HLA haplotypes, and two lines from cord blood with first-ranked HLA haplotypes (Umekage et al., 2019).

**Table 1. iPSCs that have begun to be used/are expected to be used in clinical trials**

<table>
<thead>
<tr>
<th>Disease</th>
<th>Condition</th>
<th>Cell therapy product (iPSC-derived)</th>
<th>Current status</th>
<th>Lab/company</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>AMD</td>
<td>Wet type</td>
<td>Autologous RPE cells</td>
<td>Clinical trial (ongoing)</td>
<td>Masayo Takahashi lab</td>
<td>Mandai et al., 2017a</td>
</tr>
<tr>
<td>AMD</td>
<td>Wet type</td>
<td>HLA-matched allogeneic RPE cells</td>
<td>Clinical trial (protocol approved)</td>
<td>Masayo Takahashi lab</td>
<td>Sugita et al., 2020</td>
</tr>
<tr>
<td>AMD</td>
<td>Dry type</td>
<td>Autologous RPE cells</td>
<td>Clinical trial (ongoing)</td>
<td>Bharti lab</td>
<td>Mandai et al., 2017b</td>
</tr>
<tr>
<td>Cornea disease</td>
<td>Epithelium damage</td>
<td>Allogeneic cornea epithelium</td>
<td>Clinical trial (ongoing)</td>
<td>Nishida lab</td>
<td>Hayashi et al., 2018</td>
</tr>
<tr>
<td>Cornea disease</td>
<td>Endothelium damage</td>
<td>Allogeneic cornea endothelium</td>
<td>Preclinical</td>
<td>Shimamura lab</td>
<td>Hatou and Shimamura, 2019</td>
</tr>
<tr>
<td>Retinitis pigmentosa</td>
<td>Familial retinitis pigmentosa</td>
<td>Allogeneic retinal organoids</td>
<td>Clinical trial (protocol approved)</td>
<td>Masayo Takahashi lab</td>
<td>Mandai et al., 2017b; Tu et al., 2019</td>
</tr>
<tr>
<td>PD</td>
<td>Sporadic</td>
<td>Allogeneic dopamine neurons</td>
<td>Clinical trial (ongoing)</td>
<td>Jun Takahashi lab</td>
<td>Kikuchi et al., 2017</td>
</tr>
<tr>
<td>SCI</td>
<td>Subacute</td>
<td>Allogeneic NS/PCs</td>
<td>Clinical trial (protocol approved)</td>
<td>Okano and Nakamura lab</td>
<td>Tsui et al., 2019</td>
</tr>
<tr>
<td>Aplastic anemia</td>
<td>(Transfusion) refractoriness</td>
<td>Autologous platelets</td>
<td>Clinical trial (ongoing)</td>
<td>Eto lab</td>
<td>Ito et al., 2018</td>
</tr>
<tr>
<td>Heart failure</td>
<td>Myocardial infarction</td>
<td>Autologous myocardial sheet</td>
<td>Clinical trial (ongoing)</td>
<td>Sawa lab</td>
<td>Kashiyama et al., 2019</td>
</tr>
<tr>
<td>Heart failure</td>
<td>Dilated cardiomyopathy</td>
<td>Autologous cardiomy-place</td>
<td>Clinical trial (protocol approved)</td>
<td>Fukuda and Shimizu lab</td>
<td>Kishino et al., 2020</td>
</tr>
<tr>
<td>GVHD</td>
<td>Steroid resistant GVHD</td>
<td>Allogeneic MSCs (Cynerus™)</td>
<td>Clinical trial (ongoing)</td>
<td>Cynatha Inc</td>
<td>Ozay et al., 2019</td>
</tr>
<tr>
<td>Fatal hepatic diseases</td>
<td>Metabolic liver disorder</td>
<td>Allogeneic liver buds</td>
<td>Preclinical</td>
<td>Taniguchi and Takebe lab</td>
<td>Takebe et al., 2013; Koike et al., 2019</td>
</tr>
<tr>
<td>Articular cartilage injury</td>
<td>Articular cartilage injury</td>
<td>Allogeneic cartilage cells</td>
<td>Clinical trial (protocol approved)</td>
<td>Tsumaki lab</td>
<td>Yamashita et al., 2018</td>
</tr>
</tbody>
</table>

AMD, age-related macular degeneration; GVHD, graft versus host disease; MSCs, mesenchymal stem cells; NS/PCs, neural stem/progenitor cells; PD, Parkinson’s disease; RPE, retinal pigment epithelial; SCI, spinal cord injury.
Together, these lines cover the HLA types of 32% of the Japanese population. However, it is still not clear how efficiently HLA-matched allogeneic iPSCs can prevent immunorejection in the absence of immunosuppressants – and this is likely to be context-dependent. In the case of transplantation of dopamine neurons in unlesioned brains of non-human primates, it was shown that major histocompatibility complex (MHC)-matching could improve the engraftment of iPSC-derived neurons (Kikuchi et al., 2017). Notably, however, a recent report showed that MHC-matching failed to prevent long-term rejection of iPSC-derived neurons in the lesioned brain of non-human primates (Aron Badin et al., 2019). Recently, an allogeneic cell suspension of iPSC-RPE derived from a super-donor carrying the most common HLA haplotype in Japan, which matched the HLA type of recipients, was transplanted into five patients with AMD without using immunosuppressive medications. Notably, they found that grafted cells stably remained during the 1 year observation period, although mild immune rejections were observed in one patient (Sugita et al., 2020). However, as described above, it remains to be elucidated whether HLA matching is sufficient to prevent long-term immune rejections in humans in other contexts.

**HLA non-matched allogeneic iPSCs**

Although HLA matching or engineering has clear advantages, for practical reasons most of the recent iPSC-based cellular therapies have been based or are expected to be based on the use of HLA non-matched allogeneic iPSCs. This is a more cost-effective strategy but carries the risk that transplanted cells or tissues may be rejected by the recipient, and/or that recipients will need long-term immunosuppressive therapy, depending on the context.

**Conditions being targeted for cell therapy trials**

iPSC-based cellular therapy was undertaken for the first time by Masayo Takahashi’s group in Japan in 2014 (Mandai et al., 2017a; discussed further below). Table 1 summarizes those clinical trials currently underway (or beginning shortly). In the following sections, we discuss the conditions for which PSC-based cell therapy is looking most promising.

**Type 1 diabetes**

PSC-based cellular therapy has recently been started for type 1 diabetes (T1D) – with the aim of replacing islet cells (particularly insulin-producing β-cells) that are lost in T1D patients. Effective allogeneic islet transplantation (from donors post-mortem) has demonstrated that T1D can be treated by cellular therapy (Lacy and Scharp, 1986). However, the potential of such treatment is restricted by the limited cell supply and variable quality of the cells, and the need for life-long immunosuppression. Generating pancreatic islet cells from PSCs would overcome at least some of these issues, and great progress has been made by mimicking the natural development of the human pancreas, i.e., stepwise differentiation of PSCs into the endoderm, pancreatic endoderm and endocrine progenitors that give rise to the islets, including β-cells, α-cells and δ-cells (Pagliuca and Melton, 2013; Pagliuca et al., 2014; Vegas et al., 2016; Schulz, 2015).

These stepwise protocols for inducing pancreatic islet cells from human (h) ESCs have been applied for industrial standardization by two biotech companies, Semma Therapeutics (recently acquired by Vertex Pharmaceuticals; https://www.vrtx.com/) and ViaCyte (https://viacyte.com/). Both companies have succeeded in producing large amounts of transplantable islet/β-cells that meet good manufacturing practice (GMP) guidelines. These hESC-derived islet cells were found to be capable of functional insulin secretion upon exposure to high-glucose or high-potassium stimuli (Vegas et al., 2016). Furthermore, both companies have demonstrated rescue of T1D animal models (such as rodents and larger animals) with these cells (Vegas et al., 2016; Schulz, 2015) and have developed immune-protected encapsulated islet/β-cells by immobilizing cells within semi-permeable membrane polymer shells that can provide mechanical protection and immuno-isolation (Vegas et al., 2016; Stock et al., 2020). Such encapsulated islets/β-cells can release insulin to the extracellular space in response to physiological and pharmacological stimuli such as high glucose, high K⁺ conditions, amino acids and others (Vegas et al., 2016). Importantly, this study demonstrated a key proof-of-concept that such devices protected PSC-derived islets in immunocompetent pigs and released human C-peptide (insulin gene product) in mouse blood.

With the pre-clinical work having shown positive results, clinical trials are beginning, in the hope of a cure for T1D. ViaCyte is currently conducting a clinical Phase I/II open-label study of PSC-derived islet replacements for T1D by directly transplanting hESC-derived pancreatic precursor cells (PEC-01 cells) directly (without encapsulation) under the skin (Cooper-Jones and Ford, 2017).

**Parkinson’s disease**

PD is characterized by the degeneration of midbrain dopaminergic neurons. In 1987, a group at Lund University first transplanted human fetal ventral mesencephalic (hVM) cells derived from aborted fetuses, enriched in dopamine neurons, into the striatum of a patient with PD (Lindvall et al., 1989). Such grafts were shown to survive for a decade and to release dopamine, contributing to functional recovery (Piccini et al., 1999) and providing the proof-of-concept for dopamine cell replacement therapy. However, this approach suffers from a deficiency of donor cells as well as ethical controversies. There has therefore been increasing interest in generating dopaminergic neurons from hPSCs for clinical translation.

hPSCs can be induced to differentiate into dopaminergic neurons with ventral midbrain identity using recently developed protocols (Barker et al., 2017; Kirkeby et al., 2012, Kriks et al., 2011; Doi et al., 2014, 2020). An international consortium of groups working on PD therapy using hPSC-derived dopaminergic neurons, including European (EUROPEAN STEM-PD, using ESCs), American (NYSTEM-PD, using ESCs), and Japanese (CiRA trials using allogeneic iPSCs) efforts, have started a new initiative, the GForce-PD (http://www.gforce-pd.com), for facilitating worldwide clinical translation (Barker et al., 2017). As part of this initiative, a clinical trial started in Japan in 2018 using allogeneic hiPSCs-derived dopaminergic neurons precursor cells for PD patients (UMIN000033564, JMA-IIA00384). Clinical trials of PSC-based cellular therapy for PD are also underway in Austria (NCT02452723) and China (NCT03119636, ChineseASZQ-003).

**Macular degeneration**

In 2014, Masayo Takahashi’s group in Japan performed the first clinical study involving an iPSC-derived product, using a sheet of autologous iPSC-derived retinal pigment epithelial (RPE) cells in a patient suffering from wet-type AMD. The grafting iPSC-RPE sheet remained intact with no sign of immune rejection during the 1 year monitoring period following transplantation (Mandai et al., 2017a). As discussed above, the same group has transplanted cell suspension of iPSC-RPE derived from an HLA-matched super-donor into five patients. The grafted cell location was not
sufficiently controlled to access the efficacy of this therapeutic approach, and further optimization of the surgical procedure for the human iPSC-RPE cells transplantation will be necessary in future studies (Sugita et al., 2020).

Kapil Bharti’s group at the National Eye Institute (NEI) of the National Institutes of Health in the USA is planning a clinical trial of transplantation of autologous iPSC-derived RPE cells for patients with dry-type AMD. Their clinical protocol was approved and a Phase I/IIa clinical study, aimed at confirming safety, will begin soon with 12 patients with advanced-stage geographic atrophy (https://www.nih.gov/news-events/news-releases/nih-launches-first-us-clinical-trial-patient-derived-stem-cell-therapy-replace-dying-cells-retina).

Cardiovascular disease
The first transplant of hESC-derived cardiac progenitors was performed for treatment of severe heart failure treatment by Menasché et al. (2015), with no adverse effects over a 3-month follow-up period. Following pre-clinical work confirming the safety and effectiveness of iPSC-derived cardiomyocyte sheets in animal experiments (Miyagawa and Sawa, 2018; Ishida et al., 2019), a team led by Yoshiki Sawa at Osaka University performed a world-first transplant of iPSC-derived cellular therapy for heart disease, using HLA-non matched iPSC-derived cardiomyocytes in the form of cardiomyocyte sheets (https://www.bionews.org.uk/page_147579).

This followed earlier work from the same group in which myoblasts taken from a patient’s skeletal muscle in the leg were used to prepare cell sheets that were placed on the surface of a heart in a patient suffering from dilated cardiomyopathy to restore cardiac function (Sawa et al., 2012). However, the therapeutic effect of this treatment was limited, as it was not effective in patients with severe disease. PSC-derived cardiomyocyte sheets are expected to be better suited for therapy for heart failure (Ishida et al., 2019) and it is hoped that such sheets could resolve the donor shortage common for heart therapies.

Spinal cord injury
Following SCI, ischemia induces secondary damage processes, leading to axonal degeneration and death of both neuronal and glial cells (Nagoshi and Okano, 2018), and consequently to dysfunction of motor and sensory systems. It is hoped that cell replacement therapy could be an effective treatment.

The first clinical trial for PSC-based cell replacement therapy for SCI, by Geron Corporation (now Asterias Biotherapeutics) in 2009, used hESC-derived oligodendrocyte progenitors (OPCs). A dose of two million cells was transplanted into five patients in the initial Geron’s Phase I trial, and has been followed up by the recent Asterias dose-escalation Phase I/IIa study (SCIstar study) using 10-20 million cells, with mild immunosuppression (reviewed by Irion et al., 2017).

Our own group (H.O.) is planning to transplant allogeneic iPSC-derived neural stem/progenitor cells (NS/PCs) into patients with complete SCI in the subacute phase (Tsui et al., 2019; Nagoshi et al., 2019). NS/PCs can be obtained from iPSCs by stepwise induction of neuralized embryoid bodies through inhibiting TGFβ and BMP pathways and a subsequent selective expansion of NS/PCs using a neurosphere method (Nori et al., 2011). Pre-clinical studies have shown that such NS/PCs could induce long-term functional recovery of SCI model animals without tumor formation (Nori et al., 2011; Okano et al., 2013). It is likely that these cell replacement effects contribute to the graft-mediated functional recovery of SCI animals (Okano et al., 2013; Okano and Yamanaka, 2014).

The primary objective of the planned clinical study, which has been approved (Cyranoski, 2019), is to evaluate the safety and effectiveness of the transplanted allogeneic iPSC-derived NS/PCs and of transplantation methods (Tsui et al., 2019; Nagoshi and Okano, 2018). To this end, two million clinical grade iPSC-derived NS/PCs will be transplanted into each of four SCI patients with complete motor and sensory paralysis. Surgery will be followed with immunosuppressants and rehabilitation treatment, and patients will be followed closely for 1 year.

Safety issues in iPSC-based cell therapy
The potential risks of cellular therapy would generally include malignant transformation of the cells, migration of the cells to non-target sites, induction of immune responses against the treatment, and lymphoid mediated graft-versus-host disease. Although safety issues will differ depending on the context, the following quality checks should generally be performed (Nagoshi and Okano, 2018; Doi et al., 2020; Mandai et al., 2017a). (1) Marker expression by flow cytometry and transcriptome analysis to examine the expression of remaining pluripotency markers (TRA-1-60, TRA-2-49, SSEA-4, Oct4, Nanog and Lin28) and lineage- or cell type-specific markers. Ideally, gene-expression profiling, including single cell RNA sequencing, would be used to examine the maturation status and purity/heterogeneity of the cell populations to be transplanted (Mandai et al., 2017a). (2) Genomic and epigenome analysis for copy number variations and single nucleotide variations through exome sequencing or whole-genome sequencing to exclude the presence of cancer driver mutations (Mandai et al., 2017a) including p53 (Aivor et al., 2019), and to assess DNA methylation pattern (particularly focusing on hypermethylation of tumor-suppressor genes). (3) General cellular profiles: karyotype, cell cycle and cell growth analysis. (4) Other cellular profiles specific to the cells to be transplanted e.g. differentiation capability into neurons and glia in the case of NS/PCs. (5) The possibility of microbial contamination through assays for e.g. endotoxins, Mycoplasma and particular viruses. Furthermore, tumorigenicity should be assessed in vivo: the final cell products should be transplanted into orthologous sites of immunodeficient animals and followed long-term (at least 6 months).

It should be noted, however, that although pre-clinical testing can help to prevent such adverse effects, the safety of cellular products is still hard to demonstrate definitively before clinical trials, given the difference of in vivo microenvironment between in vitro culture or animal models and the human diseases.

Conclusion and future perspectives
Recent years have seen an increasing number of potential clinical applications of cellular therapy for various diseases using stem cells. PSCs show huge potential for such therapies as they can, in principle, be differentiated to a wide range of cell types through the recapitulation of normal developmental process in a dish. The huge accumulation of knowledge over recent decades on the molecular and cellular mechanisms involved in normal development has facilitated these innovations required for PSC-based cellular therapy.

We are now at a stage where PSC-based therapies are in clinical trials, but it is still early days for these therapies, with safety and effectiveness remaining key concerns. As discussed above, a small number of diseases are leading the way with these clinical trials, but PSC-based therapies hold promise for a range of other conditions, including retinitis pigmentosa (by grafting iPSC-derived retinal tissues; Mandai et al., 2017b; Tu et al., 2019), fatal hepatic diseases.
such as metabolic liver disorder (Koike et al., 2019; Takebe et al., 2013) and solid tumors (by grafting cancer-targeting cytotoxic lymphocytes including invariant natural killer T cells; Yamada et al., 2016; Minagawa et al., 2018; Ueda et al., 2020). To proceed with these efforts in a safe and responsible manner, a deep understanding of the developmental mechanisms of cellular differentiation, multicellular cellular interactions, tissue homeostasis and repair would be extremely important in each case.

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Competing interests
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