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HARNESSING STEM CELLS AND DENDRITIC CELLS FOR NOVEL THERAPIES

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Abstract: This review presents the latest achievements in basic studies on stem-cell biology and on approaches aimed to design therapies based on stem cells and dendritic cells. Studies on stem-cell homeostasis are aimed to delineate the accessibility of these cells for therapeutic purposes. Hematopoietic stem cell transplantation has become a routine application of stem cells in the treatment of patients with cancer and with hematologic disorders. Perspectives of application of the mesenchymal stem cells for regenerative medicine and for tolerance induction following allogeneic transplantation are being extensively explored. Reprogramming of adult somatic cells to an undifferentiated pluripotent state *in vitro* by a transduction with just four genes encoding transcription factors opened the way for the generation of patient-specific pluripotent stem cells. Such induced pluripotent stem cells hold a great promise for replacement therapies of various so far incurable disorders. However, because of the tumorigenic potential of the retroviral vector transduction-induced pluripotent stem cells, before these cells become useful for clinical application, appropriate, safe methods of stem cell generation, selection, proliferation and differentiation need to be elaborated. There is a great potential for further developments of cancer immunotherapies and for controlling of post-transplantational reactions, if current basic studies on stem cells and on immunostimulatory and tolerogenic dendritic cells would be successfully translated to the clinic.

Keywords: stem cell, hematopoietic stem cell, mesenchymal stem cell, induced pluripotent stem cell, dendritic cell

Considerable progress has been recently achieved in basic studies on embryonic and somatic stem cell biology. There is a growing interest in designing new stem cell-based therapeutic strategies and in potential improvements in the established therapies that have already become part of mainstream medicine, and save lives on a regular basis. So far, the most advanced application of stem cells is hematopoietic stem-cell transplantation in cancer patients and in patients with nonmalignant hematologic disorders. Other applications, such as treatment of neurodegenerative disorders or diabetes, are still the distant goals, but ones that seem increasingly realistic as understanding of stem cell biology grows. Generally, stem cells hold great promise for the regenerative medicine. However, many technical problems as well as ethical and safety issues have to be addressed before accessibility of transplantable cells for replacement therapies will at least in part meet expectations inflated by coverage in the mainstream media. Combined cellular therapies, aimed to increase effectiveness of cancer treatment, are currently being elaborated, and various populations of stem cells and defined populations of cells propagated and differentiated *ex vivo* are being employed.

Stem-cell homeostasis

Stem cells are defined as undifferentiated cells dividing for indefinite periods with the capacity to self-renew and the ability to generate a functional progeny of specialized cell types. Pluripotent embryonic stem (ES) cells that form the inner cell mass of blastocyst are capable to form all the body's cell lineages. Further specialization results in generation of adult stem cells residing in various somatic tissues. Multipotent adult stem cells are able to form multiple cell lineages that constitute an entire tissue or tissues. Oligopotent stem cells form two or more lineages within a tissue, and their physiological function is to replenish mature cell population of a particular tissue. A hierarchical scheme that combines differentiation with proliferation, induced at subsequent steps of stem cell specialization, allows to compromise between production of large number of differentiated cells and preservation of stem cell reservoirs.

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Stem-cell homeostasis is required to secure the process of tissue renewal and regeneration throughout life, since stem cells are scarcely distributed in various somatic tissues. Sustained asymmetric stem cell divisions provide both cells that remain in dormant state and maintain pluripotency, and cells pushed to expand and differentiate. Differentiation of stem cells is governed by intrinsic signals coming from epigenetic programming and gene expression, and external signals delivered from the surrounding cellular environment, known as the stem cell niche. The external signals delivered following injury or degenerative process, first of all mobilize tissue stem cells, with potency limited to the cells of their respective tissue. Multipotent stem cells at low differentiation stage, including those retained in distant reservoirs in the bone marrow, can also be mobilized to repair the damage by external cues coaxing them back into the cell cycle after a prolonged period of dormancy.

Under normal homeostatic conditions, there is a low demand on dormant multipotent stem cells. Since these cells divide infrequently, they are protected from perils of DNA replication and mutagenesis. However, cell senescence and apoptosis, which relay on telomere shortening and the activities of p53 and $p16^{INK4a}$, may induce a decline of the replicative function of certain stem-cell types with advancing age. In this manner, the mechanisms that control cell proliferation and suppress the development of cancer, may also affect regenerative capacity of tissues and contribute to some aspects of mammalian aging [review in (1)]. Older individuals are more likely to suffer from prolonged immunosuppression in response to conventional cytotoxic chemotherapy. It indicates a reduced marrow regenerative capacity with aging. A response to DNA damage may compromise stem cell function and their persistence in the organism. In this manner, a response to DNA damage is an essential factor that determines aging and the rate of living. Humans and mice with congenital deficiencies of DNA repair and metabolism sometimes exhibit premature stem cell dysfunction and some features of accelerated aging. Likewise, exposure to DNA-damaging agents during radio- and chemotherapy induces a durable degradation of stem cell function.

An intriguing direct link between exhausted regenerative potential and accelerated aging has been demonstrated recently in an elegant study on the forced regeneration in mice (2). Regeneration was forced in mice to reconstitute proliferating tissues after inactivation of developmentally essential DNA-damage response gene *Atr*. In experiments with the use of a conditionally inactivable allele, *Atr* gene was excised in adult mouse tissues through transient somatic activation of a tamoxifen (TAM)-inducible Cre recombinase. The acute somatic *Atr* deletion induced a rapid depletion of proliferating cells in the bone marrow and a marked intestinal atrophy. Since the Cre-mediated excision of the *Atr* gene was not 100% efficient, the animals survived the transient cell loss. Few normal cells that had not recombined the *Atr* allele, quickly replaced the lost cells. The repopulated mice appeared largely normal by 1 month after conditional activation. By 3 months after TAM treatment, the mice developed a progeroid phenotype with osteopenia, hair greying, changing in skin thickness, and loss of lymphoid and hematopoietic progenitors. This marked progeroid phenotype developed in adult mice following the excessive regeneration, indicated a durable compromise of stem-cell function. These results suggest that forced regeneration in response to homeostatic demands can be toxic to stem cells across many tissues, even in the absence of extrinsic DNA-damaging agents. In contrast to other mouse models, age-related phenotype observed in this study was neither influenced by developmental abnormalities nor was a direct result of such abnormalities. In fact, it is also possible that *Atr* excision and subsequent rapid regeneration damaged the niche rather than the cells self-renewing within. Effect of forced regeneration on stem cells exhaustion and on supporting cells in the niche requires further studies.

The stem cell potential for tissue regeneration may be compromised with aging, regardless of theoretical models currently designed to understand long-term stem cell function. Stem cell damage that accumulates with age delineates a rate-of-living and sets the limits for regenerative medicine-based approaches for anti-aging therapy. It implicates that searching for alternative stem cell sources to patient's own cells will be necessary, whenever a regenerative therapy is intended for patients with an exhausted regenerative potential. Another impediment for development of regenerative medicine is a question of collection of adequate tissue sample that would provide a sufficient number of stem cells for therapeutic purposes.

Current clinical applications of system cells

So far, the most advanced application of stem cells in medicine is hematopoietic stem-cell transplantation (HSCT) in cancer patients and in patients with nonmalignant hematologic disorders. At present, there are three accessible sources of hematopoi-

etic stem cells (HSC) for transplantation, i.e.: bone marrow, peripheral blood following stem cell mobilization to periphery by granulocyte-colony stimulation factor (G-CSF) administration, and cord blood. Survey on HSCT activity in 2005 reported by European Group for Blood and Bone Marrow Transplantation listed 24168 first HSCT performed by 597 centres, in both autologous and allogeneic settings (3). Since bone marrow HSC were traditionally collected from the iliac crest under general anesthesia, mobilized peripheral blood HSC have been increasingly used.

HSCT following high-dose chemotherapy (CHT) in cancer patients secures hematologic recovery. CHT is a double edge-sword that eliminates cancer cells but results in hematologic toxicity. Many cancers are thought to arise from rare self-renewing cells (cancer stem cells) that had acquired oncogenic mutations at the stage preceding differentiation. Cancer stem cells, being biologically distinct from their more numerous differentiated progeny, may be resistant to treatments designed to target differentiated cells. As a result, a dramatic response of the bulk of cancer cells for treatment is followed by a re-growth of cancer originating from the cancer stem cell reservoir. High-dose CHT potentially improves targeting a cancer stem cell reservoir, but seriously increases morbidity, due to the delay or lack of hematologic recovery. Infusion of autologous or allogeneic HSC following high-dose CHT, secures hematologic recovery. Autologous HSC are collected for transplantation prior to high-dose CHT, either by leukapheresis following myelosuppressive mobilization CHT and G-CSF treatment or by bone marrow aspiration. The myelosuppressive mobilization CHT is aimed to deplete differentiated leukocytes that control HSC proliferation and migration, whereas G-CSF to stimulate the HSC expansion and efflux from the bone marrow into the vasculature. High-dose CHT combined with autologous HSCT increases progression-free survival and overall survival, but does not eradicate cancer stem cells. In contrast to autologous HSCT, allogeneic HSCT may produce immune-mediated graft-versus-tumor reaction that eradicates cancer. However, allogeneic HSCT is associated with a risk of graft-versus-host disease (GvHD).

Perpectives of development of cell therapies based on multipotent stem cells and progenitor cells generated *ex vivo*

Human bone marrow contains two major multipotent stem populations, i.e. HSC and mesenchymal stem cells (MSC), both responsible for the generation of bone marrow microenvironment. Human adult HSC may be induced to proliferate extensively *in vitro*, and to produce various proportions of differentiated hematopoietic lineages, depending on cytokines applied for cell expansion. So far, the attempts to establish culture conditions that enable self-renewal of human HSC *ex vivo* have been unsuccessful, what limits the therapeutic use of these cells at present. Reconstruction of the three-dimensional stem-cell-niche unit *in vitro* will not be possible unless the molecular crosstalk between HSC and cellular constituents of HSC microenvironment in the bone marrow become well recognized. Stromal cells and osteoblasts that are responsible for bone remodelling as well as fenestrated endothelium of the bone marrow sinusoids have been postulated as the cell populations involved in formation of the HSC niche. Numerous molecules mediating intercellular signaling and cell adhesion (cytokines, chemokines, cadherins, and integrins) are involved in the HSC niche functions such as storage of quiescent HSC, self-renewal, and inhibition of differentiation. It is a matter of debate whether all these functions are provided by a single niche, or whether different types of specialized niches coexist in the bone marrow (4). Multiple factors derived both from the niche and from the periphery affect HSC efflux from the bone marrow into the vasculature (mobilization), specific movement through the vasculature (homing), and lodging in the niche. Therefore, an attempt to reconstruct HSC niche *in vitro* seems challenging. The impressive progress in the HSC niche field clearly indicates that substantial clonal expansion of HSC *in vitro* requires much more than just a cytokine cocktail. Matrix- and tissue-engineering developments have to keep in pace with cellular and molecular biology progress to enable three-dimensional reconstruction of the HSC niche.

Accumulating experimental evidence indicates that the hypothesis on HSC capability to generate also progeny differentiating to all 3 germ layers, based on the over-interpretation of some experimental data, was premature. When highly purified adult mouse HSC are cultured *in vitro* in neuronal differentiation medium supplemented with retinoid acid, 50% of the cells express nestin, the neural progenitor cell marker (5). In spite of phenotypic conversion into neural-like cells *in vitro*, these cells do not trans-differentiate into neurons, and are also incapable of generating action potentials. When transplanted, the cells either differentiate to macrophages/microglia or die. According to the present knowledge, the contribution of bone marrow-derived stem cells to

organ/tissue regeneration can be ascribed to a non-hematopoietic population of the so-called very small embryonic-like stem cells. The mRNA expression pattern of such stem cells suggests their differentiation plasticity toward muscle, liver and neural cells. The number of these rare cells that are distinct from HSC by size and by the surface phenotype, can be increased in circulation in response to injury or in response to mobilizing agents, such as G-CSF (6).

Bone marrow MSC basically give rise to cartilage, bone and fat. The existence of multipotent adult stem cells with high differentiation plasticity in various tissues became a subject of debate, as it was demonstrated that MSC were able to differentiate also toward the derivatives of germ layers other than mesoderm. Unlike HSC, MSC can give rise to cultured cell strains. Adult human MSC isolated from bone marrow, liver, and heart, were induced to proliferate extensively *in vitro* (7). Transcript expression profiling by cDNA microarrays revealed that heart-, liver-, and bone marrow-derived multipotent adult stem cells exhibit a very similar gene expression profile, but distinct from that of somatic cell lines and adult tissues. Their progeny retained clonogenicity, expressed the pluripotent state-specific transcription factors OCT-4, NANOG, and REX1, and displayed telomerase activity. It was also demonstrated that cell clones generated from adult MSC, unlike HSC, can be pushed by growth factors to express features that are associated *in vivo* with derivatives of the 3 germ layers. Yet, such transgermal, multilineage differentiation potential of MSC proved *in vitro*, requires validation by *in vivo* assays of MSC survival after transplantation and self-renewal, without a loss of properties acquired *ex vivo*. Sustained expansion or clonogeneity in culture does not surrogate self-renewal *in vivo*.

The best accessible population of adult non-hematopoietic multipotent stem cells for therapeutic purposes is a population of MSC residing among bone marrow stromal cells. For allogeneic transplantation MSC can also be obtained from various compartments of umbilical cord and from the cord blood. There are MSC among primitive stromal cells in the connective tissue surrounding the umbilical vessels (Wharton's jelly) and among umbilical vein subendothelial cells. MSC can be isolated from 20-50% of freshly prepared mononuclear cell fractions from umbilical cord blood (8). There are less than 0,01% of clonogenic MSC among bone marrow mononuclear cells and among umbilical cord nucleated cell populations. Human MSC grow as adherent cells if fetal calf serum or the concentrate of human platelets is supplied in a culture medium.

Experience on MSC expansion *ex vivo* and on the use of human MSC *in vivo* has been gathered in various studies [reviews (9, 10)]. Application of MSC for repair of age-related or traumatic tissue damage and for mesenchymal cell disorders, eg. osteogenesis imperfecta, receives great interest. Immunosuppressive properties of MSC inspired the attempts to use MSC as immune modulators in autoimmune diseases and to prevent allograft rejection. Immunomodulatory functions of MSC are exerted on multiple levels. MSC not only directly inhibit alloreactive T cells, but also induce dendritic cells (DC) to tolerogenic function and stimulate proliferation of the regulatory T cells. Attempts to treat GvHD and to improve hematopoietic recovery following HSCT have advanced to the phase of clinical trials. There are preliminary data demonstrating that co-transplantation of *ex vivo* expanded donor MSC is safe and feasible (11). It has been suggested that the co-transplantation of MSC might reduce the risk of graft failure in haploidentical HSCT (12).

Different types of stem cells and progenitor cells have been considered as candidates for therapeutic delivery to treat heart disease. Clinical trials have mostly used bone marrow mononuclear cells or endothelial progenitor cell subset that differentiates into endothelial cells. These cell types do not exactly reflect stem cell population most likely capable to regenerate myocardium. Besides, the true cardiac regeneration will probably require much more than simply injecting the right cells in the right place. First, the factors that limit engraftment of transplanted stem cells in the hostile microenvironment of injured myocardium have to be defined.

Perspectives of generation of patient-specific pluripotent stem cells

Generating pluripotent stem cells for an individual patient is one of the ultimate goals in regenerative medicine. ES cell lines can be generated from human blastocyst-stage embryos. Such ES cell lines are considered as potential donor sources for cell transplantation therapies for the diseases such as Parkinson's disease and heart failure. However, tissue rejection remains a significant concern for ES cell transplantation. Another concern is the use of human embryos. Pluripotent stem cells were generated in mouse models by cell fusion between ES cells and somatic cells, by nuclear transfer from somatic cell to an oocyte or by forced expression of certain transcription factors. In addition, pluripotent stem cells were obtained from adult mouse germ cells [review in (13)]. When considering the potential of nuclear transfer and ES cell fusion strategies

for generating patient-specific ES cell lines, the availability of human oocytes or surplus human *in vitro* fertilization embryos is a significant issue. The use of human embryos as a source of ES cells or donated human oocytes for nuclear transfer is surrounded by ethical controversy. In some European countries, as in Spain, the UK and Sweden, legislation allows access to human embryos for biomedical research.

Experiments in mouse models have shown that reprogramming of a somatic genome by nuclear cell transfer or cell fusion is accompanied by epigenetic changes such as DNA demethylation of pluripotency genes at their promotor regions. Successful reprogramming of somatic cells by fusion with ES cells indicates that ES cells have factors that induce and maintain pluripotency. The analysis of 24 different candidate factors for their ability to induce pluripotency revealed that retrovirus-mediated introduction of four transcription factors (Oct3/4, Sox2, c-Myc, and Klf4) into mouse embryonic or adult fibroblasts, followed by selection for expression of Fbx15, a target of Oct3/4 and Sox2, results in the generation of induced pluripotent stem (iPS) cells (14). However, the Fbx15-selected cells were significantly different from the ES cells in gene expression and DNA methylation patterns. The iPS cells transplanted into blastocysts give rise to chimeric embryos only, but not to adult or germline competent chimeras. Thus, researchers used more stringent selection markers, i.e. endogeneous activation of Nanog or Oct4, playing a crucial role in maintaining pluripotency. The improved method of selection provided germline-competent iPS cells with an increased ES cell-like gene expression. The selected iPS cells presented the DNA methylation patterns and chromatin state similar to those of ES cells (15-17). In many aspects, ES and iPS cells are similar to tumor cells. ES cells are immortal, proliferate rapidly, and form teratomas when transplanted into immune-deficient mice. It is likely that the mechanism of induction of iPS cells from somatic cell requires transformation by c-Myc and Klf4. Klf4 might be required to suppress p53 and c-Myc-induced apoptosis. Oct3/4 probably changes the cell fate from tumor cells to ES-like cells, whereas Sox2 is required to establish pluripotency (13). The efficiency of Nanog iPS cell induction was less than 0,1% of the cells that have incorporated four retroviruses (15). Such a low efficiency suggests that in fact iPS cells were generated from the rare stem cells co-existing in mouse embryonic fibroblast culture.

The remarkable findings from mouse model have been translated to human. Adult human dermal fibroblasts or fibroblast populations isolated from synovial tissue and neonatal skin from different human donors were selected as target cell populations for reprogramming. Human fibroblasts were reprogrammed to an undifferentiated state by transduction with retroviral vectors carrying just four genes: *OCT3/4, SOX2, KLF4* and *C-MYC* (18) or, alternatively: *OCT4, SOX2, NANOG* and *LIN28* (19), and were subsequently cultured under ES cell culture conditions. The resulting ES cell-like iPS cell colonies could be further propagated and expanded. DNA microarray analyses showed that the global gene-expression patterns between human iPS cells and human ES cells were similar, but not identical. Chromatine immunoprecipitation performed at the aim to analyze the histone modification status revealed that ES cell-specific genes are active in human iPS cells. The retroviral vectors enabled silencing of all four transgenes after human iPS cell formation, as found previously in the mouse system. It indicates that the reprogrammed human iPS cells no longer depend on the expression of the inserted transgenes. Human iPS cells differentiate *in vitro* into the cell types of 3 germ layers, and *in vivo* into teratomas. In contrast to mouse iPS cells, human iPS cells were generated without any genetic selection procedures.

A success in reprogramming adult fibroblasts by certain transcription factors to the cells of embryonic stem cell characteristic, capable to become any cell-type in the body, paves the way to produce patient-specific pluripotent cell lines without employment cell nuclear transfer to either embryonic stem cells or to oocytes. Thus, it becomes possible to circumvent ethical concerns. However, before the iPS cells can be used in the clinic, additional research is required to avoid using vectors which integrate into the genome and oncogenes in the process of turning back the developmental clock. With the presently available techniques, skin cells reprogrammed into iPS cells carry multiple copies of the retroviruses used to deliver the inserted genes, what could lead to mutations at the integration site and cause tumors. Mice derived from iPS cell are more prone to cancer than their normal counterparts. A significant proportion of mice derived from mouse iPS cell develop tumors due to reactivation of the c-Myc retrovirus. So far, human iPS cells with retroviral integration are potentially useful for studying disease mechanisms and for drug testing. Once safety issue is overcome, iPS cells will be applicable in regenerative medicine. Possibly, high-throughput screening of chemical libraries might identify small molecules capable to replace products

of the four genes in the process of turning back adult human fibroblasts or other cells to the pluripotency stage. However, it has to be kept in mind that iPS cells, even obtained without the use of retroviral transduction may develop teratomas, when directly transplanted into adult organs. In contrast to a blastocyst, adult tissues may not provide the microenvironment that sufficiently controls the expansion of ES-like iPS cells and differentiation of their progeny. Therefore, in an attempt to meet safety requirements, a future strategy of iPS cell application in the regenerative medicine could adopt iPS cell expansion *in vitro*, followed by a subsequent generation of more specialized multipotent stem cells and/or generation of differentiated cell populations. Then, a custom-made progeny of iPS cells could be prepared.

The recently published data from murine studies are very encouraging. The feasibility of treating multiple parkinsonian mice with dopamine neurons derived from ES cells generated by somatic cell nuclear transfer to oocytes has been recently proved. The transplanted cells were compatible with the host tissue, since nuclei from autologous fibroblasts were used for the transfer (20). However, a method of generating dopamine neurons from iPS cells seems even more suitable for the replacement therapy in Parkinson's disease. Dopamine neurons derived from the reprogrammed rat fibroblasts synaptically integrated into the fetal mouse brain after transplantation and improved behavior of rats in model of Parkinson's disease (21).

Combination of stem cells and dendritic cells in therapy dendritic cell-based vaccines against cancer

Experience gathered for a long time in clinical studies reveals that the curative effect of allogeneic HSCT depends on the development of graft versus leukemia/lymphoma (GvL) reaction following transplantation. However, the full therapeutic potential of allogeneic HSCT cannot be exploited until GvHD is diminished while maintaining positive contribution of donor T lymphocytes to GvL reaction overshadowed by GvHD. Therefore, future approaches to increase therapeutic use of allo-HSCT need wider exploitation of DC immunomodulatory functions. Infusion of tolerogenic DC, or infusion of regulatory T cells generated *ex vivo* in the presence of tolerogenic DC, is currently examined in pre-clinical studies as an alternative therapy aimed to prevent or to treat posttransplantational GvHD, instead of MSC transplantation. Similarly, DC-mediated tolerance might be a prerequisite step in stem cell-based replacement therapies in auto-immune disorders. The great potential of DC to induce immune response to cancer antigens needs to be further verified in a clinical setting.

Dendritic cells are the only specialized, highly potent antigen presenting cells that prime naive T lymphocytes with antigen to initiate immune response. Since the role of DC as sentinels directing immune response was recognized, a new type of anti-cancer vaccines has been designed. By DC isolation from patient's peripheral blood or bone marrow, loading DC with tumor antigens *ex vivo*, and re-injection to patient, it became possible to engineer the process of direct antigen administration to DC during vaccination. Dendritic cells are a heterogeneous cell population rarely distributed in almost all tissues and organs, and thus difficult to isolate. Some vaccine studies are based on DC directly obtained from the peripheral blood or on DC generated *in vitro* from HSC collected following mobilization with G-CSF, and subsequently expanded *in vitro* in the presence of the cocktail of growths factors preferentially inducing DC growth and differentiation. However, in most vaccination studies, monocyte-derived DC have been used. Monocytes induced *in vitro* with granulocyte-macrophage-colony stimulating factor (GM-CSF), interleukin-4, and optionally with pro-inflammatory cytokines, gain DC phenotype and function.

Multiple clinical trials have been performed so far with DC loaded with tumor proteins, peptides, whole tumor cell lysates, or with DC transfected with m-RNA or DC fused with tumor cells. The majority of these trials were designed as an experimental therapy for patients with a measurable tumor mass. The observed rates of objective clinical responses to the therapeutic DC vaccines did not exceed 5-10%, in spite of the much more often observed immune response, as it has been reviewed in melanoma patients (22). Although DC-based therapeutic tumor vaccines achieved higher clinical efficiency than those not using DC as an adjuvant, the "proof of efficacy" is still not satisfactory. Tumor progression is accompanied with the phenomenon of "immune escape" caused by Darwinian type selection of less immunogenic tumor clones and by development of immunosuppression, induced by tumor itself and/or by mechanisms of immune regulation within the immune system. Therefore, some new strategies aimed to induce immune response against cancer include now attempts to break tolerance to tumor antigens.

A general concept of tumor vaccination should be revised. In contrast to the attempts to reduce tumor mass, a vaccination in an adjuvant setting per-

formed in a remission achieved following conventional therapies, seems to be a more rational strategy. At least, the disease should not be rapidly progressing in order to give the vaccines an adequate time to develop immune reaction. It has to be reminded that vaccines against pathogenic organisms are generally performed in a preventive setting. It is speculated that tumor debulking by surgery or chemotherapy followed by anti-cancer vaccination might prevent relapses. Alternatively, tumor vaccination in the early disease setting before surgery might diminish the risk of cancer recurrence, and protect against the development of invasive cancer (23).

Preparation of patient-specific "tailor-made" DC vaccines is laborious, so it might be believed that companies would not be interested in DC vaccination. However, several companies try to get approval from official regulatory authorities. The *Sipuleucel-T* vaccine, made from DC pulsed with recombinant prostatic acid phosphatase fused to GM-CSF, prolonged overall survival of hormone refractory metastatic cancer patients from 21.4 to 25,9 months in a placebo-controlled phase III trial (24). Although FDA Advisory Committee initially recommended to approve *Sipuleucel-T*, the FDA decided to await further proof of efficacy.

An existing paradigm of cancer treatment is directed solely against differentiated cancer cells, and does not target cancer stem cells thought to be responsible for cancer recurrence. Designing new therapies directed against cancer stem cells could increase treatment effectiveness, but considering cancer stem cell characteristics such a task seems challenging. DC-based cancer vaccination and graft versus tumor reaction following allo-HSCT are potentially capable to target cancer stem cell compartment.

FUTURE DIRECTIONS

The ultimate goal of stem cell-based regenerative medicine is to generate functional cell types relevant for replacement therapies. Progress in somatic reprogramming creates the possibility to generate genetically identical iPS cells for replacement therapy from the patient's somatic cells. First, new methods of delivery of the pluripotency-inducing factors have to bypass the use of retroviral vectors randomly integrating to the genome because the potential retroviral vector reactivation in the transplanted cells could lead to cancerous transformation. A task of designing DNA-free reprogramming methods, possibly based on the use of small molecules and/or engineered membrane permeable transcription factor proteins may turn out to be challenging. An appropriate selection method of undifferentiated iPS cells from the cell progeny generated for transplantation has to be elaborated, to avoid a risk of teratoma formation by the transplanted cells. Then, a therapeutic effect of transplantation of neurons, insulin-producing cells, cardiomyocytes, hematopoietic cells, and possibly other *ex vivo* custom-made cell populations has to be proved in the clinical studies.

There is a need for designing efficient methods of isolation and expansion of multipotent stem cell populations derived from adult tissues and from the umbilical cord. Further studies are required on engraftment mechanisms and barriers that prevent tissue regeneration by the endogeneous tissue stem cells and transplanted stem cells. Treatment of organ allograft rejection and prevention of GvHD by co-transplanted MSC, tolerogenic DC or regulatory T cells, needs to be verified in clinical studies.

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