Stem Cell Sources and their potential for cancer therapeutics

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Introduction

Stem cells are the natural sources of embriogenetic tissue generation and continuous regeneration throughout adult life. In embryogenesis, cells from the inner cell mass (ICM) of the gastrula are known as 'embryonic stem cells' and their multilineage potential is generally referred to as pluripotent[1]. The gastrular ICM cells commence formation of the three germ layers: endoderm, mesoderm, and ectoderm, each committed to generating specified tissues of the forming body, and thus containing stem cells with more restricted potential than pluripotent stem cells[2]. Tissue specific stem cells, such as mesenchymal stem cells (mesoderm), hematopoietic stem cells (mesoderm) and neural stem cells (ectoderm), have been identified as present and active for virtually every bodily tissue, and are hierarchically situated between their germ layer progenitors and differentiated end-organ tissues[2].

Stem cells can be isolated in three ways: from the ICM of the gastrula (embryonic stem cells), from fetal cord blood, and from adult tissues or blood (adult/somatic stem cells). It is not entirely clear whether adult stem cells harbor intrinsic differences from embryonic stem cells. Embryonic stem cells display indefinite self-renewal capacity due to high telomerase expression. In contrast, telomerase activity in adult stem cells seems to be lower, limiting their perpetuation capacity in the long run[3]. Adult stem cells have been studied extensively and are already a successful source of FDA-approved treatments for nine human diseases, such as Parkinson's disease and juvenile diabetes, currently applied in clinical centers[4]. Though not as highly pluripotent and self-renewing as their embryonic counterparts, adult stem cells are much safer with respect to post-grafting tumor formation. Further, whereas the isolation of adult stem cells from specific parts of the body-such as brain or heart-is complicated, the advent of transdifferentiation techniques and ongoing discovery of unexpectedly plastic and versatile stem cells might provide autologous stem cells resembling these clonal subtypes[5, 6]. Namely, the long held dogma of differentiation as a rigid and non-reversible process has been challenged over the past decade by a vast amount of studies claiming to show transdifferentiation or even de-differentiation of committed cells. Mesenchymal stem cells (MSC), HSC, muscle stem cells, and NSC all seem to possess the potential of converting to tissue types of other lineages, both within or across germ lines[7-9]. The

highest degree of lineage plasticity has been imputed to bone marrow derived MSC, which appear capable of giving rise to virtually all cell types following implantation into early blastocysts and are relatively easy to handle *in vitro*[8, 10]. Recent reports have showed that pluripotent stem cells could be generated from murine fibroblasts[11], as well as from several human organs, such as heart, skin[12], and bone marrow[5]. Also, researchers seem progressively to be able to guide differentiation of pluripotent stem cells into cell types of interest[13, 14]. These studies indicate that controlled transformation of naïve or committed adult cells from dispensable tissue into desired cell types for autologous transplantation might become reality in the near future.

Adult Stem cells

Mesenchymal stem cells

The ability of MSC to develop into various cell types, and the ease with which they can be expanded in culture, have led to a great deal of interest in their use as therapeutic agents to treat a wide range of diseases. They can be isolated from adult human tissues, have the capability for self-renewal and differentiation into mesenchymal lineages-osteocytic, chondrocytic, and adipogenic. They can be expanded and manipulated *in vitro*, and subsequently re-grafted. Following re-implantation, they have been found to suppress immune system, reintegrate into tissue architecture and give rise to progeny consisting of both stem cells and lineage restricted daughter cell types[15]. Most importantly, MSC exhibit potent pathotropic migratory properties, rendering them attractive for use as targeted delivery vectors in tumor therapy[15, 16].

MSC have been successfully isolated from a number of organs including brain, liver, kidney, lung, bone marrow, muscle, thymus, pancreas, skin, adipose tissue, fetal tissues, umbilical cord, Wharton's jelly, and placenta[17-20]. The highest degree of lineage plasticity has been imputed to bone marrow derived MSC, which are capable of giving rise to virtually all cell types following implantation into early blastocysts and are relatively easy to handle *in vitro*[8, 10]. Most of the pre-clinical studies to date have been performed with bone marrow derived MSC which might not be the most practical source available

for the clinical settings. The harvesting of bone marrow requires an invasive procedure which yields a small number of cells, and the number, differentiation potential, and lifespan of bone marrow-derived MSCs decline with patient age[21-23]. Two alternate sources for harvesting MSCs that have received considerable attention in recent years are adipose tissue and umbilical cord blood. Adipose tissue obtained from subcutaneous tissue represents the most abundant potential source for harvesting MSCs reliably using simple techniques. The expansion potential, differentiation capacity, and immunophenotype of MSCs derived from adipose tissue are nearly identical to those isolated from bone marrow[22]. Umbilical cord blood, obtained after removal of the placenta, is a rich source of hematopoietic stem cells[24, 25] and has been shown to be also a rich source of MSCs[26]. Mononuclear cells can be separated and cultured from the cord blood, and cells in heterogenous adherent layer have been shown to have a fibroblastoid morphology, and express same markers as bone marrow derived MSC, namely CD13, CD29, CD49e, CD54, CD90, but not CD14, CD31, CD34, CD45, CD49d, nor CD106, among others[27]. Umbilical cord blood derived MSC expand at a higher rate as compared to bone marrow and adipose-derived MSCs[22, 28], which may be due in part to higher telomerase activity[29]. All three type of cells differentiate into osteocytes and chondrocytes[22, 27, 30, 31]which is consistent with the properties of MSCs.

Neural Stem Cells

NSC isolated from both embryonic and adult human tissues have emerged as attractive candidates for delivering therapeutic proteins that specifically target glioma cells. These cells can be expanded and manipulated *in vitro*, and re-engrafed following transplantation. NSC have shown the ability to migrate extensively to sites of different pathologies and reintegrate into tissue architecture to give rise to progeny consisting of both stem cells and lineage- restricted terminal cell types[1, 32, 33]. For therapeutic purposes, NSC must be derived, in a substantial number, from safe, consistent, and reliable sources and must meet the criterion of plasticity. Both embryonic stem (ES) cell and adult NSC can be obtained in substantial amounts and have the intrinsic ability to adapt their specification fate in response to different

environmental cues[34]. Recent advances in the *in vitro* expansion of human ES culture involve the characterization of defined factors which negate the use of feeder layers (often of murine origin)- thus eliminating the problems of xenogeneic cell contamination and possible viral transmission[35, 36]. Adult NSC are multipotent cell that can be obtained from embryonic, fetal, neonatal or adult CNS tissue. These cells are found in abundance during embryonic development and their numbers and developmental potential dwindle as development progresses and exist only in small numbers and in specialized niches in the adult organism. In the adult CNS, these cells are especially enriched in the subventricular zone and the subgranular zone of the hippocampal dentate gyrus. Also, NSC have been isolated from the human postnatal cerebellum and adult brain[37, 38]. In humans, fetal NSC were originally isolated from the germinal zones in the subventricular region of a fetal telencephalon[39]. Difference in developmental plasticity between embryonic, fetal, and adult stem cells could be either due to intrinsic cellular difference or disparity in the surrounding microenvironment but most likely a combination of the two[40, 41]. This abrogation of developmental plasticity could also explain for the limited ability for tissue repair seen in the adult organism. Non-CNS derived multipotent somatic stem cells, such as mesenchymal stem cells[42], placental cord blood stem cells[43], skin stem cells[44] and adipose tissue stem cells[45] have recently been shown to have the potential to become NSC.

Therapeutic applications of NSC require a substantial number of cells which can be propagated *in vitro* in serum- free condition in the presence epidermal growth factor (EGF) and β-fibroblast growth factor (FGF) as multicellular free- floating spheres or neurospheres. Withdrawal of growth factors promotes the spontaneous differentiation into mature cells (astrocytes, oligodendrocytes, and neurons) within the neurospheres. Regular disaggregations of neurospheres ensure the healthy propagation of NSC *in vitro* and numerical expansion of NSC. This however is time- consuming and does not yield the large numbers of cells required for most experimental and clinical trials. Immortalization of primary NSC offers a solution to the above problem and can be achieved via the transduction of oncogenes such as the simian virus 40 large T antigen or the *v-myc* gene[46, 47]. These cells behave similarly as non- immortalized NSC with the capability to migrate extensively in the developing and mature CNS. Ectopic expression of

telomerase has also been shown to prolong the undifferentiated stem- like property of the NT2 neural progenitor cells[48, 49].

Apart from ethical considerations, the therapeutic use of ES cells is constrained by some key issues- such as feeder-dependent growth expansion. As mentioned previously, this vexing problem, especially in the *in vitro* propagation of human ES cells, is gradually being solved with the characterization of factors responsible for maintenance of the differentiated state of the ES cells. In addition, better understanding of developmental kinetics of stem cells help to increase the yield of ES- derived NSC. However, additional guidelines need to be instituted especially with respect to avoidance of *in vivo* teratocarcinoma formation associated with ES cells. Practical issues pertaining to these matters are discussed in a review by Martino and Pluchino[50].

Induced pluripotent stem cells

Induced pluripotent stem cells (iPSCs) are created by inducing differentiated cells to express genes that are specific to embryonic stem cells. iPSCs cells share many characteristics of embryonic stem cells, including the ability to differentiate into cells of all organs and tissues. The idea of being able to restore pluripotency to somatic cells by co-expression of specific reprogramming factors has created powerful new opportunities for modeling human diseases and offers hope for personalized regenerative cell therapies[51] [52]. IPSCs have been shown to have the capacity to re-differentiate into almost any human cell type.

Induced pluripotent stem cells (iPSCs) are a novel and practical tool for human disease modeling and correction, and in theory could serve as a limitless stem cell source for patient specific cellular therapies[53]. Pluripotency means the ability of stem cells to grow indefinitely in culture while maintaining the potential to give rise to any of the three germ layers: the endoderm, mesoderm and ectoderm. Somatic cells can be reprogrammed to a stem-cell like state by transferring their nuclear content into oocytes or by fusion with embryonic stem cells (ESCs), indicating that unfertilized eggs and ESCs

contain factors that can confer pluripotency to somatic cells[52, 53]. Takahashi and Yamanaka hypothesized that the factors that play important roles in the maintenance of ES cell identity also play pivotal roles in the induction of pluripotency in somatic cells[11]. A screen of 24 candidate genes lead to the triumphant description of a tetrade of transcription factors, Oct4, Sox2, Klf4, and cMyc, sufficient to reprogram tailtip fibroblasts of mice into iPSCs[52, 53]. This contribution stimulated an overwhelming number of follow-up studies, with successful reprogramming quickly translated to human fibroblasts[12, 54, 55] and then to a wide variety of other cell types, including pancreatic β cells[56], neural stem cells[57, 58], mature B cells[59], stomach and liver cells[60], melanocytes[61], adipose stem cells[62] and keratinocytes[63], demonstrating the seemingly universal capacity to alter cellular identity.

Other Stem Cell sources

Dental Pulp Stem Cells

The potential use of adult dental pulp as a source of MSCs has also been explored and validated. Dental pulp (DP) is a vascular connective tissue similar to mesenchymal tissue. The dental pulp derived stem cells have a phenotype similar to the adult bone marrow derived MSC and these cells also express mesenchymal progenitor-related antigens SH2, SH3, SH4, CD166 and CD29 with a cellular homogeneity of 90-95%. Also, the dental pulp and bone marrow derived stem cell populations have a similar gene expression profile[64, 65]. In contrast to BM-MSCs, DP-MSCs have presented a higher proliferation pattern and lower differentiation ability. The most evident difference is the inability of DP-MSCs to differentiate towards chondrogenesis. This may indicate either that BM-and DP-MSCs are present at different stages of commitment and differentiation, not marked by phenotypical characteristics, or that different humoral networks are involved in each microenvironment[64].

In short, the dental pulp derived stem cells are derived from a very accessible tissue resource, which is further expandable by using deciduous teeth, and possess stem cell-like qualities, including very good self-renewal and multilineage differentiation. Their capacity to induce osteogenesis[64, 66] could be of great clinical application in implantology. Moreover, these cells also could have potential clinical application in autologous *in vivo* stem cell transplantation for calcified tissue reconstruction. Their proven

immunomodulatory activity makes them suitable for suppression of T-cell mediated reaction in the setting of allogeneic bone marrow transplantation[64].

Menstrual blood stem cells

Menstrual blood from the uterine lining has been recognized as a novel source of stem cells[67, 68] with high regenerative capability after the menstrual cycle[67, 69]. Additionally, stromal cells derived from menstrual blood (MenSCs) can be acquired without invasive procedures and avoid any ethical controversies. These cells display stem cell-like phenotypic markers, a propensity for self-renewal, high proliferative potential in vitro, and the ability to differentiate towards diverse cell lineages.

The utilization of human MenSCs as a potential source for reprogramming into iPSCs offers several advantages. First, MenSCs may be more easily reprogrammed than terminally differentiated fibroblasts. Second, the procedure for isolating MenSCs is relatively simple, fast, and safe, and does not pose any ethical concerns. Third, it is convenient to obtain a large quantity of MenSCs as the starting population for reprogramming. Fourth, because the reprogramming process requires only two factors, opportunities for insertional mutagenesis are minimized. Furthermore, obviating the requirement for KLF4 and c-MYC reduces the risk of inducing tumorigenesis. However, there is one obvious limitation for MenSCs in that they are only obtained from menstrual blood samples of women of reproductive age, which may narrow their applications. However, if iPSCs indeed have memory of the donor tissue[67, 70], MenSCs-iPSCs should be the best candidate for producing MenSCs to treat uterus-related problems[67, 68].

Prospects and caveats on the way to the clinics

Stem cell research is one of the most rapidly developing areas of science and medicine.The ability adult stem cells to preferentially migrate towards local and disseminated malignant disease, interact with different tissue environments present them as most attractive candidates for cell based therapies in humans.. For translation of promising pre-clinical studies into clinics, it is critical to develop a greater understanding of stem and progenitor cell characteristics, single-cell heterogeneity and their fate in mouse models that recapitulate more closely clinical settings. The type of stem cells used for a particular

type of cancer in clinics will depend on their isolation efficiency and their pre-requirement as an allogeneic transfer. For example, the clinical translation of umbilical cord blood derived MSC will be limited by their unreliable and often low isolation efficiency and requires allogeneic transfer. In contrast, allogeneic transfer is not necessary for adipose or bone marrow-derived MSCs, in which case an autograft can easily be harvested from any patient. The advantage of using autologous stem cells is mainly their immunological compatibility, which has been shown to have a profound effect on cell survival after transplantation. For most of of the stem cell based therapeutics for cancer, genetic manipulation of cells prior to transplantation to combat the disease process will be required. Prior to manipulation of the stem cells with a tumor specific transgene, a thorugh understanding of the altered signaling pathways in different cancer types is necessary. This will ensure the specificity of the stem cell based targeted therapeutics. The safety of the transplanted stem cells is a major concern in clinical setting. Importantly, non-immortalized adult stem cells do not confer the same danger as immortalized adult stem cells and may be used without posing risk to the patient. A number of clinical trials utilizing stem cells for cancer (Add new references) have not reported any major adverse eventstill date. There are also a number of ongoing clinical trials which are utilizing stem cells for cancer therapy, and the results of any adverse effect from such trials is still awaited. When the malignant transformation of transplanted stem cells is suspected, It would therefore be desirable to selectively eradicate MSC by incorporating activatable cellular suicide genes into transplanted MSC or to selectively turn off gene expression. Possible mechanisms that allow for such controls are stem cell-conferred prodrug converting enzymes and transgenes that require additional *in vivo* cues for expression and the use of tetracyclin-regulatable promoters to turn off gene expression.

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