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Transformation of Mesenchymal Stem Cells

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1. Introduction

As part of normal organismal homeostasis, the human body loses various types of cells like hepatocytes, keratinocytes and certain types of blood cells and needs to replace them. These cells are replaced using stem cells as the source. Many different names are used for cells with stem cell-like properties, such as precursor cells, progenitor cells, somatic stem cells and adult stem cells. Stem cells are unique cells that have the ability to renew themselves through mitotic cell division and differentiate into a diverse range of specialized cell types. In addition, stem cells, with few notable exceptions, are cell types that show telomerase activity and therefore, actively maintain telomere length to some degree. The ability to maintain telomere length allows them to have an extended proliferative capacity compared to somatic cells.

There are three kinds of stem cells: Embryonic, germinal and somatic or in other terms adult stem cells. The differentiation potential of stem cells varies according to type from totipotency to unipotency. Cells such as the fertilized oocytes, up to the 8-cell blastocyst, are considered to be totipotent since they can differentiate and generate a complete organism. Embryonic stem (ES) cells are derived from the inner cell mass of a blastocyst. ES cells posses all the characteristics of true stem cells. In addition to the self-renewal capacity, they are pluripotent, being able to produce derivates of all three germ layers (endoderm, mesoderm and ectoderm) but not the complete organism (Burdon *et al*., 2002). A high telomerase activity prevents the ES cells from undergoing crisis and reaching senescence, which is an advantage for long-term culturing. ES cells have furthermore been shown to retain their developmental identity even after reintroduction into the blastocyst (Beddington & Robertson, 1989). Somatic stem cells are differentiated forms of embryonic stem cells that are known as multipotent stem cells. They are capable of self-renewal and are responsible for the regenerative property of the e.g. hematopoietic system and the gastrointestinal system. These cells can be isolated from the developing organism (the fetus and the postnatal organism) as well as from the adult organism. Depending on their origin, the offspring of the somatic stem cells is also specific to the original tissue. A stem cell is said to be unipotent like the epidermal stem cells if they can persistently give rise to only a specific cell type. It has for many years been known that stem cells – contrary to other cell types –

can perform asymmetric cell divisions, whereby one daughter cell remains undifferentiated, while the other is committed to differentiation. It is important to also note that through transdifferentiation, somatic stem cells are also able to produce progeny different from their tissue of origin. These cells can be isolated from the developing organism in fetal and postnatal stages as well as the fully developed organism. Examples of somatic stem cells will include; stem cells which are harvested from the brain which are capable of differentiating into the three lineages of the central nervous system (CNS) (neurons, astrocytes and oligodendrocytes). Another example of somatic stem cells is the bone marrow stem cells that include hematopoietic and mesenchymal stem cells which are able to repopulate the blood and the bone cell systems.

In this chapter, we will focus on Mesenchymal Stem Cells (MSCs), their therapeutic potential, risks of neoplastic transformation of Mesenchymal Stem Cells (MSCs) during expansion, issues related to the use of MSC during therapeutic use and possible ways of addressing these issues.

2. Mesenchymal stem cells

2.1 Properties and isolation of mesenchymal stem cells

Bone marrow contains a supporting tissue called the stroma which was thought of as a simple structural framework for the hematopoietic system. It is now known to have very diverse functions. One of its most important aspects is that it contains mesenchymal stem cells (MSCs). These cells are strongly adherent and therefore, they can be isolated by culturing marrow on a special substrate, which allows the other cells to be washed off (Dennis *et al*., 2004; Pittenger *et al*., 1999; Terskikh *et al*., 2006). Their properties were described as early as 1968 by Friedenstein (Friedenstein, 1968). The human mesenchymal stem cells (MSCs) are multipotent precursor cells and can differentiate into various types of mesenchymal tissue cells. Mesenchymal stem cells (MSCs) undergo self-renewing divisions but also give rise to more committed progenitor cells, which can differentiate into cells of the mesodermal lineage, such as adipocytes, osteocytes and chondrocytes, as well as cells of other embryonic lineages (Jiang *et al*., 2002).

In addition to bone marrow, MSC-like cells have been shown to be present in a number of other adult and fetal tissues, including circulating blood (Zvaifler *et al*., 2000), cord blood (Weiss & Troyer, 2006), placenta (Miao *et al*., 2006), amniotic fluid (Tsai *et al*., 2004) heart (Chen *et al*., 2008), skeletal muscle (Peault *et al*., 2007), adipose tissue (Zuk *et al*., 2001) synovial tissue (De Bari *et al*., 2001) and pancreas (Di Rocco *et al*., 2008). In addition to healthy tissue, MSC-like cells have even been isolated from pathological tissue like rheumatoid arthritic joints (Marinova-Mutafchieva *et al*., 2000). Cells that carry MSC characteristics might be present in nearly all postnatal organs and tissues (Chamberlain *et al*., 2007). Historically, the broad variety of tissue sources from which MSCs are isolated, in conjunction with disparate culture conditions like media formulations and plating density, has led to a lack of consensus regarding the phenotype of the MSC. However, recent reports are pointing to a strong consensus regarding the morphology of fresh MSCs, irrespective of the method by which they were isolated. This morphology of MSCs has been described as large cells with a prominent nucleoli and bleb-like projections. These projections extend further as MSCs adhere. The morphology is different from the spindle-shape of typically shaped MSCs (Jones & McGonagle, 2008). Despite the historic variation in reported phenotypes of MSCs, it is widely accepted that cultured cells, regardless of the methods

employed in their isolation and culture, lack expression of prototypic hematopoietic antigens including CD45, CD34, CD11b and CD14 and express SH2 (CD105), SH3/SH4 (CD73), CD29, CD44, CD90, CD71, CD106, CD166, STRO-1, GD2, and CD146 (Martinez *et al*., 2007; Pittenger *et al*., 1999; Shi & Gronthos, 2003; Simmons & Torok-Storb, 1991; Sordi *et al*., 2005).
The methodology used in the isolation and enrichment of human mesenchymal cells is essentially based on the ability of these cells to adhere to and subsequently proliferate on tissue culture plastic with 10% fetal calf serum. The cells might experience a lag phase but then divide rapidly. The doubling time of the MSCs *in vitro* depend on the donor and the original plating density (Chamberlain *et al*., 2007). Indeed, culture selection is still widely employed as a means of MSC isolation. Pre-enrichment through cell separation strategies using cocktails of antibodies that deplete the bone marrow of specific cell populations (negative selection) (Louis *et al*., 2001; Reyes *et al*., 2001) or FicollTM separation are most widely used as an initial step in MSC isolation. To date no single and unique marker allowing for MSC isolation has been reported thus a range of composite cell surface phenotypes are being used. Enriched populations of MSCs have been isolated from human bone marrow aspirates using a STRO-1 monoclonal antibody in conjunction with antibodies against VCAM-1/CD106 (Simmons & Torok-Storb, 1991), CD271 (Quirici *et al*., 2002), D7-Fib30 and CD49a.31. In order to harvest and isolate MSCs, a needle is used to aspirate the bone marrow from the trabecular of the bone. The bone marrow can then be manipulated within the laboratory to remove the red blood cells, macrophages, and other extraneous material (Boiret *et al*., 2005). MSCs can then be enriched by their adherence to a plastic culture dish. They can also be sorted via flow cytometry based on MSC surface proteins and viewed under a microscope to determine that the cells look like MSCs (Campagnoli *et al*., 2001; Li *et al*., 2006; Quirici *et al*., 2002). MSCs have also been isolated from other species such as mice, rat, cats, dogs, rabbits and baboons with varying success. However, MSCs from different species do not express the same molecules as the human cells (Javazon *et al*., 2004).

2.2 Therapeutic potential of mesenchymal stem cells

MSCs have many desirable characteristics that make them great therapeutic tools for many diverse illnesses. MSCs can be isolated from adult donors and can easily be expanded in culture without greatly compromising genetic stability; an in depth discussion on genetic stability and neoplastic transformation will be presented in the following section. Their lack of immunogenicity allows for allogenic transplantation and their homing capacity creates room for treatment with minimal invasion (Teng, 2010). Isolation of MSCs has been discussed in greater detail in the previous section. They can be isolated not only from blood tissue but also from many diverse tissues such as adipose tissue and trabecular bone and placenta.
Many studies have been performed using MSCs. These studies were mainly based on site-directed and/or systemic administration of MSCs and both delivery methods of hMSC have shown their ability of engraftment in a number of tissues after injury (Barbash *et al*., 2003; Horwitz *et al*., 2002; Orlic *et al*., 2001; Ortiz *et al*., 2003). In order for a stem cell to work as an effective therapeutic tool it must be able to access the target organ to deliver their therapeutic effect. Access to the target organ might not be a problem in some cases, however, if the illness is systemic in nature or if the target organ is not anatomically accessible effective delivery might become a problem. MSCs provide us with a very valuable tool in the latter two scenarios as they have been shown to spread to various tissues after their intravenous

administration (Devine *et al*., 2003). These systemically administered MSCs home to the site of injury and aid in functional recovery. In earlier studies, cultured MSCs have been infused into patients to support bone marrow transplant for osteogenesis imperfecta and glycogen storage disease where the therapeutic options are limited (Pittenger & Martin, 2004). They have also been used in graft versus host disease and their role in the treatment of Crohn's disease is being explored (Mittal *et al*., 2009). MSCs are also used in diverse variety of clinical trials for ischemic stroke, multiple sclerosis, acute leukaemia, critical limb ischemia, articular cartilage and bone defects.

Although the exact mechanism is not well understood, introduction of MSCs into the infarcted heart directly or through intravenous administration, have resulted in improved recovery and prevented deleterious remodeling (Pittenger & Martin, 2004). The ability of MSCs to repair damaged tissue is thought to be primarily from their capacity to secrete paracrine mediators such as interleukin-10, interleukin-1ra, keratinocyte growth factor and prostaglandin (Matthay *et al*., 2010). However, the mechanisms underlying migration/homing of hMSC have yet to be clarified, even though there are some evidence suggesting the involvement of chemokines and their receptors (Kortesidis *et al*., 2005; Von Luttichau *et al*., 2005; Wynn *et al*., 2004). In addition to cellular adhesion molecules, the following chemokine/receptor pairs have been implicated in MSC migration; SDF-1/CXCR4, SCF/c-Kit, HGF/c-Met, VEGF/VEGFR and PDGF/PDGFr. However, the migration to the tumor site is believed to be non-specific since the administered MSCs have been shown to localize to lung, bone marrow and lymphoid organs. Additionally, it was observed that whole body irradiation increases the distribution of MSCs to multiple organs (Momin *et al*., 2010).

Because of their stem cell properties, facilitation of engraftment of the transplanted hematopoietic stem cells and promotion of the structural and functional repair of damaged tissues are some of the first studies the scientists have focused in the MSC field. However, because of the immunomodulatory properties of MSCs, they might have potential uses in immune-related diseases as well (Uccelli *et al*., 2008). One of the advantages of using MSCs as therapeutic agents is their poor immunogenicity *in vitro*, in preclinical trials and in human studies. This would allow the use of MSCs from allogenic donors. There still remains the possibility of using autologous MSCs even in autoimmune conditions. (Bartholomew *et al*., 2002; Le Blanc *et al*., 2004; Murphy *et al*., 2002; Tse *et al*., 2003). Recently reported findings present the observation that MSCs derived from the bone-marrow suppress T-cell proliferation (Uccelli *et al*., 2008). MSCs have been shown to have a role in both innate and adaptive immunity. In innate immunity, MSCs can decrease the pro-inflammatory response potential of dendritic cells by inhibiting their production of tumor-necrosis factor (TNF). They have also been shown to have an inhibitory effect on the maturation of monocytes and cord-blood and CD34+ hematopoietic progenitor cells (Jiang *et al*., 2005; Li *et al*., 2008; Nauta *et al*., 2006; Ramasamy *et al*., 2007). Neutrophils, an important part of innate immunity, can also be regulated by MSCs. The MSCs, through an IL-6 regulated mechanism, can dampen and delay the spontaneous apoptosis of resting and activated neutrophils (Raffaghello *et al*., 2008). In adaptive immunity, T cells are maintained in a state of quiescence through an MSC-mediated anti-proliferative effect, which can be partially reversed through IL-2 stimulation (Zappia *et al*., 2005). It has also been suggested that MSCs are able to modulate the intensity of immune system response through an inhibition of antigen-specific T-cell proliferation and cytotoxicity and through promotion of regulatory T cell generation (Hwa Cho *et al*., 2006; Pevsner-Fischer *et al*., 2007; Tomchuck *et al*., 2008).

2.3 Limited proliferation capacity of hMSCs

Most of the human somatic cells can undergo 60-70 population doublings on average, and then enter senescence (Meyerson, 2000). Cellular senescence is the process by which normal cells lose the ability to divide. This limited number of cell divisions is called the "Hayflick limit" (Hayflick, 1976) (Figure 1, top panel).

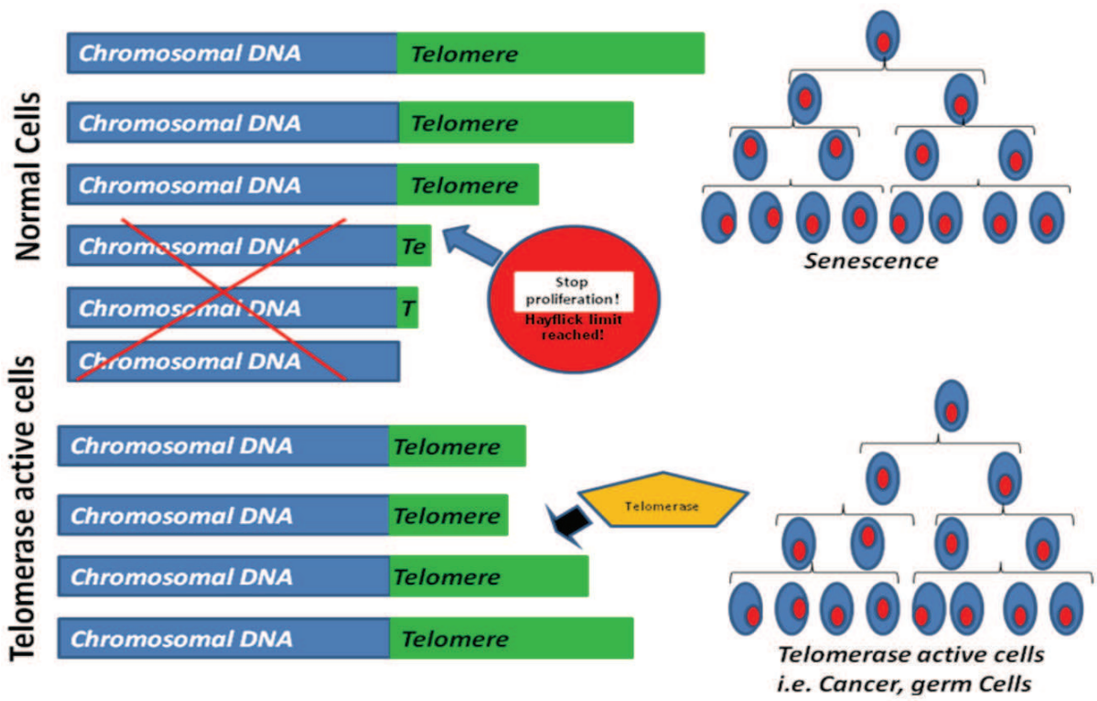

Fig. 1. Hayflick limit, senescence and telomerase reactivation

In normal cells, cell divisions are preceded by replicating the DNA to form two daughter molecules each having an original strand from parent cell and one newly synthesized strand. Replication of the leading DNA strand is simple and complete, but on the lagging strand replication uses small Okazaki fragments, to ensure 5′ to 3′ addition of bases. This results in the incomplete replication of the extreme ends of the lagging strand of chromosomes, and loss of genes. This is called the "end replication problem". The normal senescence involves the p53 and pRb pathways and leads initially to the arrest of cell proliferation (Campisi, 2005). The telomere signal that activates the senescence program operates through the Rb and p53 pathway. Rb and p53 deficient primary cells that continue cellular growth beyond the Hayflick limit exhibit severe telomere shortening, marked genetic instability and massive cell death—this period is referred to as crisis (Counter *et al*., 1992; Shay *et al*., 1991). It is thought that senescence plays an important role in the suppression of cancer emergence. Once the telomeres reach a critical short length, by means of the end replication problem, the cell enters the M1 stage (mortality 1) and goes into senescence (Kim *et al*., 1994). If the cell escapes senescence and continues to proliferate with further shortening of telomeres, it will undergo crisis or mortality 2 (M2). Cells that overcome M1 and M2 by acquiring mutational changes become immortal. Most cancers are the result of "immortal" cells that have evaded programmed cell death. At this point the cells will have acquired telomerase reactivation to maintain a constant length of telomeres (Fig 1).

Despite their stem cell characteristics when pressed to proliferate strongly, mesenchymal stem cells can also suffer from replicative senescence with critically short telomeres (Kim *et al*., 1994). This brings about certain limitations in therapeutic use of stem cells. One of these limitations is as mentioned above, the limitation in proliferative capacity, which undoubtedly can be overcome by introduction of certain genes that will enable them to continue to proliferate. It has been shown that there are numerous genes like the TERT gene that are capable of extending the proliferative capacity (Fig 1) and immortalizing the stem cells or progenitor cells, however, with the risk that malignant transformation may occur. Therefore, telomere dynamics becomes an important issue in stem cell function especially when expanding a population of stem cells is needed.

Considering the fact that stem cells in general tend to give rise to a high number of daughter cells, one would expect, that stem cells would express telomerase to maintain telomere length. But the real picture is inconsistent. In primary stem cells the replicative capacity is, however, limited. The telomere length pattern (also termed the telomere profile) can be monitored until the cells reach replicative arrest after approximately 10 population doublings (PD) and it has been shown that the telomere profile is conserved for this number of doublings. In addition, the possible conservation of profile has been studied in telomerase-immortalized mesenchymal stem cells where these cells were grown for 205 PD's and still they maintained the profile. This surprisingly long term conservation of the telomere profile clearly suggests that also in mesenchymal stem cells there is a very low degree of random fluctuation in the telomere dynamics, as previously suggested in lymphocyte progenitor cells. Overall, there is a general agreement, that adult stem cells have very low levels of telomerase, thus telomeres are slowly shortened during life. The primary mesenchymal stem cells have also been shown to obtain a diminished mean telomere length during long term culturing periods (Serakinci *et al*., 2007). (Fig2). Serakinci *et al.* have shown that the mean telomere length of the primary cells is 8 kb and the length is continuously decreasing with the population doubling level.

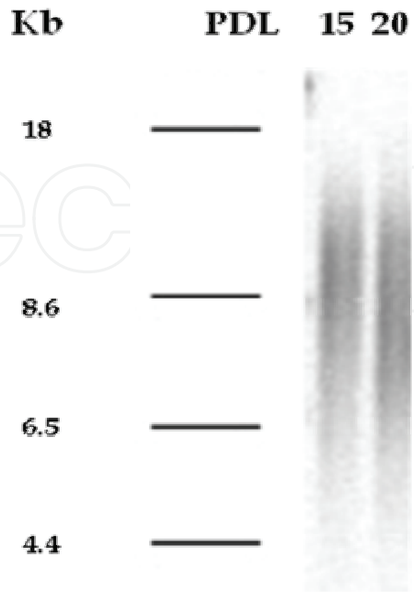

Fig. 2. Deccreased telomere length in primary hMSC with population doubling levels (PDL)

3. MSC expansion for therapeutic purposes: Neoplastic transformation

Within the last decade, human mesenchymal stem cells have been validated as potential tools in different therapeutic approaches. Many of these therapeutic potentials for MSCs have been discussed in the previous section in detail. Initial results were promising but there are some challenges for the use of MSCs in clinical applications. During the isolation process, only 1 in every 10^5 cells is MSCs and there is also the issue of low grafting efficiency and potency of MSCs. Although hMSCs have great potential in therapeutic use the main rate limiting factor is that hMSCs exhibit limited mitotic potential, especially considering that, in a human system very many cells are needed for injection. Therefore, a need for large scale MSC expansion is obvious (Momin *et al*., 2010; Teng, 2010). To overcome this proliferative limitation introduction of the TERT gene has been highlighted. Hence, telomere dynamics is an important issue in stem cell function especially when expanding a population of stem cells. Serakinci *et al.* by introducing a retrovirus carrying the hTERT gene has established an immortalized hMSC-telo1 cell-line, which maintain their stem cell characteristics and have an expanded life span (Serakinci *et al*., 2007). But extending the proliferative capacity of stem cell populations through manipulation of the telomere-telomerase system brings certain risks that are associated with the possibility that stem cells may show increased susceptibility to carcinogenesis. This manipulation bypasses the naturally built-in controls of the cell that govern the delicate balance between cell proliferation and senescence and carcinogenesis.

Given the critical role of telomere dynamics and telomerase in tumor progression and the fact that the cancer cells rely on telomerase for its survival, it is not surprising that telomeres are rather unique structures in a given cell. On one hand they have a role in protecting the chromosome ends from being recognized as DNA double strand breaks by the DNA repair machinery. On the other hand telomeres, when critically shortened, can lead to cellular senescence, which can be regarded as a barrier against cancer formation via the so-called telomere-mediated checkpoints. However, in the cells that manage to avoid being destroyed, dysfunctional telomeres can affect the genomic stability through initiating the so called Break-Fusion-Break (BFB) cycles leading to severe genomic aberrations and ultimately to cancer development (Furlani *et al*., 2009; Serakinci *et al*., 2008) (Fig 3). These events have been reported in literature in adipose-derived human MSCs (Rubio *et al*., 2005) and bone marrow-derived mouse MSCs (Miura *et al*., 2006; Tolar *et al*., 2007; Zhou *et al*., 2006). These MSCs showed phenotypic and genotypic alterations such as chromosome instability, rapid cell proliferation, loss of contact inhibition, gradual increase in telomerase activity and increased c-myc activity (Furlani *et al*., 2009).

Besides the immortalized cell line examples, there are also studies in both rodent models and human mesenchymal stem cells that have suggested that during long-term culturing mesenchymal stem cells acquired chromosomal aberrations and subsequently exhibited a malignant transformation (Rubio *et al*., 2005; Zhou *et al*., 2006). Such studies raised the concerns that hMSCs that are forced into extensive expansion can undergo spontaneous transformation. Several groups have shown results showing spontaneous transformation of human mesenchymal stem cells that are expanded long term (Serakinci *et al*., 2004; Rubio *et al*., 2005; Momin *et al*., 2010; Wang *et al*., 2005). Most studies have been done in mouse but Rubio *et al*., have shown that adipose tissue–derived human MSC populations, after a long-term *in vitro* expansion, can transform spontaneously. Rubio *et al*. further characterized the molecular mechanisms implicated in the spontaneous transformation. They have shown that

the transformation process occurred after the hMSC had bypassed senescence by up regulating c-myc and repressing p16 levels. Then, through acquisition of telomerase activity, deletion at the Ink4a/Arf locus and hyperphosphorylation of Rb followed (Rubio *et al*., 2008). It was also reported by Wang *et al*. that human MSCs derived from the bone marrow would produce a sub-population of cells when cultured. These cells would have high levels of telomerase activity, chromosomal aneuploidy and translocations and were able to form tumors in multiple organs in NOD/SCID mice (Momin *et al*., 2010; Wang *et al*., 2005). Contrary to these studies, several groups have reported no transformation of hMSC after long-term culture (Bernardo *et al*., 2007; Meza-Zepeda *et al*., 2008). Using comparative genomic hybridization, karyotyping and subtelomeric fluorescent *in situ* hybridization analysis Bernardo *et al.* performed extensive studies on the genetic changes in the hMSCs at different stages of the long-term culture but they did not find any evidence for spontaneous transformation of hMSCs during long term culture (Bernardo *et al*., 2007). In addition, there are studies demonstrating that telomerase-immortalized hMSC accumulate various genetic and epigenetic changes in spite of maintaining a normal karyotype, and ultimately showed spontaneous transformation (Burns *et al*., 2005; Serakinci *et al*., 2004). These studies supported the existence of neoplastic transformation of hMSCs during *in vitro* expansion. However, the spontaneous transformation potential of hMSCs is still a controversial issue and more evidence is needed. In addition to *in vitro* expansion studies, there are studies suggesting a role for hMSC in the carcinogenesis progression. Recent evidence thus suggests that exogenously administrated hMSCs can be recruited to the stroma of developing tumors when systemically infused in animal models for glioma, colon carcinoma, ovarian carcinoma, Kaposi's sarcoma and melanoma (Lazennec & Jorgensen, 2008).

Christensen *et al* have investigated and shown that the telomerase expression in long-term cultured telomerase-immortalized hMSCs (hMSC-telo1 cells) did not give rise to a tumor formation (Christensen *et al*., 2008). Telomerase activation most often occurs at the transition to cancer which is highly specific to cancer cells. Neoplastic transformation occurred when the cells were subjected to 2.5 Gy of gamma irradiation and subsequently cultured long-term. We, therefore, could conclude that transducing hMSC with hTERT did not unmask the neoplastic potential on its own but subsequent DNA damage such as irradiation-induced damage contributes to mesenchymal tumor development (Fig 3). These findings are in agreement with observations made by a number of other groups. Thus, it appears that using telomerase to help production of large numbers of cells is effective but it has impact on neoplastic transformation. Therefore, more information about adult stem cells and how their growth is regulated is required. Thus, close monitoring is crucial for clinical applications.

4. Two-faces of mesenchymal stem cells in cancer

It has been shown that exogenously administered hMSC may contribute to tumor stroma formation in animal tumor models by promoting angiogenesis or by creating a niche to support cancer stem cell survival (Sullivan & Hall, 2009). These tumor models include melanomas, colon adenocarcinomas, lung cancer, multiple myelomas and glioblastomas (Momin *et al*., 2010). The reason for this is thought to be the immunosuppressive ability of MSCs. As the MSCs interact with many of the cells of the innate and acquired immune system and suppress them, the tumor cells may get the chance to better evade the immune surveillance (Lazennec & Jorgensen, 2008; Momin *et al*., 2010).

Recently, Correa *et al.* has induced the development of a gastric cancer by *Helicobacter felis* infection in a C57BL/6 mouse model where normal bone marrow was replaced with bone marrow cells tagged with either beta galactosidase or GFP. With this study, they could demonstrate that in this model cancer cells are directly derived from bone marrow stem cells. During the helicobacter inflammation, these cells have been recruited to the site, where the gastric cancer later occurred (Correa & Houghton, 2007). Similar results have recently been achieved in a rat model of Barrett's metaplasia (Sarosi et al., 2008). In addition to these examples, there is substantial literature in rodent models, demonstrating the ability of MSCs' homing and transdifferentiation to inflammatory lesions at extra-medullar sites (Hayashi et al., 2008; Kahler et al., 2007; Serikov et al., 2007; Suzuki et al., 2008).

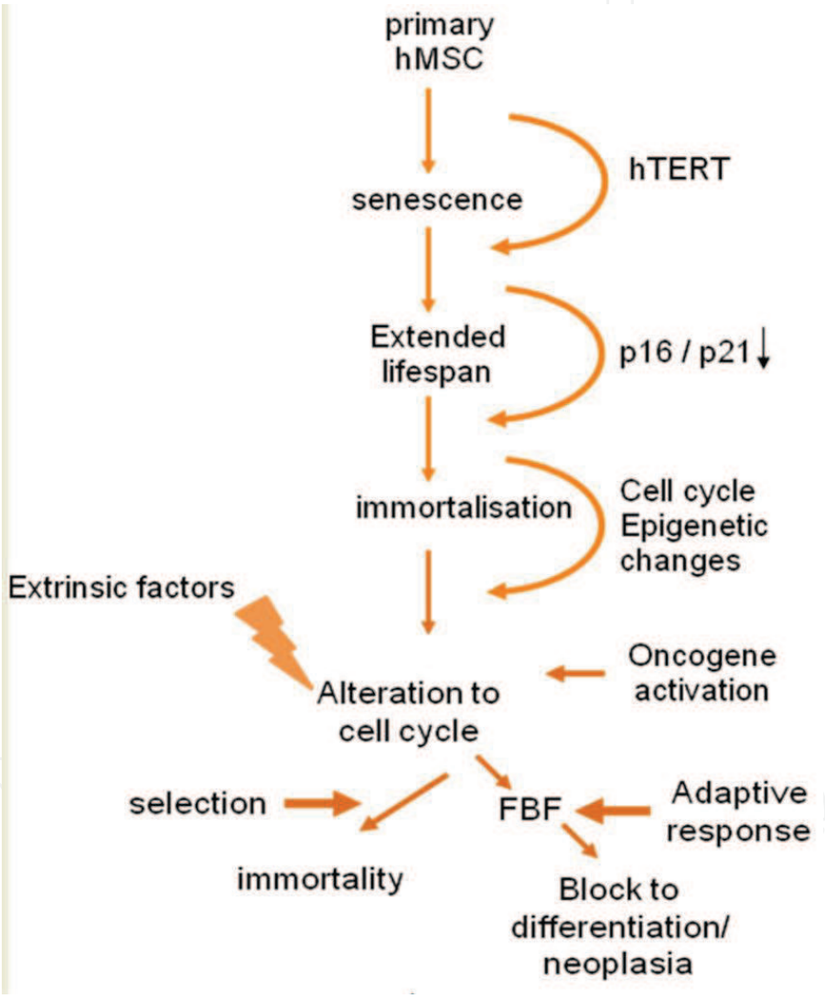

Fig. 3. Illustration of the sequence of events during the neoplastic transformation of hMSCs

Another face of the cancer and MSC interplay is that, the stromal cells of the tumor might exert transforming effects on the mesenchymal stem cells. It is thought that after a prolonged exposure to the tumor-conditioned media the MSCs can assume a carcinoma associated fibroblast (CAF)-like phenotype. CAF are known to support the growth of cells and angiogenesis in breast cancer. These observations have lead us to consider that upon

exposure to the tumor environment, MSCs could transform into CAFs and may help in tumor growth and even spread (Momin *et al*., 2010). However, the current animal models are not enough to help us understand this complex relationship that exists between MSCs and tumors. The animal models used now rely on harvesting the human tumor cells and culturing them *in vitro* before grafting them into the animal. The problem with this approach is that during the culturing process the cells are away from the tumor stroma and the epithelial cells. And even when the cells are inside the animals the stroma will be formed from the animal's own cells leading to a chimeric tumor that would not reproduce the interaction between the MSCs and the tumor stroma. Perhaps the new generation of immunocompromised mice such as the NOG mouse will be able to help in the investigation of this relationship as it is better able to accept heterologous cell populations (Hahn & Weinberg, 2002; Ito *et al*., 2002; Kim *et al*., 2004; Momin *et al*., 2010; Rangarajan & Weinberg, 2003; Rosen & Jordan, 2009).

Today, cancer still remains as one of the most challenging diseases with regards to treatment. One of the biggest problems is that there is really no selective killing towards tumor cells. Thus, therapies that are more specifically directed towards cancer stem cells might result in much more durable responses and even might also cure the metastatic tumors. While hMSCs play a role in supporting tumor formation, based on their homing abilities hMSC have been used as cellular vehicles for local delivery of biological agents to brain tumors. Human MSCs were transduced with a lentivirus expressing secretable TRAIL (S-TRAIL) and mCherry (red fluorescent protein) and injected into established intracranial glioma tumors in mice. The genetically modified hMSCs were able to inhibit tumor growth, resulting in significantly longer animal survival. Thus, the study demonstrated the therapeutic efficacy of hMSC S-TRAIL cells and confirmed that hMSCs can serve as a powerful cell-based delivery vehicle for the site-specific release of therapeutic proteins (Menon *et al*., 2009). In earlier experiments looking at targeted delivery, MSCs genetically engineered to secrete IFN-β were able to successfully home and engraft at melanomas growing in mice lungs and locally deliver IFN-β. When delivered locally, IFN-β was able to inhibit the growth of malignant cells both *in vivo* and *in vitro*. The same effect could not be achieved with systemically delivered IFN-β or IFN-β produced away from the tumor site (Studeny *et al*., 2002; Studeny *et al*., 2004). In a recent experiment, MSCs were shown to be recruited to and incorporated into the prostate epithelium during prostate regrowth (after testosterone reintroduction). The incorporated MSCs were used to deliver frizzled related protein-2 (SFRP2) to antagonize the Wnt-mediated cancer progression by reducing tumor growth, increasing apoptosis and causing potential tumor necrosis (Placencio *et al*., 2010).

hMSC having the potential to home to the tumor stroma allows them to be a promising tool for the delivery of anticancer drugs to the tumor microenvironment. This strategy has been shown to work by the observation of specific homing of intravenously administered hMSCs, engineered to produce interferon-β (IFN-β), to tumors with subsequent tumor regression in a xenogenic mouse model (Studeny *et al*., 2004). This study showed that mesenchymal stem cells expressing IFN-β could inhibit the growth of tumor cells *in vivo*. The approach required integration of the hMSC at the tumor site, because non-tumor site integrated or systemic delivery of IFN-β did not have enough tumor regressing effect. These findings have recently been supported by Serakinci *et al.* where they have shown that hMSC can home to tumor site and furthermore could deliver a therapeutic agent to the site (Serakinci *et al*, in press).

Besides the abovementioned studies, Seo *et al*. have demonstrated that IL-12M-expressing MSC injection directly into the tumor had the strongest antitumor effect compared with other injection routes such as intravenous or subcutaneous. In addition to the inhibition of solid tumor growth, the same study also demonstrated anti-metastatic effects for MSCs/IL-12M when embedded in the Matrigel (Seo *et al*., 2011). Furthermore, Correa *et al*. have demonstrated that gastric cancers may originate from hMSCs (Correa & Houghton, 2007). Based on these and similar studies, we can suggest that hMSCs can modulate the tumor growth, although this issue still remains controversial and not fully understood.

It seems that to incorporate cancer-fighting genes inside stem cells grown from a patient's bone marrow mesenchymal stem cells are promising therapeutic and newly emerging approaches. Success of such approaches is dependent on well designed vectors or viruses carrying therapeutic agents. Then those vectors or viruses can be inserted into stem cells e.g. mesenchymal stem cells, which are grown from a sample of a patient's bone marrow. Such stem cells would not be rejected as foreign objects by the immune system when they are injected back into the patient. Once inside the patient's bloodstream, the stem cells would migrate to tumor site and release the anti-carcinogenic agent. The basic approach would be to harvest MSCs, modify them such that they secrete an anti-neoplastic compound and as the last step, administer them into an animal that has a tumor. To date, various stem cells have been modified to secrete several different compounds that would either reduce the tumor size or prolong the survival of the organism. These compounds had varying degrees of success (Aboody *et al*., 2008; Momin *et al*., 2010).

Gene and viral therapies for cancer have shown some therapeutic effects, but there has been a lack of real breakthrough. Based on others and our studies it seems that the identification of reservoirs of multipotential stem cells within adult tissue provides exciting prospects for developing novel vehicle for stem cell-mediated gene therapy. This new strategy seems to produce stronger and more specific anti-tumor effects. But considering that one of the hallmarks of cancer development is continued cell growth, which is most often correlated with activation of telomerase, the question must be raised if there is a potential cancer risk of genetically engineered cells. Critical to such approaches will be an ability to remove or inactivate the genetically engineered stem cells that homed to tumor site at the time of delivery of the targeted treatment.

One approach might be to use telomerase inhibitors that may be a way to stop proliferation of these particular cells. Since the telomerase is essential to the life of a cell, treatments with modified structures called telomerase inhibitors will eventually lead to cell death. But this approach might face another problem namely that some of these cells will escape the cell death and this will lead to genomic instability, ultimately causing development of a new cancer. Another and perhaps safer approach might be that after delivery of the targeted treatment, the vehicle cells should be engineered to commit suicide. Such a strategy has been used in connection with the tumor-selective viruses that mediate oncolytic effects on tumors due to genetically modified viruses, which is engineered to replicate in and kill targeted cancer cells. Such viruses have been engineered with tumor-specific transcriptional response elements based on the telomerase promoter sequence thereby attacking telomerase-positive cells (Abdul-Ghani *et al*., 2000; Bilsland *et al*., 2005; Komata *et al*., 2001b; Plumb *et al*., 2001). These studies have suggested that combinational therapy approaches (genetically engineered vehicle stem cell therapy and the suicide gene therapy) might improve targeted therapies and at the same time reduce the risk of secondary tumors.

Numerous clinical trials have been published involving MSCs. In many of these studies the MSCs were evaluated only in a non-cancerous context like myocardial infarctions or the role of MSCs in graft versus host disease (Momin *et al*., 2010). No acute or long-term effects have been reported so far including any reports of carcinogenesis. The longest follow up reported in these studies is 3 years. However, more information and studies are needed regarding these studies as carcinogenesis might be a long process. Another point to consider is that most of these studies were carried out on patients with poor prognosis which might be masking any adverse effects from MSCs. Furthermore, there has been no appropriate imaging methods used in clinical trials to look for carcinogenesis in the body. The last point we will need to keep in mind regarding the use of MSCs in therapy is that so far not many genetically-modified MSCs have been used in clinical trials published to date (Momin *et al*., 2010). Carcinogenesis issues remain a hot topic with regards to MSCs and will always need to be carefully evaluated. The risk from the illness, the risk of not getting a treatment will have to be weighed against the risk of transformation of MSCs, which may lead to carcinogenesis.

5. Future directions

Considering the neoplastic transformation risk of genetically engineered hMSC and their role in tumor formation different systems should be devised to address this risk during the conduction of therapeutic approaches. It is critical to place a fail-safe system that will remove or inactivate the genetically engineered stem cells once they have homed to the tumor site and delivered the therapeutic agent. To overcome the expansion related risks Momin *et al*. have suggested using MSCs from different sources to reach to a larger number of cells with fewer number of passages. Another approach might be to use telomerase inhibitors in an effort to stop the proliferation of the genetically modified MSCs once they were done with delivery. Since the telomerase is essential to the life of these telomerased cells, treatment with telomerase inhibitors will eventually lead to cell death. However, this approach might raise yet another problem; namely, some of these cells might escape cell death which will lead to genomic instability and ultimately cause the development of a new cancer. Alternatively, another and perhaps safer approach might be to cause the vehicle cells to commit suicide after delivery of the targeted treatment. This strategy has been used in connection with tumor-selective genetically modified viruses that mediate oncolytic effects on tumors by replicating in and killing cancer cells in a targeted manner. These viruses contain transcriptional response elements based on the telomerase promoter sequence thereby attacking telomerase-positive cells, which includes the genetically modified MSCs in this case (Abdul-Ghani *et al*., 2000; Bilsland *et al*., 2005; Komata *et al*., 2001a; Plumb *et al*., 2001).

Taken together, studies on the impact of neoplastic transformation of the hMSC and the studies on impact of using telomerase to help production of large numbers of cells, one could conclude that caution is warranted for stem cell-based therapies. However, this does not mean that these studies need to be put off. Thus, considering their susceptibility to neoplastic transformation and potential to home to tumor site one of the key questions that still needs to be addressed is; how safe is to use hMSC for targeted cancer therapy? And the answer to this question is that there is to date good indications that human bone marrow MSC, which will be isolated from individuals and *in vitro* expanded, can be used for cancer therapy especially as delivery vehicles targeting tumor stroma after a careful safety

monitoring and preclinical tests. Still, for successful clinical applications more information about human mesenchymal stem cells and their growth regulation is still required.

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Cancer Stem Cells - The Cutting Edge

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Over the last thirty years, the foremost inspiration for research on metastasis, cancer recurrence, and increased resistance to chemo- and radiotherapy has been the notion of cancer stem cells.The twenty-eight chapters assembled in Cancer Stem Cells - The Cutting Edge summarize the work of cancer researchers and oncologists at leading universities and hospitals around the world on every aspect of cancer stem cells, from theory and models to specific applications (glioma), from laboratory research on signal pathways to clinical trials of bio-therapies using a host of devices, from solutions to laboratory problems to speculation on cancersâ€™ stem cellsâ€™ evolution. Cancer stem cells may or may not be a subset of slowly dividing cancer cells that both disseminate cancers and defy oncotoxic drugs and radiation directed at rapidly dividing bulk cancer cells, but research on cancer stem cells has paid dividends for cancer prevention, detection, targeted treatment, and improved prognosis.

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