Histol Histopathol (2004) 19: 505-509

http://www.hh.um.es

Histology and Histopathology

Cellular and Molecular Biology

Review

Relationships between stem cells and cancer stem cells

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Summary. Stem cells have been shown to exist in a variety of tissues. Recent studies have characterized stem cell gene expression patterns, phenotypes, and potential therapeutic uses. One of the most important properties of stem cells is that of self renewal. This raises the possibility that some of the clinical properties of human tumors may be due to transformed stem cells. Similar signaling pathways may regulate self renewal in normal and transformed stem cells. These rare transformed stem cells may drive the process of tumorigenesis due to their potential for self renewal. There are important ramifications for clinical cancer treatment if the growth of solid tumors is at least partially dependent on a cancer stem cell population. In the cancer stem cell model, tumor recurrence may be due to the non-targeted stem cell compartment repopulating the tumor. If cancer stem cells can be prospectively identified and isolated, it should be possible to identify therapies that will selectively target these cells.

Key words: Embryogenesis, Plasticity, Transformation, Tumorigenesis, Cell lineage

Introduction

In its simplest form, a stem cell renews itself and gives rise to differentiated progeny (for review see Morrison et al., 1997; Blau et al., 2001). Self renewal has been demonstrated in clonogenic assays and transfer to recipients. Other stem cell characteristics have been proposed including the ability to divide asymmetrically, exist in a mitotically quiescent state, and regenerate. Stem cells are believed to undergo divisions that produce one differentiated and one stem progeny. In longer lived organisms, certain subsets of stem cells may begin division at different ages. Regulation of self renewal may be controlled by extrinsic factors, such as the available space in the stem cell niche. Stem cell proliferation has been shown to increase in response to injury (Forge et al., 1993). Certain proteins such as epidermal growth factor and basic fibroblast growth factor can promote stem cell self renewal (Johe et al., 1996). Other proteins such as transforming growth factor ß can inhibit stem cell renewal (Podolsky, 1993). Not all stem cells have the same capacity for self renewal, which may be an intrinsic property (Harrison and Zhong, 1992). Despite evidence for asymmetric division, symmetrical cell division also occurs in fetal liver hematopoietic stem cells (Morrison et al., 1995). It is not entirely clear whether stem cells exist in a G_0 state or progress slowly through G_1. Differentiation may be induced when the microenvironment no longer promotes maintenance of the uncommitted state, either by default or due to specific factors.

Embryonic stem cells are believed to be pluripotent, and these cells have been shown to differentiate into a variety of cell types. Adult stem cells are more restricted in their proliferation and differentiation, being limited to the tissue in which they reside. Recent studies have suggested greater plasticity in adult stem cells (for review see Blau et al., 2001). Fate changes may be a property of adult stem cells in order to repair damaged tissue. However, some of this plasticity has been attributed to fusion of putative stem cells with diverse differentiated cell types (Terada et al., 2002). Recruitment of specific cells from diverse tissues to distant sites has been suggested as a newly recognized property of stem cells, but has long been recognized as a characteristic of metastatic cancer cells. Indeed a well developed model of cancer cell metastasis proposes that discrete subpopulations are responsible for this phenotype (Reya et al., 2001). The clinical phenotypes of certain cancers such as leukemia may be due in part to small groups of transformed stem cells (Bonnet and Dick, 1997). In other types of cancers such as squamous cell carcinomas, stem cells may be more likely to undergo transformation than proliferating basal layer cells since the latter divide for relatively short periods before terminally differentiating (Watt, 2001). It is less likely that a large proliferating population could acquire

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the self renewal potential of stem cells in order to accumulate additional mutations leading to tumorigenesis. It appears then that oncogenic mutations could be inherited from transformed stem cells and thus give rise to the diverse cell populations observed in many solid tumors. This review examines properties of embryonic and adult stem cells and considers whether these features are potentially important for tumorigenesis.

Embryonic stem cells

Embryonic stem cells are derived from early stage mammalian embryos and are believed to have broad developmental potential. These cells generate multiple cell types found in diverse tissues. Among these tissue types are blood, muscle and brain. Using a five step process embryonic stem cells were shown to differentiate into dopamine neurons that functioned in animal models of Parkinson's disease (Kim et al., 2002). The Nurr1 transcription factor was used as a marker of dopamine neuron differentiation. Nurr1 induced expression of tyrosine hydroxylase, another neuronal marker. Addition of leukemia inhibitory factor to the cultures promoted differentiation of neurons. Fibroblast growth factor 8 and sonic hedgehog dramatically increased numbers of serotonin positive neurons. Other neuron specific genes were upregulated in the differentiated population such as amino acid decarboxylase and the homeobox gene Ptx3. These cells were successfully engrafted into an animal model of Parkinson's disease with improvement of signs.

Embryonic stem cells have also been shown to differentiate into motor neurons (Wichterle et al., 2002). Retinoic acid treatment of embryoid bodies caused caudalization of neuronal progenitors as determined by expression of Hoxc5 and Hoxc6. Motor neuron differentiation was dependent on sonic hedgehog signaling. High levels of sonic hedgehog produced large numbers of motor neuron progenitors. Differentiation of stem cell derived motor neurons was shown in the embryonic spinal cord using fluorescent protein labeling. Proper segregation of these cells was observed even when initial placement was not controlled. Expression of mouse specific markers provided evidence against somatic cell fusion. The axons of stem cell derived motor neurons projected along normal trajectories and formed junctions with muscle targets.

Embryonic stem cells have been shown to differentiate into other tissue types such as pancreatic islets (Lumelsky et al., 2001). Using a multistage differentiation procedure, insulin secreting cells were isolated from mouse embryoid bodies. These cells also produced glucagon, somatostatin, and pancreatic polypeptide. Expression of these markers coincided with cessation of cellular proliferation. Insulin secretion was shown to respond to glucose levels in the cultures. A number of agonists and antagonists of insulin secretion appropriately regulated production of this hormone. However, engraftment of these cells into diabetic mice did not show sustained correction of glucose levels.

Adult stem cells

Neural stem cell properties have been characterized in brain ependymal cells (Johansson et al., 1999). Olfactory bulb neurons were shown to derive from ependymal cells. Single ependymal cells were capable of forming neurons, astrocytes, and oligodendrocytes. Neural stem cells were purified utilizing an ependymal cell marker Notch 1. These cells exhibited a slow proliferation rate and generated an amplifying progenitor population which increased in response to injury. The astrocyte differentiation pathway also was stimulated following injury. Oligodendrocyte precursors have been shown to revert to a neural stem cell phenotype in response to specific extracellular signals (Kondo and Raff, 2000). The neural stem cell phenotype was observed following culture in PDGF, serum, basic FGF, and EGF. Neural and glial cell markers were detected under various growth conditions.

A separate study purified a pluripotent neural stem cell from mouse brain by expression of a variety of cell surface markers (Rietze et al., 2001). These cells formed neurospheres which contained astrocytes positive for glial fibrillary acidic protein, neurons positive for ß-tubulin type III, and oligodendrocytes. The neural stem cells also expressed nestin which is a marker of this lineage and were predominantly found in ependymal and subventricular zones. Adult astrocytes from the hippocampus can also regulate neurogenesis by allowing stem cells to adopt a neuronal fate (Song et al., 2002).

Functional human hematopoietic stem cells have also been characterized (Berardi et al., 1995). In this protocol, human bone marrow cells that responded to Kit ligand and interleukin 3 were forced to undergo chemotherapy mediated apoptosis. The surviving fraction represented approximately 1 in 100,000 bone marrow mononuclear cells and were quiescent by cell cycle analysis. These cells expressed genes characteristic of stem cells by immunohistochemistry. The population was highly enriched in cells which could initiate long term culture, were capable of secondary colony formation, and produced both myeloid and lymphoid progeny. A subsequent study generated self renewing and multipotent stem cells from adult human bone marrow stromal cells (Colter et al., 2001). Bone marrow stromal cells were shown to contain three morphologically distinct types: spindle shaped, large flat cells, and very small round cells that rapidly self renewed. Samples enriched for small cells had greater potential for differentiation. The small cells possessed surface epitopes and other markers that could be used to distinguish this subpopulation. A subpopulation of cells co-purifying with marrow mesenchymal stem cells were shown to differentiate at the single cell level into mesenchymal, visceral endoderm, neurectoderm, and endoderm (Jiang et al., 2002). When injected into an

early blastocyst, these cells contributed to all somatic tissue types. When transplanted into a non-irradiated host the cells engrafted and differentiated into hematopoietic, liver, lung, and gut cells. Hematopoietic and gastrointestinal engraftment was increased when the cells were transplanted into an irradiated host. These cells proliferated extensively without senescing or loss of differentiation potential. Wnt signaling has been shown to regulate the self renewal process of hematopoietic stem cells (Reya et al., 2003). Overexpression of activated ß-catenin expanded the pool of stem cells in long term culture. Hematopoietic stem cells also activated a LEF-1/TCF reporter, indicating that these cells respond to Wnt signaling. Inhibition of Wnt signaling led to growth inhibition and reduced reconstitution in vivo. Wnt signaling also induced HoxB4 and Notch1 expression, which are self renewal markers in hematopoietic stem cells.

There has been substantial interest in characterization of stem cells from human epidermis for many years (for review see Jones, 1997; Lavker and Sun, 2000; Slack, 2000; O'Shaughnessy and Christiano, 2001; Watt, 2001; Potten and Booth, 2002; Alonso and Fuchs, 2003). A number of studies have attempted to identify markers to identify epidermal stem cells. Among these markers are ß1 integrin (Jones and Watt, 1993; Jones, 1996). Keratinocytes with characteristics of stem cells were isolated from cultured human epidermal cells based on high ß1 integrin expression and adhesion to extracellular matrix. ß1 integrin expression correlated with proliferative capacity in culture. Increased population doublings has been confirmed as a property of keratinocyte stem cells in culture (Bickenbach and Chism, 1998; Dunnwald et al., 2001). Cells with highest ß1 integrin expression adhered rapidly to type IV collagen and fibronectin. Cells with low ß1 integrin expression underwent rapid terminal differentiation. Similar conclusions were reached using α6 integrin as a cell surface marker (Li et al., 1998). These results establish integrins as promising marker proteins for identifying epidermal stem cells.

The p53 homologue p63 has been proposed as a marker of epidermal stem cells (Pellegrini et al., 2001). Nuclear p63 expression was identified in basal cells of limbal epithelium which were highly proliferative in culture. In contrast, telomerase expression was decreased in epidermal stem cells (Bickenbach et al., 1998). Calcium treatment which induces terminal differentiation of keratinocytes downregulated telomerase expression in a dose dependent manner. These studies have provided additional candidate markers with which to identify putative epidermal stem cells.

Hair follicular stem cells located in the bulge region have been shown to give rise to several cell types in the follicle and upper follicular cells (Taylor et al., 2000). Expression of putative epidermal stem markers were localized to the entire hair germ and subsequently to the outermost cells and bulge region (Akiyama et al., 2000). These cells showed undifferentiated morphologic features and expressed high levels of epidermal growth factor receptor and keratin 19. These findings indicate that the hair follicle represents a major repository of epidermal stem cells that are potentially pluripotent.

Human breast epithelial cell lines with stem cell properties have also been isolated (Gudjonsson et al., 2002; Dontu et al., 2003). By using cell surface markers and immunomagnetic sorting, two epithelial cell populations were isolated from the luminal compartment of reduction mammoplasties. The major population expressed sialomucin and epithelial specific antigen. The minor population expressed epithelial specific antigen but no sialomucin. The cell lines were established by transduction of the E6/E7 genes from human papillomavirus type 16. Both cell lines maintained a luminal phenotype as determined by expression of claudin 1 and occludin. In clonal culture, the double positive cell population was restricted in its differentiation. However, the epithelial specific antigen positive and sialomucin negative population was able to regenerate itself, double positive cells, and myoepithelial cells expressing smooth muscle actin. The epithelial specific antigen positive and sialomucin negative cells also expressed keratin 19 found in terminal duct lobular units in vivo. The double positive population formed cellular spheres resembling acini in reconstituted basement membrane. However the single positive population formed branching structures resembling terminal duct lobular units. These structures were also obtained by inoculating the cells into nude mice. The generation of mammospheres was also demonstrated during in vitro culture. These cells were able to proliferate in suspension and were enriched in early progenitor stem cells. These cells were able to differentiate into all mammary epithelial lineages and clonally regenerate complex functional structures in vitro. Gene expression analysis revealed overlapping patterns with other stem and progenitor cells and identified new potential stem cell markers. These studies may uncover pathways that govern normal mammary development and breast carcinogenesis.

The isolation of clonogenic fibroblastoid cells from peripheral blood of different species has been reported (Kuznetsov et al., 2001). This population was notable for its lack of hematopoietic and endothelial marker protein expression. The population expressed collagen, fibronectin, osteonectin, and smooth muscle actin. Bone deposition was demonstrated under specific conditions upon transplantation into immunodeficient mice. Some of the bony transplants also formed complete marrow including adipocytes. Osteoblasts and osteocytes in the bone matrix were demonstrated to be of donor origin. These results indicated that cells with osteogenic potential could be isolated from the circulation.

Hepatic stem cells have also been identified by flow cytometry and clonogenic assays (Suzuki et al., 2002). These cells could be propagated in culture where they gave rise to hepatocytes while some clones were

maintained in the stem cell state. Transplanted cells also gave rise to functional hepatocytes and bile duct structures. These cells also differentiated into pancreatic ducts and acini or intestinal epithelium when implanted into these anatomic sites. These results indicated that self renewing stem cells were present in the developing mouse liver. These cells could be induced to differentiate into other endodermal organs in the appropriate microenvironment.

Cancer stem cells

If signaling pathways that normally regulate stem cell renewal become dysregulated, it is possible that stem cells become the target of transformation in cancer (for review see Reya et al., 2001). Since stem cells are capable of self renewal, maintaining this phenotype is likely less difficult than inducing it in differentiated cells. Stem cells also persist for long time periods rather than dying or undergoing terminal differentiation. Therefore there is more opportunity for mutations to accumulate leading to cancer. Progenitor cells are less likely than stem cells to undergo transformation because they proliferate for a shorter time period. Progenitor cells would have to acquire the extensive self renewal potential of stem cells to accumulate the additional mutations leading to transformation. However, progenitor cells could inherit mutations from stem cells so that fewer new genetic insults would be required for transformation. The cells which can produce acute myeloid leukemia in immunocompromised mice express stem cell markers (Bonnet and Dick, 1997). Leukemia cells without stem cell markers are incapable of producing disease in these mice, suggesting that stem cells are the target for transformation. The chromosomal translocations that occur in these cells were found in the stem cell population, and subsequent generations acquired additional mutations leading to leukemia. Corresponding findings have also been observed in lymphoid and chronic myeloid leukemia (George et al., 2001; Mauro and Druker, 2001).

Both normal stem cells and tumorigenic cells have extensive proliferative potential and ability to produce new cells. Tumors and normal tissue are composed of different cell types with divergent phenotypic characteristics (Fidler and Hart, 1982). Many tumors have a clonal origin, therefore these cells must give rise to progeny with varying degrees of proliferative potential. This suggests that tumor cells undergo processes that are analogous to those of stem cells.

When human breast cancer cells were grown in immunocompromised mice, only a fraction of the cells were able to form new tumors (Al-Hajj et al., 2003). Tumorigenic breast cancer cells were distinguished from nontumorigenic ones based on cell surface protein expression. These cells were CD44 positive and CD24 negative, and as few as 100 were able to form tumors in mice. In contrast, tens of thousands of cells with the alternate phenotype failed to form tumors. The tumorigenic cells could be serially passaged and formed both groups of cell types. The ability to identify these cells will facilitate their molecular characterization and possibly lead to more effective therapies.

Clinical implications

There are important ramifications for clinical cancer treatment if the growth of solid tumors is at least partially dependent on a cancer stem cell population. Clinically, malignant tumors are often treated as largely homogeneous groups of cells with similar replicative and invasive potential (Reya et al., 2001). However, cancer cells have been shown to disseminate from primary tumors without development of metastatic disease suggesting that only rare clonogenic stem cells can invade and replicate (Salsbury, 1975). Additionally, most nonsurgical therapy targets dividing cell populations to shrink solid tumors (Stockler et al., 2000). Often these effects are temporary and may not increase patient survival (Lippman, 2000). In the cancer stem cell model, this recurrence may be due to the non-targeted stem cell compartment repopulating the tumor, or that stem cells are inherently more resistant to chemotherapy (Harrison and Lerner, 1991). If cancer stem cells can be prospectively identified and isolated, it should be possible to identify therapies that will selectively target these cells.

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Accepted November 5, 2003