Current views on stem cell biology and plasticity

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ABSTRACT
Multipotent stem cells are found in mature tissues and are formed by the body to replace worn out cells in tissues and organs. Hematopoietic stem cells (HSCs) are present in peripheral blood, bone marrow and cord-blood and are capable to give rise to blood and immune system cells. HSCs can be enriched using several techniques and can be cultured in clonogenic or expansion culture-assays. Embryonic stem cells (ESCs) are pluripotent cells derived from blastocysts that can be propagated indefinitely undifferentiated in vitro, can differentiate to all cell lineages in vivo and can be induced to differentiate to all cell lineages in vitro, and can be induced to differentiate to most cell types in vitro. Mesenchymal Stem Cells were also identified and it was shown that when bone marrow is plated in fetal calf serum containing medium, colonies of adherent fibroblast like cells develop that differentiate into bone and adipocytes. Embryonic stem cells have been utilized in applied research to provide a source of stem cells for the amelioration and treatment of human diseases which can not be treated by conventional techniques and modalities. The ability of tissue-specific stem cells to differentiate to cell types different from the tissue of origin has been defined as "stem cell plasticity". The use of embryonic stem cells in regenerative medicine raises ethical, legal and social concerns among the public. These stem cell-based therapies can be broadly implemented for diseased individuals with Parkinson’s disease, Alzheimer’s disease, diabetes mellitus, spinal cord-injuries, osteoarthritis. These new approaches may also offer another advantage of overcoming the problem of tissue rejection in transplantation. [Turk J Cancer 2003;33(2):69-74]

KEY WORDS:
Stem cell plasticity, embryonic stem cells, mesenchymal stem cells

INTRODUCTION
Multipotent stem cells are found in mature tissues and are formed by the body to replace worn out cells in tissues and organs (1,2). Stem cells in bone marrow, called hematopoietic stem cells form the various kinds of blood cells. Neural stem cells form the brain and central nervous system. Mesenchymal stem cells form fat, bone, muscle and cartilage. Multipotent stem cells are sometimes called somatic or adult stem cells (1-5).

Within last twenty years several in-vitro techniques have been developed to isolate, clone and grow stem cells in soft agar or methylcellulose-based media. Utilizing special Petri dishes into which the stem cells are seeded virtually all three hematopoietic lineages can be grown including erythroid, myeloid and megakaryocytic colonies (6).
Types and features of stem cells

Hematopoietic stem cells (HSCs) are present in peripheral blood, bone marrow and cord-blood and are capable to give rise to blood and immune system cells. HSCs can be enriched using several techniques and can be cultured in clonogenic (colony-assay) or expansion culture-assays. The culture of choice will depend on the goal of the scientist to fully characterize the stem cells or simply increase the number of progenitors. Colony assays have been widely used for a wide variety of clinical and research applications. HSCs can be induced in-vitro to form stem cell colonies provided that the appropriate growth factors or colony-stimulating factors (CSFs) have been incubated and present in the culture media (7) (Figure 1).

The potential uses of hematopoietic stem cells (HSCs) in the therapy of human diseases continue to progress very rapidly. In last three decades, HSCs are utilized to treat the chemotherapy or chemo-radiotherapy induced myelosuppression as well as in the treatment of hematologic malignancies, solid tumors and non-malignant diseases (8-10).

Cord blood stem cells

Umbilical cord blood contains neonatal and a rich concentration of stem cells. These cells are pluripotent and are capable of differentiating into various cell types such as hematopoietic cells (blood and immune system-forming cells) (11). These properties of cord blood stem cells are similar to those shown by embryonic stem cells. However, unlike embryonic stem cells, umbilical cord blood stem cells are abundant, easily collected and are not highly immunogenic. This source is abundant, easily collected and is free from moral and ethical concerns. Stem cells from cord blood have been used in stem cell transplantation procedures in the treatment of life-threatening or debilitating diseases (12). Parents choose to preserve umbilical cord blood for many reasons. They may have an immediate family member who requires a stem cell transplant and their baby’s cord blood may be an appropriate source of stem cells for treatment of the disease. Parents may decide to store cord blood because their family’s medical history may indicate there is an increased risk of disease. Finally, some parents decide to preserve their child’s cord blood as a protective precaution should it ever be needed as a stem cell source for the child from which it was obtained or another family member (10-12).

Embryonic stem cells

Embryonic stem cells (ESCs) are pluripotent cells derived from blastocysts that can be propagated indefinitely undifferentiated in vitro, can differentiate to all cell lineages in vivo and can be induced to differentiate to all cell lineages in vivo, and can be induced to differentiate to most cell types in vitro (13-16). ESCs are derived from the inner cell mass of the blastocyst which is the early developmental stage of the embryo prior to its implantation in the uterus wall. As well studied and widely recognized ESCs are able to differentiate into cells from all three embryonic germ layers which is defined as pluripotency. ESCs similar to other stem cell types are highly capable of self-renewal, maintain a full diploid karyotype and generate any tissue when introduced into an embryo (4-8) (Figure 2).
ESCs are unrestricted in their pattern of differentiation mainly to the somatic and germ cell lineages. ESCs can also be manipulated to differentiate into a diversity of cell types (17,18). It is commonly practiced that ESCs be cultured on feeder cells and the cells pile up maintaining discrete colonies on top of the feeder layers. The ESC lines usually are maintained at relatively high densities to obtain high growth rate in order to minimize spontaneous differentiation. The initial choice of cells is from the blastocyst stage embryo. Blastocysts are grown in culture in standard tissue-culture media and usually do not require special reagents. The recovery of stem cells usually is in the range of 10 to 30%, and commonly many embryos are lost and wasted. Following the use of best methods for the culture of blastocysts, pluripotential stem cell colonies have to be accurately identified using cellular morphology and careful observation of growth patterns (19-21).

Mesenchymal stem cells

Mesenchymal Stem Cells (MSCs) were identified and it was shown that when bone marrow (BM) is plated in fetal calf serum (FCS) containing medium, colonies of adherent fibroblast like cells develop that differentiate into bone and adipocytes (24). Since then, several investigators have shown that these cells can also differentiate into chondrocytes, adipocytes and at least in rodents, skeletal myocytes. MSCs can be purified on the basis of their ability to adhere to plastic. Expansion of cells from postnatal BM that can differentiate at the single-cell level not only into MSCs, but also into cells of visceral mesodermal origin, such as endothelium, which are defined as mesodermal progenitor cells (MPCs) (25-27).

Embryonic stem cells have been utilized in applied research to provide a source of stem cells for the amelioration and treatment of human diseases which can not be treated by conventional techniques and modalities. In view of their pluripotency many investigators initiated very promising research projects exploring therapeutic potentials of ESCs. It was soon reported that when ESCs are exposed to appropriate “cocktail” of growth factors, and “inducers” in the in-vitro cultures, they were able to differentiate into myocytes, neural cells, hepatocytes, endothelial cells and even to adipocytes (28-30) (Figure 3).
In principle, ESCs can make every cell in the body and they can make a differentiated cell (28-30). Researchers at the Columbia University showed directed differentiation of mouse ESCs into spinal progenitor cells and motor neurons. In recent experiments it has been demonstrated that working with ESCs have advantages over adult stem cells in which researchers can use normal developmental patterns as a guide in developing strategies for controlled differentiation.

The high potential of stem cells for generating non-hematopoietic cells for tissue repair and in regenerative medicine several studies are underway to explore these issues (31,32).

**Definition of stem cell plasticity**

The ability of tissue–specific stem cells to differentiate to cell types different from the tissue of origin has been defined as “stem cell plasticity” (33). Some explanations have been proposed for stem cell plasticity: a) persistence of multipotent stem cells in post-natal life, b) de-differentiation and re-differentiation of stem cells, c) presence of multiple tissue specific stem cells in different organs, and d) fusion of donor cells and resident cells (34,35).

Most studies of adult stem cell plasticity have used mouse models. Using (immune-deficient) SCID/beige mice, human adult stem cells derived from bone marrow or cord blood when injected in various tissues human donor cells repopulated both the bone marrow and liver tissue of recipient mice. Recent research studies have demonstrated that adult stem cells can exhibit a much wider potential for development and differentiation.

The extensive capacity of HSCs to maintain their numbers by self-renewal mechanisms clearly support the notion that stem cell population does not go through senescence process. Adult HSCs which constitute approximately 0.01% of the bone marrow population are long-lived cells.

**Applications of stem cells**

In last five years it has been demonstrated that adult stem cells can differentiate in various lineages. Mouse ESCs were noted to have the ability to produce new strains of animals with specific genetic changes, and their capacities to differentiate into multiple lineages in in-vitro cultures. The differentiation of embryoid bodies develops into a variety of cell types including endothelial, muscle, neuronal, hematopoietic and hepatic with high potential of adult and embryonic features (29,30).

Nowadays most clinicians and basic scientists are trying to develop techniques for application of human ESCs in cell-based replacement therapies. This type of approach can replace diseased or “damaged” tissue or cell populations. Although, the research in this area is in its infancy, long-term outcome of such experiments could be highly revolutionary in regenerative medicine (31-35).

The notion of using adult stem cells in some therapies is very attractive. Methods for transplanting stem cells need to be developed. It will be crucial to ensure that the transplanted cells are located in the tissues and function properly in heart, neural tissues and other organs. Studies done last four years provided the evidence that transplanted mouse ESCs can relieve Parkinson’s disease and partially restore neural function in animals with spinal-cord injuries.

**Regulatory issues in stem cell research**

The use of embryonic stem cells in regenerative medicine raises ethical, legal and social concerns among the public. Regenerative medicine to overcome various chronic diseases will involve the implantation of normal tissue cells in patients with damaged organs. These stem cell-based therapies can be broadly implemented for diseased individuals with Parkinson’s disease, Alzheimer’s disease, diabetes mellitus, spinal cord-injuries, osteoarthritis. These new approaches may also offer another advantage of overcoming the problem of tissue rejection in transplantation. Somatic-cell nuclear transfer technique could produce a lineage of stem cells which are genetically identical to the donor (34-38).

The wide spectrum of social, political, legal, economical, philosophical and ethical topics must be discussed in detail by policy makers and specially formed admin-
istrative bodies. When President Bush announced their federal policy in 2001, it became quite clear that embryonic stem cell lines will be restricted for research and a national advisory or regulatory committee should be established to oversee policies in stem cell research (39-41).

In the United States, the NIH is implementing a policy permitting Federal funding of research on ongoing and existing human embryonic stem cell lines which fulfill President Bush’s eligibility criteria (39):

1) NIH will not fund derivation of embryonic stem cells,
2) Embryonic stem cells will only be obtained from in vitro fertilization embryos which are in excess of clinical need,
3) No profit is to be made by donors or clinics on the donation of embryos, and
4) Documentation of full informed consent by the donors is necessary.

The Canadian Institutes of Health Research (CIHR) released guidelines allowing publicly funded stem cell research on “surplus” embryos created at fertility clinic and donated with informed written consent of the donors. In 2001, United Kingdom became the first country clearly to allow the creation of embryos as a source of stem cells. Regulations were approved in 2001, enabled scientists to use embryonic stem cells for practical uses other than reproduction (39-46).

The American Society of Hematology (ASH) published a report urging that therapeutic cloning techniques and research avenues not be limited to ban human reproductive cloning. Given the potential of stem cells United States Congress and the administration should allow scientists to pursue research in animals and in cultured cells (40).

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