Biotechnology of Extracellular Matrix

Potential use of stem cells from bone marrow to repair the extracellular matrix and the central nervous system

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Abstract
A subset of stem-like cells from bone marrow that are referred to as marrow stromal cells (MSCs) have been shown to be capable of differentiating into osteoblasts, chondrocytes, adipocytes, myocytes, astrocytes and perhaps neurons. Recently, conditions have been developed where human MSCs can be expanded almost without limit in culture without apparently losing their multipotentiality for differentiation. The cells appear to be potentially useful for the repair of extracellular matrix and the central nervous system.

Introduction
One of the remarkable features of the extracellular matrix is that it undergoes dramatic degradation and re-synthesis during early development and postnatal growth. The metabolic turnover of matrix diminishes with age but still occurs at an impressive pace throughout life in bone and in many other tissues following traumatic or pathological injury. For example, collagen was initially thought to be metabolically inert, but isotopic studies on urinary excretion of [14C]hydroxyproline demonstrated that metabolic turnover of collagen continues even in aged rats [1]. Since the work of Cohnheim in the nineteenth century [2], there has been controversy as to the source of the cells involved in the re-synthesis of matrix such as the fibroblasts that appear during wound repair. Some observations suggested that the fibroblasts, the pericytes of capillaries or related matrix cells arose from local stem cells found near the site of tissue repair. Other observations suggested that

Figure 1
FISH (fluorescence in situ hybridization) assay for Y chromosome to detect male cells in primary cultures of tissues from female mice that received systemic infusions of isogenic male MSCs

The recipient was a 3-week-old female transgenic mouse that had a phenotype of osteogenesis imperfecta because it expressed a mutated gene for type-I collagen. The mouse was irradiated with 700 cGy to enhance engraftment to cells. Because of the marrow ablation, the male MSCs (11 x 10^6) were infused intraperitoneally together with whole bone marrow (2 x 10^6) from an osteogenesis-imperfecta-transgenic mouse in order to provide haematopoietic stem cells. Primary cultures of various tissues were prepared 2.5 months after the infusion, and the cells assayed by FISH for presence of the Y chromosome. Taken with permission from [16]. Copyright (1998), National Academy of Sciences, U. S. A.
the cells came from the blood stream and by implication from the bone marrow. In the past several years, there has been increasing evidence that the bone marrow is a source of cells that participate in normal metabolic turnover and repair of extracellular matrices. In effect, the data suggest that, in addition to haematopoietic stem cells, bone marrow contains stem cells that are precursors of non-haematopoietic mesenchymal tissues.

The first direct evidence that bone marrow contains precursor cells for non-haematopoietic mesenchymal tissues came from the work of Friedenstein and co-workers in Moscow [3]. The observations were confirmed and extended by Piersma et al. in the Netherlands [4], Owen et al. in Oxford [5,6] and Caplan in Cleveland [7,8], and then by a large number of other investigators [9-14]. The precursor cells were referred to initially as colony-forming-units-fibroblasts. Subsequently they were referred to as mesenchymal stem cells, or marrow stromal cells (MSCs). MSCs are readily isolated by their adherence to tissue-culture surfaces and can be differentiated in culture into osteoblasts, chondrocytes, adipocytes and beating myotubes (for review, see [14]). After systemic infusion of MSCs, progeny of the cells appeared in a variety of tissues (Figure 1), including bone [15-18], cartilage [16], lung [15,16,19], spleen [19] and thymus [19]. Also, engraftment of donor MSCs into repairing muscle was observed after either local injection or systemic injection [20]. In addition, engraftment into muscle of a dystrophin-deficient mouse was seen after systemic infusion of rare marrow cells, defined as a 'side population' or SP cells, that may be precursors of MSCs [21,22].

Because of their unusual properties, MSCs have been investigated as a potential means of direct cell therapy or as vectors for gene therapy [7,14]. A preliminary clinical trial [23] has been initiated in children with severe osteogenesis imperfecta. In the preliminary trial, the patients underwent marrow ablation followed by a stan-

Figure 2

Line drawings of rat forebrain to demonstrate the extent of integration and migration of human MSCs after implantation at the level of the bregma into the striatum of adult rats

The drawing is a composite from brains examined 4, 14, 30 and 72 days after cell infusion. Symbols: ○, clusters of human MSCs that were pre-labelled with the nuclear dye bis-benzimide; O, clusters of infused paraventricular astrocytes that have many of the properties of neural stem cells; also prelabelled with bis-benzimide. Reproduced with permission from [24].

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standard allogeneic transplant from an histocompatibility-locus-antigen (HLA)-compatible brother or sister [23]. The preliminary trial was based on the possibility that whole bone marrow may contain adequate numbers of MSCs containing wild-type collagen genes that would engraft, home-in to bone, and replace the osteoblasts in the child that are expressing a mutated collagen gene. The first three children admitted to the trial all had a decrease in fracture rate, an increase in linear growth and an increase in total body bone mineral. However, only 1–2% of the osteoblasts were of donor origin. Therefore, the trial was encouraging but not conclusive. In a second stage of the trial, the same children are receiving large numbers of isolated MSCs from the same donors. Since the recipients have the immunity of the donors, no further marrow ablation is undertaken. Several of the children are currently being evaluated to see if a larger number of their osteoblasts become donor in origin and whether the therapy will convert a severe variant of osteogenesis imperfecta to a milder phenotype (E. Horwitz, personal communication).

To test the full potential of MSCs to differentiate, we infused MSCs directly into the basal ganglia of rats [24]. Surprisingly, the infused cells did not aggregate and invoke immune responses as is seen with infusion of fibroblasts. Also, there was no evidence that the cells differentiated into mesenchymal cells. Instead, the cells integrated and migrated along known pathways in a manner that was similar to the integration and migration seen with infusion of paraventricular astrocytes that have many of the properties of embryonic neural stem cells (Figure 2). Essentially the same pattern of integration and migration was seen with both rat MSCs and human MSCs infused into the brains of adult rats. In subsequent experiments, murine MSCs were infused into the brains of newborn mice [25]. Extensive migration of the cells was again seen. Some of the pre-labelled cells differentiated into astrocytes. Others appeared in neuron-rich regions of the brain and probably differentiated into neurons. Therefore, the results suggested that the cells may be promising vectors for gene therapy and repair of both the extracellular matrix and the central nervous system.

One of the major barriers in using stem cells for cell and gene therapy is the difficulty of expanding the cells in culture without their differentiating. Without the ability to expand the cells, it is difficult to generate enough of the cells for effective therapy. Also, it is difficult to introduce exogenous genes in the cells without being able to expand the cells under selective pressure. We have found recently [26] that human MSCs will expand rapidly in culture if they are plated initially at a very low density of 1.5 or 3.0 cells/cm² (Figure 3). By plating the cells at low density, the cells were amplified by an average of 600-fold in 12 days. With three passages, the cells were amplified about 10³-fold in 6 weeks. Under the conditions of low plating density, one bone-marrow aspirate obtained under local anaesthesia from one subject can generate over 10¹³ cells, a number that approaches the total number of cells in the adult body. After extensive expansion, the cells retained their ability to generate single-cell-derived colonies in culture, a parameter that was shown previously to be closely correlated to the ability of the cells to differentiate into both osteoblasts and adipocytes [27]. Therefore, the results suggested that the expanded cells maintained their multipotentiality. The ability to expand the cells rapidly suggests that the cells can readily be gene engineered by physical methods such as electroporation and without the use of viral vectors. Also, it may be possible to expand the cells sufficiently
Marrow stromal cells

Bone marrow aspirate
local anesthesia

THERAPY OF SAME PATIENT

Skeletal and Related Disease

Diseases of CNS

under selective pressure to obtain homologous recombination whereby mutated genes in the cells are replaced directly by wild-type genes. Based on these observations, we and others are currently pursuing the potential of MSCs for cell and gene therapy of a variety of diseases of the musculoskeletal system and central nervous system (Figure 4).

References
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Morphogenetic messages are in the extracellular matrix: biotechnology from bench to bedside

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Abstract

The origin and evolution of multicellular metazoa was accompanied by the appearance of extracellular matrix. The demineralized extracellular matrix of bone is enriched in morphogenetic proteins that induce bone. Bone morphogenetic proteins (BMPs) are intimately bound to collagens. BMP-4 has high affinity for type-IV collagen, and other binding proteins such as noggin and chordin. Soluble morphogens are kept in the solid state by extracellular matrix. In this sense Nature used the principles of affinity matrices long before humans patented the principle of affinity chromatography.

Introduction

Morphogenesis is the developmental cascade of pattern formation, body-plan establishment and interpretation and differentiation of the pattern. Morphogenesis is induced by morphogens. Morphogens are generally first identified in fly and frog embryos by genetic approaches, differential displays, subtractive hybridization and expression cloning, and this information is then extended to mice and men. This article will demonstrate an alternative biochemical approach based on regenerative potential of adult mammalian bone.

Bone grafts have been used by orthopaedic surgeons to aid in recalcitrant bone repair for many years. Decalcified extracellular bone matrix has been used to treat patients with osteomyelitis [1]. Lacroix [2] hypothesized that bone contains a substance, osteogenin, that initiates bone growth. Urist [3] made the key discovery that demineralized, lyophilized, segments of rabbit bone when implanted intramuscularly induced new bone formation. Bone induction is a sequential multistep cascade [4–6] that mimics embryonic bone morphogenesis. The key steps in this cascade are chemotaxis, mitosis and differentiation. Chemotaxis is the directed migration of cells in response to a chemical gradient of signals released from the insoluble demineralized bone matrix. The demineralized bone matrix is predominantly

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