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Human mesenchymal stem cells generate a distinct pericellular zone of MMP activities via binding of MMPs and secretion of high levels of TIMPs ☆

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ABSTRACT

Mesenchymal stem cells (MSCs) are attractive candidates for inclusion in cell-based therapies by virtue of their abilities to home to wound sites. However, in-depth characterization of the specific effects of MSCs on their microenvironments is needed to realize their full therapeutic potentials. Furthermore, since MSCs of varying properties can be isolated from a diverse spectrum of tissues, a strategic and rational approach in MSC sourcing for a particular application has yet to be achieved. For example, MSCs that activate their proteolytic environments may promote tissue remodeling, while those from different tissue sources may inhibit proteases and promote tissue stabilization. This study attempts to address these issues by analyzing MSCs isolated from three adult tissue sources in terms of their effects on their proteolytic microenvironments. Human bone marrow, adipose, and traumatized muscle derived MSCs were compared in their soluble and cellular-associated MMP components and activity. For all types of MSCs, MMP activity associated with the cell surface, but activity levels and MMP profiles differed with tissue source. All MSC types bound exogenous active MMPs at their surfaces. MSCs were also able to activate exogenous proMMP-2 and proMMP-13. This is in marked contrast to the MSC soluble compartment, which strongly inhibited MMPs via endogenous TIMPs. The exact TIMP used to inhibit the exogenous MMP differed with MSC type. Thus, MSCs saturate their environment with both MMPs and TIMPs. Since they bind and activate MMPs at their surfaces, the net result is a very controlled pericellular localization of MMP activities by MSCs.

1. Introduction

Adult mesenchymal stem cells (MSCs) were first discovered in bone marrow and described as mononuclear cells that culture ex vivo as adherent, colony-forming-unit fibroblasts (CFU-F) (Friedenstein et al., 1968, 1970, 1987). In the decades since, MSCs have been identified from mesoderm-, endoderm-, and ectoderm-derived tissues, including mesodermal (trabecular bone (Noth et al., 2002), synovium (De Bari et al., 2001; Djouad et al., 2005), cartilage (Hiraoka et al., 2006), fat (Zuk et al., 2001; Sekiya et al., 2004), muscle (Mauro, 1961; Nesti et al., 2008), blood vessels, and tonsil (Janjanin et al., 2008)), endodermal (thymus (Rzhaninova et al., 2005)), ectodermal (skin (Shih et al., 2005), hair follicle (Cotsarelis et al., 1990)), dura mater (Petrie et al., 2008), and dental pulp (Perry et al., 2008), prenatal and perinatal tissues (umbilical cord (Sarugaser et al., 2005), umbilical cord blood (Lee et al., 2004), and placenta (Yen et al., 2005)). Because they have been isolated from a wide range of tissues, MSCs are known by many different names in addition to the original 'mesenchymal stem cells' coined by Arnold Caplan (Caplan, 1991), including mesenchymal stromal cells (Horwitz et al., 2005), bone marrow stromal cells (Gronthos et al., 2003), marrow-isolated adult multipotent inducible cells (D'Ippolito et al., 2004), and multipotent adult progenitor cells (Belema-Bedada et al., 2008). MSCs have traditionally been thought of in terms of their multi-lineage differentiation potential, including osteogenesis, chondrogenesis, and adipogenesis (Kolf et al., 2007). Since their initial description, however, the inherent cell biology of MSCs has come into focus due to their emerging roles in a variety of physiological and pathological processes.

There is strong recent evidence to suggest that MSCs occupy a perivascular niche in a variety of vascularized tissues, affording them a prime location for regulating vascular events such as angiogenesis (Shi and Gronthos, 2003; Gruber et al., 2005; Crisan et al., 2008; Jones and

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McGonagle, 2008; Traktuev et al., 2008). Tightly wrapped around the vessels, pericytic MSCs interact with another critical regulator of the vascular environment, the vascular basement membrane (VBM). The VBM is a specialized extracellular matrix that surrounds the blood vessels of the body and is regulated through a control system involving proteases, which alter and degrade the matrix, and protease-inhibitors, which maintain and protect the VBM from disruption (Kalluri, 2003). This interplay between proteases and protease-inhibitors and its effects on the VBM profoundly influences vessel stability and, hence, many physiological and pathological processes. For example, disruption of the VBM is an early step in angiogenesis (Stetler-Stevenson, 1999; Coussens et al., 2000; Annabi et al., 2003; Kalluri, 2003; Worley et al., 2003; De Becker et al., 2007; Ries et al., 2007). During tumor growth and metastasis, cancer cells secrete proteases that degrade the VBM, allowing new blood vessels to sprout and nourish the growing tumor (Coussens et al., 2000; Kalluri, 2003; Matrisian et al., 2003). These extrinsic factors potentially tip the balance between proteases and protease inhibitors towards vascular disruption. As residents of the perivascular niche, MSCs are in a prime location to influence and alter their local environment by affecting this balance.

A specific class of extracellular matrix degrading metalloenzymes, the matrix metalloproteinases (MMP)s and their endogenous inhibitors, the tissue inhibitors of metalloproteinases (TIMPs), are specifically linked with VBM remodeling (Ray and Stetler-Stevenson, 1994). Of the approximately 26 currently recognized MMPs, several are of particular relevance to the perivascular environment (Ray and Stetler-Stevenson, 1994). For example, MMP-2 and MMP-9 are unique among MMPs in that they contain type II fibronectin domains, allowing them to bind gelatin, collagens, and laminin (Visse and Nagase, 2003). This allows MMP-2 and MMP-9 to bind intact matrix, where they degrade gelatin as well as laminins and collagen type IV, the main matrix components of the VBM (Ries et al., 2007). Furthermore, membrane type-1 MMP (MT1-MMP), working pericellularly, degrades a wide range of matrix molecules, including those of the VBM (Visse and Nagase, 2003). The TIMPs are the main MMP inhibitors, binding 1:1 stoichiometrically with the MMP active-site cleft (Visse and Nagase, 2003). Four TIMPS (TIMP-1, TIMP-2, TIMP-3, and TIMP-4) have been identified (Visse and Nagase, 2003), and each TIMP is composed of distinct N- and C-terminal domains (Brew et al., 2000). The N-terminal domains take part in the inhibitory actions, while the C-terminal domains mediate non-inhibitory complexes with MMPs. While the TIMPs as a group are largely specific in their inhibition for MMPs over other proteases, the four TIMPs exhibit important differences among their binding properties for specific MMPs. For example, the N-terminal domains of TIMP-2, TIMP-3, and TIMP-4, but not TIMP-1, are potent inhibitors of MT1-MMP (Brew et al., 2000; Bigg et al., 2001). Furthermore, the C-terminal domains of TIMP-2 and TIMP-4 bind the hemopexin domain of MMP-2, while TIMP-1 and TIMP-3 do not (Brew et al., 2000; Bigg et al., 2001; Worley et al., 2003). All MMPs are initially expressed as inactive zymogens and require proteolytic removal of N-terminal inhibitory pro-peptides for activation (Ries et al., 2007). For example, proMMP-2 activation occurs at the cell surface in a process that requires TIMP-2 and active MT1-MMP (Emmert-Buck et al., 1995). The N-terminal domain of TIMP-2 binds to the active cleft of MT1-MMP on the surface of the cell, while the C-terminal domain binds to the proMMP-2 hemopexin domain (Worley et al., 2003). ProMMP-2 is then activated though proteolytic processing by other, non-complexed MT1-MMP. Only TIMP-2 is able to mediate the ternary complex proMMP-2/TIMP-2/MT1-MMP. TIMP-4, which binds the hemopexin domain of proMMP-2 and potently inhibits MT1-MMP but does not support the ternary complex, competes with TIMP-2 for proMMP-2 and MT1-MMP binding.

We have previously shown that bone marrow derived MSCs secrete high levels of TIMPs that stabilize vessels and protect the VBM from MMP-induced degradation (Lozito and Tuan, 2011). This study focuses on interactions between MSCs and MMPs, including activation, inhibition, and binding. Special attention is paid to the role of cell-proximity (i.e., pericellular versus soluble compartments) on MSC–MMP interactions. Finally, the role of MSC tissue origin (i.e., bone marrow versus adipose tissue versus traumatized muscle) in MSC–MMP interactions is considered.

2. Results

2.1. Despite secreted MMPs, the majority of MMP activity is associated with the MSC surface

To compare the MMP activity of MSCs isolated from three different tissue sources, cultures of bone marrow derived (BMD-), traumatized muscle derived (TMD-), or adipose derived (AD-) MSCs were tested for their abilities to cleave fluorogenic MMP substrates. Identically treated cultures of the highly metastatic fibrosarcoma cell line HT1080 were included as positive controls. To determine if MMP activities were secreted or associated with the MSCs themselves, conditioned media (CM) was collected from day 4 cultures of BMD-, TMD-, and AD-MSCs and replaced with fresh serum-free (SF) media. Three groups (cells, CM, and cells + CM) were then tested for their abilities to cleave fluorogenic MMP substrates (Fig. 1A). Significantly higher MMP activity levels were exhibited by cell samples than by CM samples for all MSC types tested. This is in contrast to the control HT1080 cells, which exhibited high MMP activity levels in both the cellular and CM compartments and significantly higher MMP levels than MSC samples from all tissues and patients tested. TMD-MSC and AD-MSC samples exhibited significantly higher MMP levels than BMD-MSC samples. Differences among CM samples collected from all patients were insignificant regardless of MSC type. These results suggest that the majority of MMP activity associated with MSCs is associated with the cells themselves rather than secreted. TMD- and AD-MSCs have more MMP activity associated with their cell compartments than do BMD-MSCs.

In an effort to determine the specific MMPs responsible for the MMP activities detected in MSC cultures, proteins in the cell and CM samples collected from BMD-, TMD-, and AD-MSCs were analyzed by western blot for MMP-1, 2 and 10 (Fig. 1B). APMA treatments were used to validate MMP bands. APMA converts MMPs from their pro- to active forms by cleaving inactivating propeptides. Thus, following incubation with APMA, proMMP bands are diminished while active MMPs bands are intensified, thereby allowing positive identification of pro and active MMP bands. (In this way, the 3 MMP-1 bands detected in all cell protein samples were shown to be nonspecific as none of these bands exhibited any changes in intensity in response to APMA treatment.) All three MSC types secreted MMP-2 and MMP-10 at similar levels. Only TMD-MSCs secreted detectable levels of MMP-1. All MSC types exhibited MMP-2 and low levels of MMP-10 in their cell protein samples. Only TMD-MSC protein samples contained detectable levels of MMP-1. Cell protein samples were also analyzed by western blot for MT1-, MT2-, MT3-, MT4-MMPs (Fig. 1C). All three MSC types expressed MT-MMPs at similar levels. Thus, the higher levels of MMP activity exhibited by AD- and TMD-MSCs may be due to their binding secreted MMPs at their surfaces.

To investigate the cellular localizations of endogenous MMPs at the surfaces of the MSCs, cultures of BMD-, AD-, and TMD-MSCs were analyzed by MT1-MMP and MMP-2 immunofluorescence one or four days post-seeding onto glass coverslips (Fig. 2). For Day 1 samples of all MSC types, both MT1-MMP and MMP-2 were distributed along the entire cell. By Day 4, both MT1-MMP and MMP-2 localized to the cell body in the vicinity of the nucleus. However, MMP-2 also tended to localize to specific surface regions including cellular extensions from the cell body, giving MMP-2 staining in these regions a more punctate appearance. No consistent differences in expression levels or cellular localizations were observed based on MSC passage number or patient age/sex (data not shown).