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β2-Microglobulin as a potential factor for the expansion of mesenchymal stem cells

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Abstract

Multipotent mesenchymal stem cells (MSCs) hold great promise in regenerative medicine, but one of the biggest challenges facing for their application is the ex vivo expansion to obtain enough undifferentiated cells. Fetal bovine serum (FBS), which can elicit possible contaminations of prion, virus, zoonosis or immunological reaction against xenogenic serum antigens, still remains essential to the culture formulations. There is an urgent need to identify potential factors for the undifferentiated expansion of MSCs to reduce the use of FBS or eventually replace it. A previously recognized housekeeping gene, β2-microglobulin (β2M), is demonstrated to act as a novel growth factor to stimulate the undifferentiated ex vivo expansion and preserve the pluripotency of adult MSCs from various sources. The use of β2M might have promising implications for future clinical application of MSCs.

Keywords

β 2-Microglobulin; Ex vitro expansion; Mesenchymal stem cells

Introduction

Mesenchymal stem cells, also known as multipotent mesenchymal stromal cells (MSC), have the capacities to self-renew and to differentiate into multiple cell lineages. MSCs have been isolated from various adult tissue sources, such as cord blood, placenta, adipose and dermal tissues, synovial fluid, deciduous teeth, and amniotic fluid. This broad distribution of sources combined with their pluripotency has led them to be very attractive therapeutic candidates for tissue engineering and regenerative medicine (Uccelli et al. 2008). Although cell transplantation therapies with MSCs hold great promise for clinical application, one of the biggest challenges facing the advancement of this technology is the ex vivo expansion of MSCs to obtain enough undifferentiated cells to enable therapeutic applications because MSCs in adult tissue are very low (Horwitz 2004). Currently, the clinical protocol using MSCs underscores the need of serum as essential supplement. Serum-free media so far cannot support the proliferation of MSCs in the absence of growth factors (Stute et al. 2004; Mannello and Tonti 2007). There is thus a strong motivation to identify novel factors that might be used in serum-free formulations to expand MSCs ex vivo without influencing differentiation capacity or to release locally in vivo to influence growth of MSCs within the therapeutic site. Here, we describe an interesting finding in our lab that a previously recognized housekeeping gene, β 2-microglobulin (β 2M), could promote proliferation and preserve pluripotency of MSCs from various sources, suggesting remarkable implications of β 2M for ex vivo expansion of MSCs.

Growth regulatory properties of β 2M

β 2-microglobulin is a non-glycosylated protein with a molecular mass of 11,800 Da that is synthesized by all nucleated cells and forms a small invariable light chain subunit of major histocompatibility complex (MHC) class I antigen (also known as human leukocyte antigens (HLAs) in humans) through non-covalent linkage on cell surfaces. It is part of the major histocompatibility complex (MHC) class I molecule on the cell surface. Free β 2M is found in body fluids as a result of shedding from cell surfaces or intracellular release (Shi et al. 2008; Strominger 2002). Although the best-characterized function of β 2M is to interact with and stabilize the tertiary structure of the MHC class I α -chain to present antigenic peptides to cytotoxic (CD8+) T lymphocytes, the complex roles of β 2M remain largely unknown. The association between β 2M protein and cell growth has received many attentions in the past decades because high serum levels of β 2M are present in some autoimmune disorders (such as rheumatoid arthritis) and human malignancies (Xie and Yi 2003; Shi et al. 2009), suggesting an important, yet unidentified, role of β 2M. In the late 1980s, β 2M was reported to have mitogenic activity on bone cells, such as osteoblasts and was proposed as a potential bone growth factor (Balint and Sprague 2001). Later studies using a wide range of new molecular approaches to assess the mitogenic role of β 2M in cells have provided strong evidence to show that β 2M acts similarly to a prototypical factor capable of stimulating the growth of a wide spectrum of different cell types (Shi et al. 2009). It has been reported that β 2M could increase the proliferation of osteoblasts, stromal cells, osteoclasts, and a wide series of human cancer cells (Canalis et al. 1987; Evans et al. 1991; Rowley et al. 1995; Huang et al. 2006; Nomura et al. 2006; Menaa et al. 2008). In addition, it has been indicated that the growth regulatory effects of β 2M seem to depend on the nature of cells since it also could play as a negative growth factor in some different types of cells, such as dendritic cells (DCs) derived from peripheral-blood monocytes and several leukemic cell lines (Min et al. 2002; Xie and Yi 2003). Despite the complexity, these data strongly suggest that β 2M may represent a novel

growth regulatory factor for both normal and abnormal cell regulation. The selected studies of β 2M on the growth of representative cell types are presented in Table 1.

Proliferation-stimulating activities of β 2M on MSCs

We continue to explore the potential roles of β 2M on the growth regulation of MSCs and identified β 2M as a novel growth factor for the ex vivo expansion of MSCs (Shi et al. 2007; Zhu and Shi 2008). MSCs from different species (human and mouse) and various sources (bone marrow, skin dermis and cord blood) have been isolated and identified according to the protocol recommended by The International Society for Cellular Therapy (Dominici et al. 2006). The proliferation, differentiation and morphological characteristics of MSCs were evaluated after exposing to β 2M in the regular α -DMEM culture medium with or without fetal bovine serum (FBS). The functional activities of β 2M in MSCs were also validated by transfection of the target gene and by knockdown the target gene using sequence-specific siRNA. It was observed that physiological concentrations (1–20 μ g/ml) of β 2M could stimulate a dose-dependent mitogenic response in MSCs from different species and various sources (Fig. 1a). Moreover, exogenous over-expression of β 2M protein induced the growth of MSCs, while β 2M blocking antibody blocked the proliferation-promoting effect on MSCs. In addition, β 2M siRNA effectively blocked the endogenous level of β 2M mRNA and significantly inhibited the growth of MSCs (Fig. 1b). We next examined whether β 2M could replace serum in supporting MSCs proliferation and showed that β 2M could support the proliferation of MSCs in the culture medium with 2% FBS, but could not rescue MSCs from apoptosis by serum lower than 2%. Although expansion of the MSCs is necessary, it must be accomplished in a manner that does not alter cell phenotypes, induce differentiation or interfere with the subsequent ability to differentiate into the desired cell type. The expression of several surface markers in MSCs as CD29, CD44, CD90, CD73 were analyzed by flow cytometry and showed no marked differences after treatment with β 2M. Although β 2M functionalized with MHC-I, it did not affect the expression of MHC-I molecule in MSCs. We further determined whether the MSCs could still be differentiated by routine methods after β 2M exposure and demonstrated that β 2M pretreatment did not interfere with mouse and human MSC differentiation by adipogenic or osteogenic medium. In addition, β 2M alone did not drive MSCs into adipogenic or osteogenic lineages. We also confirmed that β 2M did not induce the expression of the endogenous osteocalcin and its promotor activity using promotor activity assay. Regarding the potential signaling pathways involved in β 2M-mediated mitogenic effects on MSCs, we identified that β 2M stimulated the proliferation of MSCs at least partially through increased phosphorylated cAMP-responsive element binding protein (Shi et al. 2007). Taken together, these data suggest that the addition of β 2M to differentiation media does not interfere with pluripotency of MSCs or drive MSCs into specific lineages and thus it holds promise for β 2M to be used in ex vivo and in vivo expansion of MSCs.

Potential applications of β 2M in MSC processing

With rapid advances on the therapeutic implications, the development of efficient culture systems for MSC expansion is an urgent need. Currently, fetal bovine serum (FBS) is still an essential component for the large-scale expansion of cells to be used in cellular therapy (Mannello and Tonti 2007). There are many reported clinical trials with MSCs cultured with FBS and no marked secondary effects were shown, but the possible contamination (prion, virus, zoonosis) or immunological reaction against xenogenic serum antigens should be carefully assessed. Although this a regulatory issue, there are increasing number of clinical trials using MSCs underscores the need for serum supplements other than FBS (Sotiropoulou et al. 2006). Since serum-free medium without growth factors still have some problems, the identification of novel factors for expansion of MSCs is important to reduce the use of FBS if it can not replace the FBS. β 2M could stimulate the undifferentiated expansion of MSCs

suggesting that β 2M or its analogs can be used as a novel factor to culture medium to facilitate the undifferentiated expansion of MSCs prior to cell transplantation. β 2M may also have in vivo therapeutic implications via controlled release to influence MSC proliferation in situ. For example, high levels of β 2M are present in auto-immune and inflammatory disorders, like rheumatoid arthritis. Recent studies also revealed that MSCs possess potent immunosuppression and anti-inflammation effects which make MSCs the ideal candidate for cell therapy to treat these diseases with inflammatory features (Chen and Tuan 2008). In such cases, β 2M may act as a mediator to promote the growth of MSCs in vivo to inhibit inflammatory responses. This potential therapeutic implication needs to be assessed.

A potential concern is whether long-term ex vivo exposure to β 2M will alter the propensity of MSCs to undergo malignant transformation. In our study, the stable transfected MSCs with β 2M did not show any transforming activity after long-term culture and indicates that β 2M is not a transformation inducing factor. So far, the molecular mechanisms of β 2M in cell growth are still not fully characterized. Recent studies indicate that β 2M is a promising therapeutic target for various cancers (Shi et al. 2009). However, a very interesting finding is that β 2M therapeutic antibodies were shown to be selective to tumor cells, without damaging to normal cells (Yang et al. 2006, 2007). Surprisingly, anti- β 2M antibodies do not block the effect of β 2M when β 2M serves as a negative growth regulator in myeloma cells, and also are synergistic with β 2M to induce cancer cell apoptosis (Min et al. 2002). These data strongly suggest that β 2M has different intrinsic mechanisms to distinct cancer cells and normal cells. It is unclear why β 2M antibodies display different effects on cancer cells and normal cells. A very recent report showed that there was a differential expression of β 2M/MHC class I molecules by normal and cancer cells. In normal B cells that Lyn was not associated with lipid rafts and β 2M-specific monoclonal antibodies (mAbs) did not trigger MHC class I relocation to the rafts, and that JNK, PI3K/Akt, and ERK activities remained unchanged after the mAb treatment (Yang et al. 2007). These data provide a plausible explanation for the selectivity β 2M-specific mAb-mediated apoptosis of normal versus malignant cells, but more extensive studies need to be performed. And this is an important issue needs to be well defined which may result in the development of novel therapeutic strategy of β 2M.

Concluding remarks

We describe here a novel nonimmunological function of β 2M to be used for the expansion of MSCs, but there are still several questions need to be defined. Although β 2M has been shown as a growth stimulating factor in various sources of MSCs, the mechanisms underlying this activity are not fully understood. As molecules of the MHC complex commonly do, β 2M has been suggested to possibly interact with hormonal and/or growth factors, like epidermal growth factor receptor (EGFR), insulin receptor, and IGF-I and IGF-II receptors, that may enhance cell growth (Balint and Sprague 2001). Recent findings have suggested that EGFR ligands could be used for ex vivo expansion and direction of MSCs (Tamama et al. 2006). It will be very interesting to investigate the synergistic effects of β 2M and EGF on the expansion of MSCs.

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Fig. 1. β 2M stimulates the proliferation of bone marrow MSCs. **a** β 2M-mediated mitogenic effect on human bone marrow MSCs in vitro after treatment with β 2M for 48 h. **b** β 2M siRNA significantly inhibited the proliferation of MSCs in vitro

Table 1Selected studies of β 2-microglobulin as a growth regulator

Cancer type	Materials	Effects on cell growth	References
Calvariae	Purified β 2M	Positive	Canalis et al. (1987)
Osteoblasts			Evans et al. (1991)
Osteoclasts	Purified β 2M	Positive	Menea et al. (2008)
Prostate cancer cells and renal cell carcinoma	Purified β 2M	Positive	Huang et al. (2006)
	Recombinant β 2M		Rowley et al. (1995)
	β 2M cDNA		Nomura et al. (2006)
Mesenchymal stem cells	Recombinant β 2M	Positive	Shi et al. (2007)
	β 2M cDNA		Zhu and Shi (2008)
Prostatic stromal cells	Purified β 2M	Positive	Rowley et al. (1995)
Leukemia and myeloma cells	Purified β 2M	Negative	Mori et al. (1999)
			Min et al. (2002)
Dendritic cells	Purified β 2M	Negative	Xie et al. (2003)