



Expression of CD146 on Human Placenta-Derived Mesenchymal Stromal Cells and their Osteogenic Differentiation Capacity are Modulated by Factors Contained in Platelet Lysate

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Abstract

Expression of CD146 on bone marrow-derived mesenchymal stromal cells (bmMSCs) is considered a marker for their multipotent differentiation potential and *in situ* localisation in the stem cell niche. We recently observed an elevated expression of CD146 on human bmMSCs upon expansion in medium enriched with platelet lysate. We therefore investigated if CD146 expression followed comparable paths on human placenta-derived mesenchymal stromal cells (pMSCs). In contrast to bmMSCs, pMSCs expanded in medium with platelet lysate expressed significantly less CD146 and failed to undergo osteogenic differentiation. We conclude that the expression of the stem cell marker CD146 on pMSCs and their osteogenic differentiation potential are modulated by factors contained in platelet lysate.

Keywords

Mesenchymal stromal cells; CD146; Osteogenic differentiation; Expansion media; GMP-compliant media

Introduction

Placenta is a rich source for mesenchymal stromal cells (MSCs) [1,2]. Placenta-derived MSCs (pMSCs) share many features with MSCs from bone marrow (bmMSCs) or other sources [3-5]. However pMSCs have a significantly different transcriptome than bmMSCs, highlighted by a significant decrease in expression of osteogenic factors [6-8]. Osteogenic differences correlate with expression of the adhesion molecule CD146 [9-13]. Hence, separating CD146⁺ from CD146⁻ pMSCs produces pro-osteogenic pMSCs vs. cells having a significantly reduced ability to undergo osteogenesis, respectively [9]. This is contrast to CD146⁻ bmMSCs which demonstrate a pro-osteogenic potential by generation of bone *in vivo* [13].

Expression of CD146 on other types of MSCs or MSC-like cells has also resulted in conflicting results with respect to its expression

and function. In contrast to adipose tissue-derived (adMSCs) and umbilical cord-derived MSCs, CD146^{low} dental pulp-derived MSC-like cells grew significantly faster compared to the CD146^{high} population [14-16]. Moreover, loss of CD146 on MSCs has been associated with senescence [14,17]. In short term cultures expression of CD146 on pMSCs did not change considerably, but CD146 was reduced after prolonged expansion of umbilical cord-derived MSCs [9,14,18].

Part of the reason for these contradictory results may be due to the MSC source (e.g., bone marrow vs. adipose tissue), but a key elemental difference can be highlighted by a difference in the methods for isolation and/or expansion of these cells. Adipose tissue-derived MSCs (atMSCs) express significantly less CD146 *ex vivo* compared to cells after expansion in media enriched with 10% FBS, suggesting that expression of CD146 can be modulated *in vitro* by the serum source [19].

In many studies MSCs were expanded in media containing foetal bovine serum (FBS) [1,5]. In some studies, especially in (pre-) clinical investigations, FBS was replaced by human serum (HS) and sometimes enriched with platelet lysate (PL) [20-22]. Hence different laboratories used quite diverse culture conditions when investigating the expression and function of CD146 on MSCs [9,13,23,24]. The importance of phenotypic changes in MSCs during *in vitro* GMP expansion and their clinical implications have been recently reviewed [18]. But the effect of a GMP-compliant medium on expression of CD146 by pMSCs was not investigated. Therefore in the present study we investigated if some of the differences in the expression of CD146 on pMSCs and their osteogenic differentiation potential properties could be attributed to PL utilized to enrich MSC growth media [20,25].

Materials and Methods

Human pMSCs were isolated from full-term placenta from elective caesareans that were free of any medical/obstetrical complications (n=5, ethics #453/2011B02) [7-9]. Cells from the same donor were expanded either in a GMP-compliant medium enriched with 5% human serum plus 5% human platelet lysate (HS+PL medium) [22], or in commercially available MSC medium containing FBS (Lonza, MSCGM). Second passage cells were harvested to confirm their MSC phenotype [1,26]. The expression of CD146 (R&D Systems) was determined by flow cytometry as described recently [7]. Expression of CD73 (BD Pharmingen) and CD90 (R&D Systems) served as controls. Statistics were computed by a two-sided t-test. Osteogenesis was induced by incubation of the pMSCs in 6-well plates in differentiation media over 28 days (DMEM-HG, 10% FBS, enriched by 1 µmol/l dexamethasone, 10 mmol/l β-glycerophosphate, 170 µmol/l ascorbate). Osteogenic differentiation was visualized by von Kossa staining [5]. Cells in media without osteogenic stimuli served as controls (DMEM-HG, 10% FBS).

Results

Modulation of expression of CD146 by MSC expansion media

All pMSCs were characterized as described in our recent studies [7,9,27] (not shown). As the next step we investigated if CD146

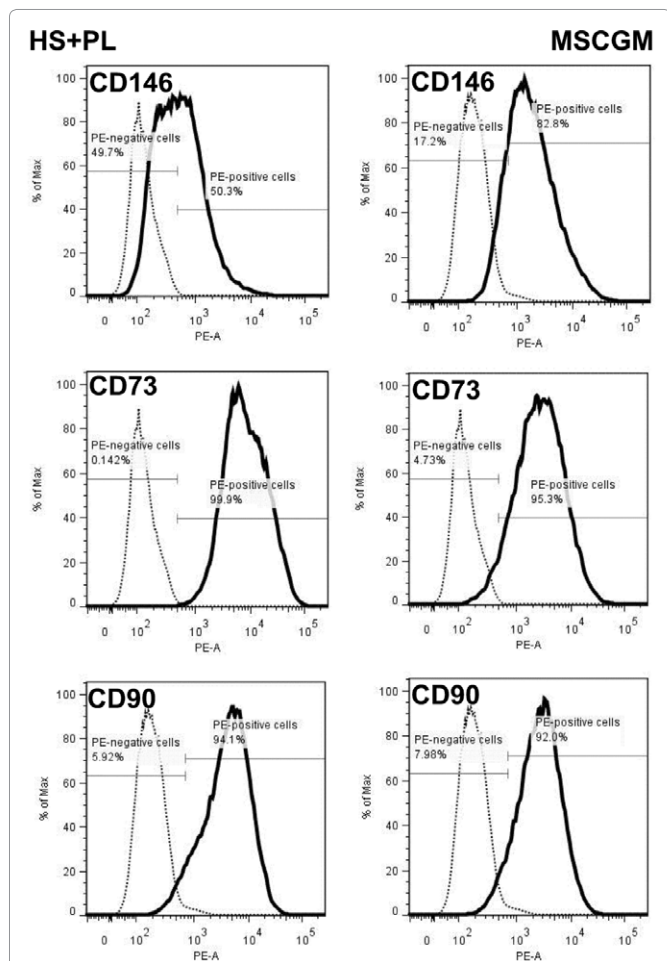
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was regulated by PL. When expanded in HS+PL medium, 58.8% of pMSCs expressed moderate levels of CD146 with a mean of fluorescence intensities (MIF) of 779 **Figure 1**. When expanded in MSCGM complemented by FBS, significantly more pMSCs expressed CD146 (average 75.1%; $p < 0.028$) with significantly higher signal intensities (MIF 3622; $p < 0.004$). In contrast, expression of CD73 and CD90 was not modulated by PL **Figure 1**. This confirmed that human pMSCs display a variable expression of CD146 [7]. We conclude that expression of CD146 is significantly reduced by complementing the expansion medium with PL (**Figure 1**).

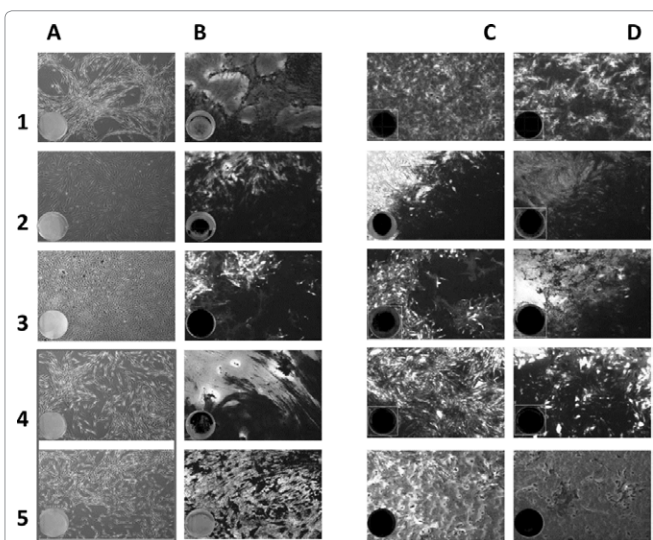
Osteogenic differentiation of pMSCs

Next we investigated if PL influenced the osteogenic differentiation capacity of pMSCs (**Figure 2**). Human pMSCs incubated in HS+PL medium did not undergo spontaneous osteogenic differentiation *in*



The pMSCs were expanded to the second passage in medium containing 5% human serum plus 5% platelet lysate (HS+PL; left panel) or in a commercial medium containing FBS but no platelet lysate (MSCGM; right panel). After expansion in HS+PL not only was the mean of fluorescence intensity reduced, but also the percentage of pMSCs expressing CD146 when compared to pMSCs from the same donor but expanded in MSCGM. In contrast, expression of mesenchymal markers CD73 or CD90 was not modulated by medium containing PL. The flow cytometry analyses are presented as representative histograms (y-axes, % of max; x-axes, signal intensities) from 3 pMSC donors; donor #p115 is shown. Bold histograms display staining with the antibodies as indicated; dotted histograms represent the controls (secondary antibodies only).

Figure 1: Expression of CD146 on pMSCs after expansion in different media.



The pMSCs (n=5) were expanded to passage 2 in HS+PL-medium (columns A, B) or in MSCGM (columns C, D). The MSCs were then placed in control medium (A, C) or osteogenic differentiation medium (B, D) for 28 days. Expansion of pMSCs in HS+PL-medium prevented spontaneous osteogenic differentiation in the controls (column A). But the pMSCs maintained an osteogenic differentiation capacity (column B). In contrast, expansion of pMSCs in MSCGM containing FBS without PL yielded cells that spontaneously generated a mineralized matrix in control medium without the presence of osteogenic stimuli (column C), which upon addition of osteogenic differentiation medium, was somewhat enhanced in some cases (e.g. in donor 3; column D). The figure presents micrographs of the cells and an overview of the total well of the same culture inserted in the lower left corner of the individual micrograph, respectively.

Figure 2: Osteogenic differentiation of pMSCs *in vitro* after expansion in different media.

in vitro, but a weak (2/5 populations), a moderate (2/5 populations), or a robust mineralization (1/5 population) was observed after 28 days of differentiation. In contrast, the same pMSCs spontaneously produced a mineralized extracellular matrix after incubation in MSCGM (5/5 populations), and osteogenesis was somewhat enhanced by addition of the differentiation cocktail to the pMSCs in all samples investigated (**Figure 2**). We conclude that osteogenic differentiation capacity of human pMSCs can be modulated by addition of PL to the expansion media.

Discussion

CD146 expression has been attributed to osteogenic differentiation and bone formation and is considered a marker for MSC “stemness” and multipotent differentiation potential capacity [10,14,17,24]. In contrast to these studies, others reported no clear-cut difference in the osteogenic potential of CD146^{high} versus CD146^{low} bmMSCs [10,12,13,24,28]. We showed that CD146 was associated with osteogenesis of pMSCs [7-9]. Taken together this suggests that the role of CD146 in osteogenic differentiation of human MSCs seems a matter of debate [13,24,28]. Reasons for these seemingly conflicting findings may include differences in the MSC sources and in the methods for isolation and/or expansion of the cells.

In this short communication we show that expression of CD146 and osteogenesis are reduced in pMSCs after expansion in HS+PL medium compared to the same cells expanded in a commercial standard MSC medium enriched by FBS without PL. This data shows that the expression of CD146 on pMSCs and its effect on osteogenic differentiation is therefore modulated by factors contained in PL.

This is in sharp contrast to another of our recent publications investigating human bmMSCs upon expansion in GMP-compliant media [22]. In contrast to pMSCs, bmMSCs showed an enhanced the expression of CD146 upon expansion in media containing PL. In another study we showed that factors contained in PL did not compromise the osteogenic differentiation of bmMSCs nor the expression of CD146, but it facilitated their smooth muscle differentiation [22,29]. To the best of our knowledge, this is the first study to demonstrate how platelet lysate regulates osteogenesis in placenta-derived MSCs.

In contrast to bmMSCs [22,29], pMSCs may respond in a different way to factors contained in PL. Key components of PL include PDGF, bFGF and TGF- β 1 [25] and MSCs express the corresponding receptors [30]. bFGF accelerated proliferation and reduced expression of CD146 in bmMSCs, whereas TGF- β 1 retarded proliferation and enhanced CD146 [12]. Overexpression of PDGF-BB and bFGF enhanced proliferation of bmMSCs and facilitated osteogenesis, whereas TGF- β 1 blocked osteogenesis but had no effect on proliferation [30]. Based on these studies we hypothesize that, the amount of TGF- β 1 in HS+PL was possibly not sufficient to activate expression of CD146 on pMSCs, which may have been reduced by bFGF, but that TGF- β 1 may be adequate to block osteogenesis of pMSCs. Alternatively, processing of TGF- β 1 signals may follow different routes in pMSCs than in bmMSCs. Expression of CD146 on bmMSCs is reduced in microsphere 3D pellet cultures, but not in monolayer cultures, indicating that a 3D environment may also regulate this adhesion molecule [31]. Differences in expression of CD146 on MSCs from distinct sources and variations reported with respect to the differentiation potential of MSCs could therefore be in part explained by deviations in the blend of PDGF, bFGF and TGF- β 1 in the media employed in the different studies.

These results may be important for studies that use PL for regenerative medicine with potential for clinical applications. Because PL is more commonly being used as an alternative growth supplement as opposed to xenogenic (e.g. FBS) supplements in MSC expansion medium, this finding bears considerable importance for physicians and their partners in research and health industry using PL or platelet extract to produce MSCs for clinical studies or cellular therapies. It has been noted, for instance, that MSCs expanded in media complemented with FBS displayed in about 30% of the tissue samples a spontaneous osteogenesis and mineralization *in vivo* when injected in a pre-clinical animal study in healthy or infarcted hearts [32]. In other studies MSCs generated bone and bone marrow upon subcutaneous transplantation *in vivo* [24]. Therefore, complementing the MSC expansion media with PL could ameliorate in a clinical situation the risk of *in situ* mineralization.

We conclude that in contrast to bmMSCs, pMSCs respond in a different way to factors contained in platelet lysate: In pMSCs the osteogenic differentiation capacity and expression of CD146 are reduced by expansion of the cells in GMP-compliant media enriched by PL [22].

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