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Human Bone Marrow Stromal Cells Lose Immunosuppressive and Anti-inflammatory Properties upon Oncogenic Transformation

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SUMMARY

Because of their immunomodulatory properties, human bone marrow stromal cells (hBMSCs) represent promising stem cells for treatment of immune disorders. hBMSCs expansion precedes their clinical use, so the possibility that hBMSCs undergo spontaneous transformation upon long-term culture should be addressed. Whether hBMSCs retain immunosuppressive and anti-inflammatory properties upon oncogenic transformation remains unknown. Using sequentially mutated hBMSCs and spontaneously transformed hBMSCs, we report that, upon oncogenic transformation, hBMSCs lose immunosuppressive and anti-inflammatory properties in vitro and in vivo. Transcriptome profiling and functional assays reveal immune effectors underlying the loss of immunomodulation in transformed hBMSCs. They display a proinflammatory transcriptomic signature, with deregulation of immune and inflammatory modulators and regulators of the prostaglandin synthesis. Transformed hBMSCs lose their capacity to secrete the immunosuppressive prostacyclins prostaglandin E2 (PGE2) and PGI2 but produce proinflammatory thromboxanes. Together, the immunoregulatory profile adopted by hBMSCs largely depends on intrinsic genetic-molecular determinants triggered by genomic instability/oncogenic transformation.

INTRODUCTION

Human bone marrow stromal cells (hBMSCs) are a rare subset of bone marrow cells and constitute a promising source of multipotent progenitors for mesodermal tissues (Pittenger et al., 1999), being used worldwide in many clinical applications including tissue repair, treatment of graft-versus-host disease, and autoimmune diseases (García-Castro et al., 2008). The clinical potential of hBMSCs relies on key properties such as (1) multipotent differentiation, (2) long-term ex vivo expansion, (3) homing ability to damaged tissues, and (4) robust immunomodulatory properties (Bernardo and Fibbe, 2012, 2013; García-Castro et al., 2008). The mechanisms through which hBMSCs display reparative effects include the capacity to home to sites of damage, the ability to release anti-inflammatory factors, and the capacity to modulate immune responses (Bernardo and Fibbe, 2012; Marigo and Dazzi, 2011). hBMSCs secrete immunosuppressive factors including prostaglandin E2 (PGE2), indoleamine 2,3-dioxygenase (IDO), transforming growth factor (TGF)-β, and nitric oxide (NO), thus modulating immune responses by inhibiting T cell activation and natural killer cell activity and inducing type II macrophage and dendritic cell differentiation and regulatory T cell (Bernardo and Fibbe, 2013; English, 2013; Herrero and Pérez-Simón, 2010; Ma et al., 2014; Yagi et al., 2010). However, it has been demonstrated that hBMSCs are not intrinsically immunoprivileged (Nauta et al., 2006), but they acquire immunosuppressive properties after exposure to an inflammatory environment (Prockop and Oh, 2012).

The immunosuppressive properties of allogeneic hBMSCs might be a double-edged sword. On one hand, they constitute the rationale for hBMSCs-based potential therapeutic approaches. On the other hand, they might enhance the ability of tumors to evade immune surveillance (Lazennec and Jorgensen, 2008; Momin et al., 2010). hBMSCs have been reported to inhibit or promote tumor growth, depending on yet undefined conditions (Momin et al., 2010; Stagg, 2008). Likewise, the experimental transformation of hBMSCs by different mechanisms gives rise to sarcoma formation in vivo, hence placing stromal mesenchymal stem cells as the cell of origin for certain sarcomas (Mohseny and Hogendoorn, 2011; Rodriguez et al., 2012). Practically, ex vivo expansion of stromal mesenchymal stem cells is a prerequisite for their clinical use (Barkholt et al., 2013) so that, when considering the use of ex vivo expanded hBMSCs, the possibility that they undergo senescence, genomic instability, and spontaneous transformation after long-term culture should be addressed (Barkholt et al., 2013; Estrada et al.,

2013; Pan et al., 2014; Wang et al., 2005). Although in vitro spontaneous transformation seems rare, no information exists about the homeostasis of long-term cultured hBMSCs regarding the donor age, underlying disease, and source of stromal mesenchymal stem cells. Furthermore, although hBMSC-based clinical trials should represent the optimal source of evidence on the potential in vivo tumorigenic capacity of hBMSCs, current trials rarely focused on parameters relevant for assessing the transformation potential of allogeneic hBMSCs because they rarely evaluate long-term safety and efficacy of mesenchymal stem cells (MSCs) (Mishra et al., 2009; Momin et al., 2010). Additionally, stromal mesenchymal stem cells exposed to the tumor milieu could differentiate into carcinoma-associated fibroblasts, enhancing tumor growth (Mishra et al., 2009; Momin et al., 2010). Together, although it is an important concern for realizing the full clinical expectative of hBMSC, the oncogenic potential of hBMSCs remains poorly explored. Consequently, whether hBMSCs retain differentiation and immunosuppressive and anti-inflammatory properties upon oncogenic transformation remains unknown. Here, we take advantage of a collection of sequentially mutated hBMSCs ranging from wild-type to fully transformed hBMSCs (targeted with up to six oncogenic mutations; Funes et al., 2007; Rodriguez et al., 2013) to address whether hBMSCs at different stages of a well-characterized oncogenic process (normal, immortalized, and transformed; Funes et al., 2007; Rodriguez et al., 2013) retain immunomodulatory properties in vitro and in vivo. We describe an oncogenic-transformation-associated loss of the immunosuppressive and anti-inflammatory properties by hBMSCs and identify candidate immune effectors underlying this loss of immunomodulation capacity. These data have enormous implications not only in ex vivo expansion of hBMSCs but also in microenvironment tumor biology.

RESULTS

Impaired In Vitro Homeostasis of Transformed hBMSC

We have very recently developed and characterized sarcoma models using several sequentially mutated hBMSCs (Funes et al., 2007; Rodriguez et al., 2013). This collection of hBMSCs ranges from wild-type (WT) (hBMSC-0H) to fully transformed hBMSC (Figure 1A; Funes et al., 2007; Rodriguez et al., 2013). The combination of oncogenic hits include p53 inactivation (hBMSC-1H), hBMSC-1H plus Rb inactivation and hTERT overexpression (hBMSC-3H), hBMSC-3H plus c-*myc* stabilization (hBMSC-4H), and hBMSC-4H plus H-RAS^{v-12} (hBMSC-5H). In addition, the fusion oncogene FUS-CHOP was ectopically expressed in all the hBMSC genotypes (Funes et al., 2007; Rodriguez et al., 2013). Figure 1A summarizes the main features and tumorogenic potential of all the different hBMSCs. Briefly, hBMSCs harboring less than three oncogenic hits are non-immortalized; hBMSC-3H-GFP, hBMSC-3H-FUS-CHOP (FC), and hBMSC-4H-GFP are immortalized, but not transformed; and hBMSC-4H-FC, hBMSC-5H-GFP, and hBMSC-5H-FC are transformed and originate sarcomas in vivo (Funes et al., 2007; Rodriguez et al., 2013). All hBMSC types, regardless of the number/nature of oncogenic hits, display a typical hBMSC phenotype (Menendez et al., 2009; Table S1 and Figure S1 available online). As expected, immortalized and transformed hBMSCs grow in vitro much faster than nonimmortalized hBMSCs (~22, ~28, and ~4 doubling populations in 30 days, respectively; Figure 1B). Additionally, sequential acquisition of oncogenic hits in hBMSCs impairs their adipogenic differentiation ability but does not compromise osteogenic potential (Figures S2A and S2B). The accumulation of oncogenic events in transformed hBMSC-5H cells induce striking alterations in the expression of many genes involved in adipogenic differentiation, and consequently, these cells fail to activate the master regulators of adipogenic differentiation peroxisome proliferator-activated receptor γ and CCAAT/enhancer-binding protein alpha (Rodriguez et al., 2013). Thus, transformation of primary hBMSC is coupled to a differentiation impairment and proliferation advantage, hallmark properties of oncogenesis.

Transformed hBMSCs Lack Immunosuppressive and Anti-inflammatory Properties In Vitro and In Vivo

It remains elusive whether transformed hBMSCs retain immunomodulatory properties. We investigated the ability of immortalized and transformed hBMSCs to inactivate T cell responses and to inhibit inflammatory responses. The potential immunomodulatory activity of immortalized (hBMSC-3H) and transformed (hBMSC-4H and hBMSC-5H) hBMSCs was compared with WT-hBMSC (hBMSC-0H) and nonimmortalized hBMSC (hBMSC-1H). The addition of hBMSC-0H, hBMSC-1H, and hBMSC-3H hBMSCs to mixed lymphocyte culture (MLC) of peripheral blood mononuclear cells (PBMCs) from different donors significantly reduced the number of total cells in the culture and specifically decreased the number of cycling CD4 T cells (Figure 2A), suggesting they were efficient inhibiting the proliferative response of activated T cells. Because they lack class II major histocompatibility complex (MHC) and CD80 and CD40 costimulatory molecules, hBMSC-0H/ hBMSC-1H/ hBMSC-3H did not stimulate the proliferation of allogeneic PBMCs, supporting their "immune-privilege" status. On the other hand, hBMSC-4H and hBMSC-5H failed to inhibit cell proliferation in the MLC assays (Figure 2A). Moreover, hBMSC-0H/hBMSC-1H/hBMSC-3H significantly inhibited the production of