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Despite differential gene expression profiles pediatric MDS derived mesenchymal stromal cells display functionality *in vitro*☆

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Abstract Pediatric myelodysplastic syndrome (MDS) is a heterogeneous disease covering a spectrum ranging from aplasia (RCC) to myeloproliferation (RAEB(t)). In adult-type MDS there is increasing evidence for abnormal function of the bone-marrow microenvironment. Here, we extensively studied the mesenchymal stromal cells (MSCs) derived from children with MDS.
MSCs were expanded from the bone-marrow of 17 MDS patients (RCC: n = 10 and advanced MDS: n = 7) and pediatric controls (n = 10). No differences were observed with respect to phenotype, differentiation capacity, immunomodulatory capacity or hematopoietic support. mRNA expression analysis by Deep-SAGE revealed increased *IL-6* expression in RCC- and RAEB(t)-MDS. RCC-MDS MSC expressed increased levels of *DKK3*, a protein associated with decreased apoptosis. RAEB(t)-MDS revealed increased *CRLF1* and decreased *DAPK1* expressions. This pattern has been associated with transformation in hematopoietic malignancies. Genes reported to be differentially expressed in adult MDS-MSC did not differ between MSC of pediatric MDS and controls.
An altered mRNA expression profile, associated with cell survival and malignant transformation, of MSC derived from children with MDS strengthens the hypothesis that the micro-environment is of importance in this disease. Our data support the understanding that pediatric and adult MDS are two different diseases. Further evaluation of the pathways involved might reveal additional therapy targets.

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Introduction

Pediatric myelodysplastic syndrome (MDS) represents a range of disorders characterized by dysplastic morphology comprising in total less than 5% of pediatric hematological malignancies (Hasle et al., 2004). The spectrum of MDS ranges from refractory cytopenia of childhood (RCC) to advanced MDS with excess of blasts (RAEB) with increasing risk of leukemic transformation (Hasle et al., 2003). Survival has increased from 30 to 60% since hematopoietic stem cell transplantation (HSCT) is applied (Strahm et al., 2011; Sasaki et al., 2001; Woods et al., 2002). The pathophysiology of MDS is not fully elucidated. However, genetic predisposition, acquired cytogenetic abnormalities and abnormal immune responses have been linked to MDS (Strahm et al., 2011; Hasle and Niemeyer, 2011). These aspects do not explain the entire range of disease in pediatric or adult MDS. Recently, it has been suggested in adult MDS that impaired interaction between hematopoietic precursor cells and their bone-marrow microenvironment might contribute to the disease (Zhang et al., 2012). In children, no conclusive data is yet available.

Mesenchymal stromal cells (MSCs) have been identified as supporting cells of hematopoietic stem cells (HSC) *in vivo* and *in vitro* (Morikawa et al., 2009; Mendez-Ferrer et al., 2010; Sugiyama et al., 2006) and linked to disease, as aberrant MSC function was shown to contribute to the pathophysiology of malignant disorders in murine models (Raaijmakers et al., 2010; Schepers et al., 2013). Characteristics of MSCs from adult MDS patients have been extensively studied focusing on cytogenetic and molecular abnormalities (Blau et al., 2011; Lopez-Villar et al., 2009; Flores-Figueroa et al., 2008) as well as gene and protein expressions (Flores-Figueroa et al., 2008; Marcondes et al., 2008; Santamaria et al., 2012). In addition, abnormal immunomodulation (Marcondes et al., 2008; Wang et al., 2013; Zhao et al., 2012a) as well as decreased hematopoietic support (Zhao et al., 2012a; Ferrer et al., 2013) by MSCs has been reported in MDS. However, these data remain conflicting with other studies reporting no abnormalities in stromal function (Flores-Figueroa et al., 2008; Klaus et al., 2010; Alvi et al., 2001). Differences in results may be explained not only by a variety of MSC expansion protocols and experimental set-up, but also by the heterogeneity of the disease (Aizawa et al., 1999). Studies reporting on (cyto)genetics and function of MDS-MSC have been summarized in Supplementary Tables S1 and S2.

Pediatric MDS is a very rare disease and publications on the role of stroma in the ontogeny and maintenance of pediatric MDS are limited to a case report on aberrant hematopoietic support by MSC derived from an MDS patient with trisomy 8 (Narendran et al., 2004), a study using stroma cells of 7 MDS patients (Borojevic et al., 2004), and a gene-expression analysis of the stromal compartment by the same research group (Roela et al., 2007). Nevertheless these scarce reports suggest an aberrant support of hematopoiesis associated with an altered gene expression profile of MSCs.

In the present study we compared MSCs derived from children with RCC and RAEB(t)/MDS-AML to MSCs expanded from age-matched healthy controls. Biological characteristics, *e.g.,* differentiation capacity and phenotype were analyzed. MSC function *in vitro* was evaluated by immunomodulatory and hematopoietic assays. In addition, genome wide gene-expression profiles were studied using Deep-SAGE sequencing.

Materials and methods

Patients and MSC expansion

Children referred to our center for HSCT were included in this study according to a protocol (P08.001) approved by the Institutional Review Boards on Medical Ethics. Next to the bone-marrow of 10 healthy controls (HC, median age 7.4, range 1.1–16.4 years) being HSCT donors, bone-marrow of 17 MDS patients (10 RCC, 2 RAEB, 4 RAEBt, 1 MDR-AML) was collected at diagnosis and prior to treatment initiation. The WHO classification adapted for children was used for the classification of patients. (2) MSCs from children with RAEB, RAEBt and MDR-AML were grouped as advanced MDS to enable the comparison between advanced and RCC-MDS. In addition, bone-marrow after HSCT was collected from 9 children (4 RCC, 1 RAEB, 1 RAEBt, 3 MDR-AML) including 6 paired samples (Table 1).

MSCs were expanded and characterized as previously described (Calkoen et al., 2013). Briefly, bone-marrow mononuclear cells (MNCs) obtained after Ficoll separation were cultured in DMEM (Invitrogen, Paisley, UK) containing 100 U/mL penicillin/100 μg/mL streptomycin (P/S; Invitrogen) and 10% (v/v) fetal bovine serum (FBS; VWR International, Bridgeport, NJ, USA). Non-adherent cells were removed by refreshing the medium twice weekly. Upon reaching confluency MSCs were harvested, pooled and passaged for further expansion resulting in non-clonal MSCs. Phenotype (CD73, CD90, CD105 positive; CD3, CD31, CD34, CD45, CD86, HLA-DR negative) and differentiation capacity towards osteoblasts and adipocytes were investigated at passages 2–3 and 5–7, respectively. All but anti-CD105 (Ancell Corporation Bayport, MN) antibodies were derived from Becton Dickinson Biosciences (BD), San Diego, CA, USA. Culture supernatant was collected after reaching 80% confluency at passages 3–5 for measurement of cytokine production.

Cytogenetics

To exclude common chromosome abnormalities in MSCs and malignant cells, interphase fluorescence *in situ* hybridization (FISH) for chromosomes 7 and 8 was performed on MSCs from patients with known monosomy 7 or trisomy 8 using the following probes: Vysis LSI D7S486/CEP7 and LSI IGH/LSI MYC, CEP8 (Abbott Laboratories, Abbott Park, IL,USA) (Bronkhorst et al., 2011).

Chimerism analysis

Chimerism (donor or recipient origin) was studied by cytosine adenine (CA)-repeat analysis in MSCs cultured from the bone-marrow harvested after HSCT as previously described (Lankester et al., 2010).