C12. **Adhesion-related abnormalities of mesenchymal stromal cells as part of pathological process evaluation in myelodysplastic syndromes**

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The present work is dedicated to the argumentation of the morpho-molecular roles of mesenchymal stromal cells (MSCs) in myelodysplastic syndromes (MDS) pathogenesis, with regards to their ability to generate a microenvironment suitable to hematopoietic cells development.

The understanding the mechanisms by which neoplasia manage to integrate the stromal components in tumorigenesis represents also a major source of progress. The hematopoietic stem cells (HSC)-MSC relationship is a critical point in the hematopoietic malignancies pathogenesis, and the current technical approaches provide limited and rather slow progresses.

Previous work has shown that adherent layers of stromal cells from bone marrows (BM) of MDS patients achieved confluence at significantly slower rate than normal donor-MSC [Boudard D, Haematologica 2003].

In line with these data, we noticed the growth deficiencies and spontaneous lysis in primary cultures of MDS stromal cells compared to normal controls, especially in refractory cytopenia (RC) cases. Moreover, morphological assessment of Refractory Anaemia with Excess Blasts (RAEB) primary layers depict dysplastic changes related to altered actin organization, such as thin and flat cells, as Ilić et al. have already reported in mouse FAK (-/-) fibroblasts [Ilić D, Nature 1995]. Morphometric evaluation highlights the different distribution of the three morphotypes (rounded-shaped, with the appearance of undifferentiated cells; thin, spindle-shaped cells; and large, flat cells) of MSC in MDS layers compared to normal settings, as well as size differences which could indicate maturation defects.

Thereof we were interested to find whether the MSC maturation abnormalities affect equally the functionality of the hematopoietic compartment, and to evaluate the adhesion-related processes, such as cell proliferation and clonogenic growth.

In this order, a first step was to achieve standard-compliant MSC preparations from reduced quantities of BM aspirates and from cells showing significantly reduced abilities to reach confluence.

Thus, we used an initial enrichment in primary cultures and an immunomagnetic double selection based on the different expression of STRO-1 and CD73, two specific markers for MSC. Technically, we noticed that these two fractions could be exploited differently, STRO-1$^+$ cells being more robust for carrying out *in vitro* MSC growth assays, whereas CD73$^+$ cells have proven their utility in the evaluation of adhesion profiles.

Of note is the fact that under the MDS condition, a higher number of STRO-1$^+$ cells which co-expressed CD106 and CD31, were noticed between 20 and 30 days in primary cultures, and were persistent until 60 days. Two hypotheses can be evoked from the expression of these molecules in relation to MDS physiopathology: the former is related to CD106 upregulation induced by TNFα stimulation [Xing L, Asian Biomedicine 2012], and the second is related to CD106 function as a major ligand for selective CD29-mediated hematopoietic precursor cells (HPC)-to-MSC adhesions, and thus, to its influence on the HPC mitotic rate and division kinetics [Kohase M, Cell 1986]. In addition, the increased expression of CD31$^+$ could be an imprint of the neoformation of blood vessels in MDS settings, as Boudard D et al. showed in a previous study [Boudard D, Haematologica 2003]. Likewise in MDS settings, the CD73$^+$ fractions of MSC displayed a significant reduction of adhesion markers, CD29, CD54, CD44, and CD49e.

Furthermore, the functional tests revealed MSC growth abnormalities in the absence of any contact with or stimulation by soluble molecules from HPCs and proved the pathological nature of stromal precursors in MDS settings.

Thus, MSC production in STRO-1$^+$ and CD73$^+$ cell cultures from refractory cytopenia with unilineage dysplasia (RCUD) and refractory dysplasia with multilineage dysplasia (RCMD) marrows was deficient, and, in addition, the clonogenic ability of these fractions was strongly diminished. We have concluded that the relative proliferation in MSC cultures from RC is the result of a division process that is continuous, but occurs at a low rate and without the ability to generate the normal functional progenitors required to form colonies. By contrast, in RAEB settings, the proliferation rate is moderately improved due to the reduced doubling time (DT) of STRO-1 cells. However, at the end point, this was not accompanied by complete functional maturity as reflected in the CFU-F number. Of note is the fact that MSCs from the RAEB in transformation (RAEB-T) cases shows a highly rate of proliferation, both in rounded, and spindle shaped STRO-1$^+$ MSCs, as well as in large STRO-1$^-$ CD73$^+$ MSCs.

Thereafter, we observed a diminution of CFU-F capacity of CD73$^+$ fractions in MDS settings which directly correlated with the CD44 mitigate on their surface. In addition, the doubling time of MSCs from MDS inversely correlate with their expression for CD49e (α_5-integrin). In conclusion, the MSC proliferation and clonogenic potential are adhesion-dependent processes.

Then, we have explored the focal adhesion (FA) signalling pathways in order to understand whether adhesion-mediated processes contribute to transduction of intrinsic proliferative signals, as well as their impact on HPC-to-MSC interactions.

Thus, we have observed that the large proliferation differences occurring in RAEB-T cultures compared to normal settings can be attributed both to smaller (S-MSCs), as well as to large cells (L-MSCs), which present qualitative defects of FA proteins (focal adhesion kinase [FAK], and paxillin) and of the chaperone heat shock protein 90 (HSP90), such as intensity differences, nuclear localization, and their association in complexes. In normal cells, HSP90 plays a number of important roles, which include assisting protein folding and DNA damage response, transcription, and degradation of proteins as well as facilitating cell signalling. Recent evidences shows that HSP90 control the histone code through regulation of KDM4B demethylase stability [Ipenberg I, J. Biol. Chem. 2013].

The MSCs from RAEB cultures highlight a strong overlapping of FA proteins to HSP90 in nuclear area, which could explain the increases proliferative capacity of these cells. A possible explanation of this is the cessation of proteasome-mediated recycling of the proteins co-located to HSP90, which confer them a proliferative advantage, as Dong JM et al. showed in a previous study [Dong JM, Biochem J 2005]. Moreover, recent evidences support the FAK direct contribution to cell growth both by influencing the proliferation rate as well as apoptosis also. FAK binding at paxillin induce its phosphorylation and conformational modification, blocking its nuclear export [Dong JM, Biochem J 2005]. It has been proven that this particular nuclear localization of paxillin stimulate DNA synthesis and cell proliferation [Dong JM, Biochem J 2005], [Kasai M, Cancer Research 2003], by suppression of H19 (a tumor-suppressor gene) transcription and promotion of Igf2 expression at the translational level [Dong JM, Biochem J 2005].

Another hypothesis that arises from our study is the putative role of FAK [Y397] expression on MSCs in HPC-to-MSC interactions and thus its implication in modulation of HPC clonogenic capacities. There is recent evidence that sustain that FAK regulates integrin expression in human fibroblast [Michael EK, Mol Biol Cell 2009]. In this study we find that the increased levels of pFAK [Y397] reversely correlate with the CD49e expression on MSC cells, and this reduction significantly correlate with the diminution of clonogenical potential of HPCs selected from MDS patients.

In conclusion, these data prove that MSCs selected from MDS patients are intrinsically pathological and that they could influence HSC behaviour by their direct interactions via FA proteins signalling. The perception of the stroma-related disease mechanisms may underlie the development of alternative therapeutic approaches, e.g. if considering that FAK is one of HSP90αβ-client proteins, HSP90αβ inhibitors may be used as adjuvants in MDS therapy.