Effects of hypoxia on the Extracellular Matrix produced by the mouse MS-5 Mesenchymal Stromal cell line

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Introduction

The extracellular matrices (ECMs) are an important component in cell signaling as well as in defining the shape and stability of tissues. ECM promotes cell recruitment, adhesion, migration, proliferation and differentiation, thereby emphasizing the importance of the biological role of ECM [1]. It has been shown that ECM is capable of directing the differentiation fate of cells cultured on top of ECM. It was also shown that ECM prepared in normoxia (21% O_2) and hypoxia (2% O_2) conditions alters the differentiation of cells [2].

MS-5 cells represent a continuously growing clone of mouse mesenchymal stromal cells (MSC). They have been extensively used in the literature as a model of MSC because a) their proteome is enriched in pro-angiogenic factors and b) their extracellular matrix (ECM) supports human hematopoietic stem and progenitor cell survival and differentiation. We have recently shown that a) the differentiation of MS5 and other continuously growing mouse MSC lines [3] and b) the DNA damage response of MS-5 [4] are both influenced by hypoxia.

Aim of the study

To better understand the biological role of MS-5 ECM. We intend to study the differences between MS-5 ECM prepared in the presence of 21% or 2% O_2. The long-term goal of these studies is to identify ECM molecules that signal MS-5 cells to retain their stemness.

Methods

Seed MS-5 (23 000 cells/cm^2) in pre-coted plates with 0.1% Gelatin

Normoxia 21% O_2; 5%CO_2 / Hypoxia 2% O_2; 5%CO_2

After 3 days treat with Mitomycin C (10µg/mL) for 3 hours at 37°C
Wash carefully and add fresh medium

"Grow" 4 days

Cell lysis (10mM Tris, 1mM EDTA, pH7.4) overnight at 4°C
Wash carefully

ECM was decellularized by 10mM DTT in 5M guanidine hydrochloride for 1 hour at 4°C

Scraped the matrix, centrifuged and stored at -80°C

Run sample in a gel, cut the gel into slices and enzymatic digestion

Peptides are analyzed by mass spectrometry and the results were compared with a library

Analysis of the results

Results

Preliminary evidence has indicated that when MS-5 cells are plated on their own ECM, there is up-regulation of transcripts encoding stem cell genes and an improvement in their differentiation capacity. In order to investigate this further, we have compared the proteomic profile of MS-5 ECM prepared from cells grown in normoxia (20% O_2) versus hypoxia (5% O_2). Results obtained indicate significant changes in the ECM composition in hypoxia. Proteomic data will be confirmed biochemically and immunologically. Glycomic profiling of MS-5 ECM is also being carried out.

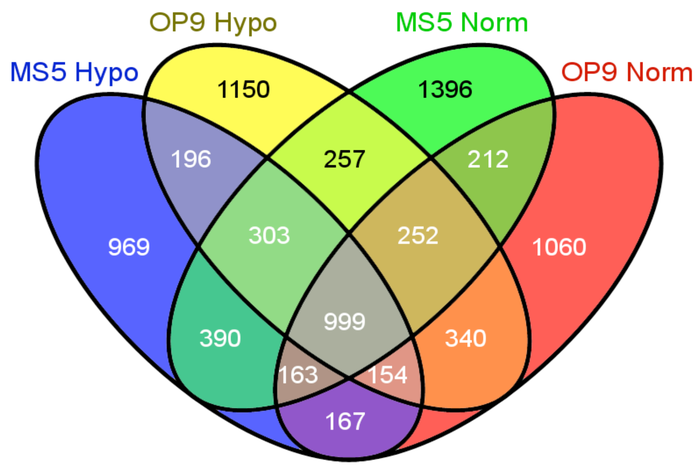

Figure 1 – Results of proteomic data. Overall distribution of proteins in two cell lines MS-5 and OP9.

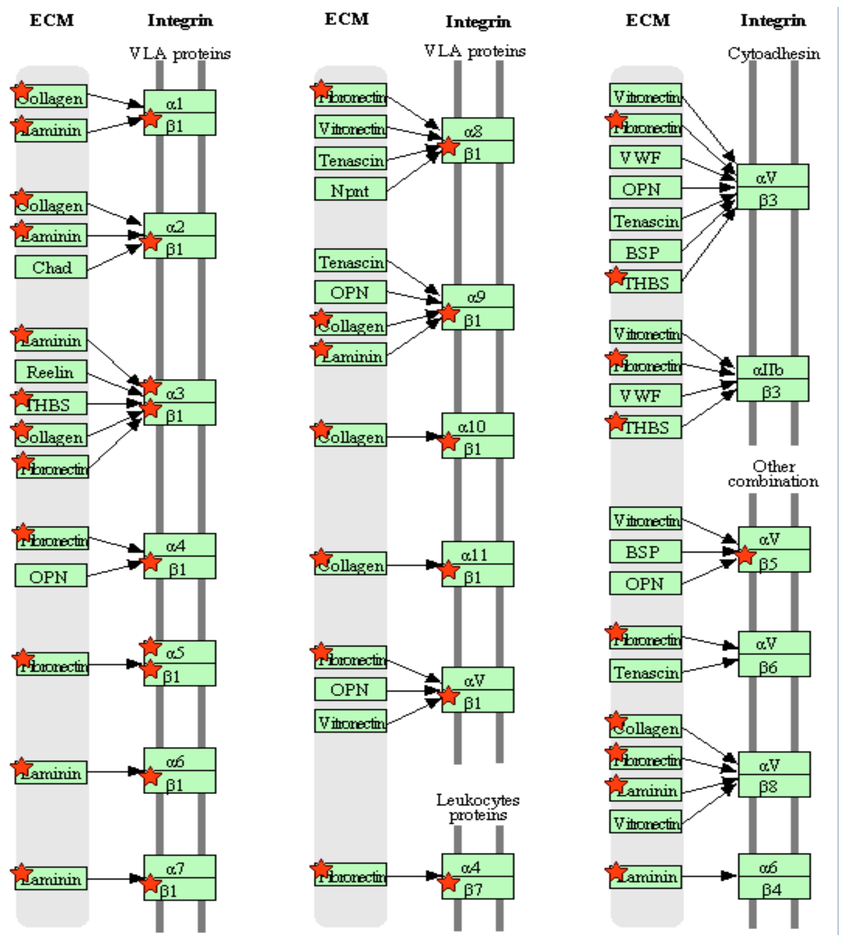

Figure 2 – ECM-receptor interaction pathway is one of the 3 pathways with high relevance. Red start means that these proteins are present in our sample.

Conclusions

Proteomic data show that MS-5 ECM prepared in normoxia has more proteins present however there are more proteins with higher significance in hypoxia. Proteomic results also show that one of the most relevant pathways affected by hypoxia is ECM receptor interaction. Parallel glycomic profiling will focus on modifications to molecules involved in cellular signaling. Finally, attempts will be made to modify the proteomic and/or glycomic profiles of ECM to determine how its biological activity is mediated.

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