Qualitative effects of hypoxia on the composition of extracellular matrix produced by a cloned mouse mesenchymal stromal cell

Andrelia Ribeiro1, Satbir Gill2, Lokesh Joshi 3, Rhodri Ceredig4

1 Regenerative Medicine Institute, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland
2 Glycoscience group, National Centre for Biomedical Engineering Science, National University of Ireland, Galway, Ireland

Introduction

Extracellular matrices (ECMs) are important components 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 ECM1. It has been shown that ECM is capable of directing the differentiation fate of mesenchymal stromal (MSC) cells cultured on top of ECM. It was also shown that ECM prepared in normoxia (21% O2) and hypoxia (5% O2) conditions alters the differentiation of cells2. MS-5 cells represent a continuously growing clone of 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 recruitment, adhesion, migration, proliferation and differentiation, thereby extensively used in the literature as a model of MSC because a) the differentiation of MS5 and other continuously growing mouse MSC lines3 and b) the DNA damage response of MS-5 are both influenced by hypoxia.

Aim of the study

To study the differences between two different methods to prepare ECM produced by MS-5, in the presence of 21% or 5% O2. Method 1 is by cell lysis, Method 2 by inducing apoptosis. The long-term goal of these studies is to identify ECM molecules that signal mouse stromal cells to retain their stemness.

Methods

Methods to prepare ECM:

Method 1 - Lysis:
Seed MS5 21% or 5% O2
Mitomycin C treatment
3 days
Lysis by osmotic shock (Tris-EDTA buffer)
4 days

Method 2 – Apoptosis:
Seed MS5 iDS 21% or 5% O2
Change media
3 days
Induce Apoptosis
4 days

Quantitative characterization of ECM:

• Immunocytochemistry (ICC)
• Proteomic analysis
• RT-PCR

Qualitative characterization of ECM:

• Culture of Balb/c (mouse stromal cells) on top of ECMS or plastic
• Differentiation assays of Balb/c on top of ECMS or plastic

Acknowledgements

This project has received funding from the European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no 315962. Within the Marie Curie Initial Training Network DECIDE (Decision-making within cells and differentiation entrapments). A special thank you to Professor Ivan Martin and Sébastien Pigeot from Institute for Surgical Research, Basel, Switzerland.

References

5. European Union’s Seventh Framework Programme for research, technological development and demonstration under grant agreement no 315962.