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3D Extracellular Matrix derived model of Alveolar Rhabdomyosarcoma

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INTRODUCTION: Rhabdomyosarcoma is the most common soft tissue sarcoma in childhood, among the subtypes the Alveolar (ARMS) is the more aggressive with a higher tendency to metastasize [1]. Integrins are a class of transmembrane adhesion molecules that mediate survival, differentiation, migration and differentiation [2]. Here we investigate the role of integrins in ARMS metastatic migration in an engineered 3D scaffold.

METHODS: ARMS xenografts are obtained from subcutaneous injection of RH30 cells in immunodeficient mice. Composition of the ECM is determined by proteomic analysis. The main components of the ECM are used to enrich a 3D collagen scaffold cultured in a perfusion bioreactor. Cells are analyzed by qPCR for the expression of a panel of integrins. Presence of the protein is confirmed by flow cytometry immunofluorescence.
MMPs expression is evaluated by zymography.

RESULTS: Verified the expression of human and ARMS marker and typical tumor morphology in xenografts, they are processed for proteomic analysis. Proteomic data analysis is currently under investigation. Preliminary data culturing RH30 cells in 3D bioreactor show upregulation of ITGα5 and CXCR4 receptor compared to 2D condition. Localization and quantification at protein level will be assessed respectively by immunofluorescence and cytofluorimetry.
Expression of MMP-9 and MMP-2 has been assessed by zymography comparing the expression of these MMPs in 2D vs 3D bioreactor and RH30 isolated from the xenograft (Figure 1).
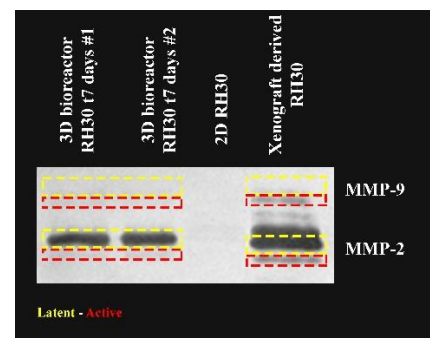

Figure 1: zymography from RH30 supernatant isolated from U-Cup 3D bioreactor (sample #1 and #2) from 2D and RH30 isolated from xenograft.

DISCUSSION & CONCLUSIONS:
Preliminary data on ITG expression show that in 3D scaffold the expression of ITGα5 and CXCR4 is upregulated. In parallel the active form of MMP-2 is more present in 3D models compared to 2D. Other groups reported a mechanical interaction between ITGα5 and MMP-2 [3]. This interaction will be studied in a more representative engineered 3D scaffold to shed light on the complex interaction between ECM and metastatic progression.

REFERENCES
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