Profiling biomolecules at cell-biomaterial interface by quantitative proteomics

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Introduction

Implant surface structure and chemistry determines the contacting cell's fate. Therefore, the fate of those cells directly affect bone-implant incorporation in clinical practice[1-5]. However, how these chemical and mechanical signals translating to cellular responses are not yet known. The major drawback is a lack of systematic study of cell-biomaterial interaction in terms of protein expression, specifically, at the attachment interface between the cell and biomaterial (adherence surface, AS). Therefore, we have proposed to unbiasedly identify the biomolecules at the interface by proteomics. This method combines the use of a subcellular fractionation with quantitative mass spectrometry-based proteomics to characterize the biomolecules at cell-material interface *in-vitro*. In the initial study we hypothesize that attachment of cells to a biomaterial in a 2D environment results in the localization of specific proteins at the interface between cells and biomaterial. Through proteomics of the interface, we aimed to discover novel proteins, which are highly localized between the cells and biomaterials.

Materials and Methods

A simple cell-biomaterial attachment model involving Madin Darby canine kidney (MDCK) cells on tissue culture polystyrene was used in this study. To label the proteins at the cell-biomaterials interface, all proteins of cells were labeled isotopically during culture via SILAC (Stable Isotope Labeling of Amino acids in Cell culture). AS of heavy isotope labeled cells were isolated (Fig. 1B), and were made lysate. Light isotope labelled cells was made whole cell lysate. "Heavy" and "Light" samples were mixed at a 1:1 ratio, followed by 1D gel separation. In-gel digested proteins were identified with LC/MS/MS. (Fig. 1. A) Western blot, confocal and atomic microscopy were utilized to demonstrate the purity of the isolation.

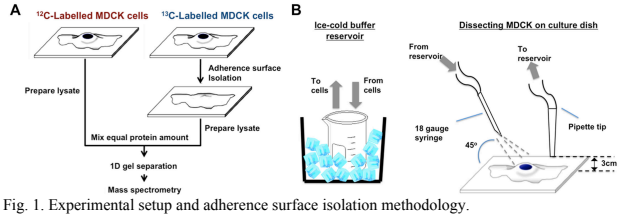

Fig. 1. Experimental setup and adherence surface isolation methodology.

Results

All imaging suggested that the apical part of adhered cells were removed, retaining its ventral layer on biomaterial. Western-blot suggested that ECM, stress fibers, and focal adhesion proteins were highly enriched in the layer. Proteins identified with high reliablility were quantified via SILAC ratio (H/L). Proteins with ratio >1 implicated they were localized at the interface, while <1 implicated it was from the apical portion. Gene ontology analysis confirmed the subcellular location of interfacial proteins were ECM, stress fibers and membrane proteins.

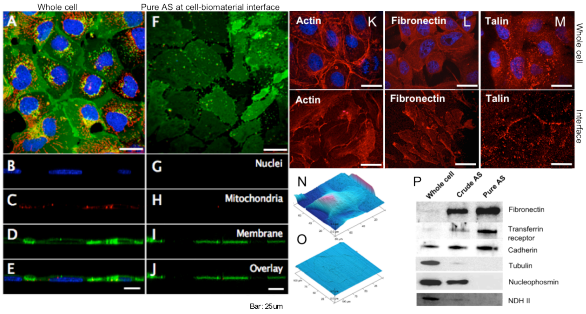

Fig. 2. [A-E] Adhered cells and cross-sections. [F-J] Isolated interface and cross-sections. [K-M] Images of Actin, Fibronectin, Talin (Top row: Cells; Bottom row: Isolated interface)[N, O] AFM of cells and isolated interface. [P] Western-blottings of Fibronectin, CD71, Cadherin, Tubulin, Nucleophosmin and NDHII.

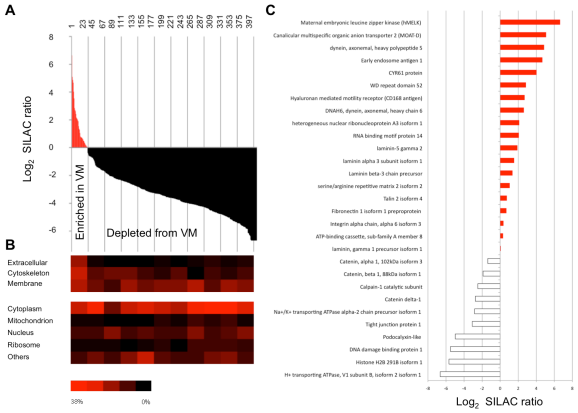

Fig. 3. [A] Quantified proteins in descending order of SILAC ratio. [B] Heat map of proteins subcellular origin of [A]. [C] Note worthy proteins and its SILAC ratio.

Discussion and Conclusions

Apart from quantifying classical adhesion proteins, proteins not previously known to be cell-substrate interaction related were identified at interface, suggesting possible new linkage to several cellular responses to substrate. Such as Cep 350, which involves in mitosis, centriole growth and maintaining microtubule network, was found. A specific subset of RNA-binding proteins was also highlighted, which agrees with the recent discovery of its association with the "spreading initiation centres", a novel structure important for the attachment of cells[8]. Their localization at the interface suggests new roles in the regulation of proliferation on the biomaterial surface. Such discovery could be utilized in designing smart implants to guide desirable cell fate. Proteomics study at the interface enables unbiased and high resolution biochemical investigation at interface between cells and biomaterials. While our study is based on a well-characterized mammalian cell line, we confirm it can be adopted on other cell lines, such as osteoblast and mesenchymal stem cells on different substrata.

References List

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Disclosures

Authors have nothing to disclose.