LC/MS identification of 12 intracellular cytoskeletal and inflammatory proteins from monocytes adherent on surface-adsorbed fibronectin-derived peptides

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Statement of Purpose: The monocyte-mediated host response to implanted devices is critical yet poorly understood. We hypothesize that the identity and conformation of adsorbed proteins on a biomaterial surface mediates the extent and duration of the host response. Therefore U937 promonocytic cells adherent to fibronectin-derived peptide-adsorbed tissue culture polystyrene (TCPS) were analyzed by nanospray HPLC-coupled mass spectrometry to gain insight into the surface-mediated monocyte response. LC/MS has been used by cell biologists for over a decade; however, the technology has not been widely applied to the field of biomaterials. We have used LC/MS to identify tyrosine phosphorylated proteins from monocytes adherent to peptide-adsorbed tissue culture polystyrene (TCPS) to obtain proteins of interest for further research into their effect upon surface mediated monocyte adhesion and activation.

Methods: Adherent U937 were harvested from G_6-, G_3RGDG- & G_3PHSRNG-adsorbed TCPS after 24 hr. Cells were lysed with detergent and brief sonication followed by immunoprecipitation of phosphotyrosine proteins via anti-phosphotyrosine monoclonal antibodies. Proteins were separated by SDS-PAGE, stained with Coomassie Brilliant Blue dye and excised according to previously performed densitometry[1]. Proteins were digested with trypsin. Peptides were extracted from the gel fragments and de-salted prior to analysis by LC/MS. All samples were analyzed in duplicate (n = 2) with a minimum peptide length of 9 amino acids detected. Sequenced peptides were searched against the National Center for Biotechnology Information database using Mascot, which uses ion scoring based on the Mowse algorithm to assess significance. Since only proteins from adherent cells are of interest, proteins identified in both adherent U937 and U937 in suspension were considered not relevant. The identified proteins were searched against both the **Ex**pert **P**rotein **A**nalysis **Sy**stem (Expasy) and the Human Protein Reference Database for protein function, protein-protein interactions and post-translational modifications.

Results/Discussion: LC/MS analysis of U937 on peptide-adsorbed TCPS identified 12 cytoskeletal and inflammatory related proteins. Moesin, heat shock protein 90β (Hsp90β), vimentin and β-actin were found only in U937 adherent to G_3RGDG-adsorbed TCPS. Moesin cross links cytosolic proteins such as RhoGTPases to the actin cytoskeleton near activated integrins that link monocytes to extracellular matrix (ECM) proteins such as fibronectin. Moesin can affect cellular polarization, migration and immunological synapse formation through these RhoGTPases. Moesin can also be phosphorylated on tyrosine145 with currently unknown biological significance. Hsp90β has been observed to inhibit tubulin polymerization aiding in cellular migration, rearrangement and polarization. β-actin and vimentin are involved in actin filaments and intermediate filaments, respectively. Only α-tubulin, which comprises part of microtubules, was identified from U937 on G_3PHSRNG-adsorbed TCPS. Both elongation factor 1α (Ef–1α) and plasminogen activator inhibitor 2 (PAI-2) were identified from cells on G_3RGDG- and G_6-adsorbed TCPS. Ef-1α localizes F- and β-actin mRNA to the plasma membrane by binding actin filaments. PAI-2 aids in extracellular matrix remodeling. Heterogeneous ribonuclear protein A2 (hnRNP A2) was found in U937 on G_3RGDG- and G_3PHSRNG-adsorbed TCPS and has been observed to stabilize collagen mRNA.

Two inflammatory proteins, glycoprotein 96 (gp96) and heterogeneous nuclear ribonucleoprotein D0 (hnRNP D0) were identified from U937 on G_3RGD-adsorbed TCPS. gp96 stimulates antigen presenting cells such as monocyte-derived macrophages to present antigen stimulating a $CD8^+$ T-cell response. hnRNP D0 acts as a transcription factor for complement receptor 2, which can increase the B cell response to antigen but its effect in macrophages is currently unknown. No inflammatory proteins were identified in U937 adherent to G_3PHSRNG-adsorbed TCPS. High mobility group box 1 (HMG1) and caspase recruitment domain 5 (CARD 5) were identified in U937 on G_6-adsorbed TCPS. HMG can be released into the extracellular milieu to stimulate release of pro-inflammatory cytokines while CARD5 increases cellular processing of pro-inflammatory cytokine interleukin 1β.

Conclusions: A total of 12 cytoskeletal and inflammatory proteins were identified by nanospray LC/MS from monocytes adherent to G_6-, G_3RGD- and G_3PHSRNG-adsorbed TCPS. The exact role these peptides play in mediating the expression and phosphorylation state of cytoskeletal proteins such as moesin and inflammatory proteins such as gp96 and HMG1 in adherent monocytes requires further research.

References: [1]Zuckerman ST. Biomat. 2005;26(8):873-882.

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