Purpose of Study This study aimed at characterizing the secretome of HMEEC-1 and to evaluate its regulation in response to NTHi lysates and better understand the pathogenesis of Otitis Media (OM).

Methods Used HMEEC-1 were labeled with heavy isotopes of arginine and lysine to obtain a spike-in standard that was mixed with the conditions of interest (control or treated 24 hrs, secretions recovered 24 hrs or 48 hrs) and separated by SDS-PAGE. Peptides generated by in-gel digestion were analyzed by LC-MS/MS. Middle ear effusions (MEEs) from patients having chronic OM were analyzed to validate the results obtained with HMEEC.

Summary of Results 767 proteins were detected by MS in HMEEC secretions. The more abundant proteins detected were components of the extracellular matrix, proteins implicated in the innate immune response, and surprisingly proteins implicated in the processing and packaging of RNA. These proteins were heterogeneous nuclear ribonucleoproteins A2/B1 (hnRNPA2B1) and K (hnRNPK) enriched at the 24 hrs time point (1.99 and 1.78 fold change respectively) and Q at 48 hrs (hnRNPK, fold change 4.76) in response to NTHi lysates. We then hypothesized that these proteins were implicated in the packaging of miRNAs in exosomes in response to NTHi lysates. An exosome marker assay showed the presence of exosomes in both the cell secretions and MEEs. A western blot analysis of MEE exosome proteins showed the presence of hnRNPs as in cell secretions. Finally, a Nanostring chip assay demonstrated the presence of 8 miRNA in MEEs, mostly reported to be produced by epithelial cells and neutrophils.

Conclusions We characterized the secretome of HMEEC in response to NTHi lysates treatment that show a potential implication of exosomes in the pathogenesis of OM. We demonstrated the presence of exosomes in HMEEC secretions and MEEs, transporting miRNAs packaged by hnRNP proteins.

MP5

STABLE ISOTOPE LABELED BY AMINO ACID IN CULTURE (SILAC) STRATEGY TO ANALYZE HUMAN MIDDLE EAR EPITHELIAL CELLS (HMEEC) SECRETOME IN RESPONSE TO NTHI LYSATES: EVIDENCE OF THE IMPLICATION OF EXOSOMES IN OTITIS MEDIA

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