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Isolating and Characterizing Salivary Exosomes

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SIGNIFICANCE

•Approximately 30,000 Americans are diagnosed with oral cancer each year (NCI, 2012).

•Diagnosing oral cancer early in the pre-malignant lesion stage instead of the oral squamous cell carcinoma stage is key to survival and recovery (Kooren et al., 2011).

•The current standard for accurate diagnosis of oral lesions requires an invasive and painful biopsy followed by labor-intensive analysis (Rhodus, N.L., 2005, Lingen et al., 2008).

•Exosomes—small membrane bound vesicles secreted in saliva and many other biological fluids—that contain nucleic acids and proteins have been of great interest to researchers for their potential as disease biomarkers (Mathivanan, S., et al., 2010; Bandhakavi, S., et al., 2011; Gonzalez-Begne, M., et al., 2009).

•Project goal: To isolate exosomes from whole saliva and to characterize their protein content using tandem mass spectrometry.

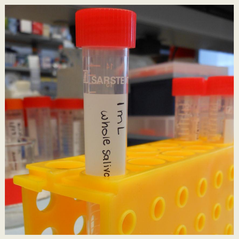
MATERIALS AND METHODS

•1-2 ml of whole saliva was collected and treated with acetic acid

•Treated saliva underwent serial ultracentrifugation and filtration in order to isolate the exosome pellet (Théry, C., et al., 2006).

•Samples were prepared using the Filter-Aided Sample Preparation Technique (FASP) for mass spectrometry (Wiśniewski , et al., 2009).

•The mass spectrometry parameters used for this experiment were the Orbitrap Velos setting as described in (De Jong, E., et al., 2012).



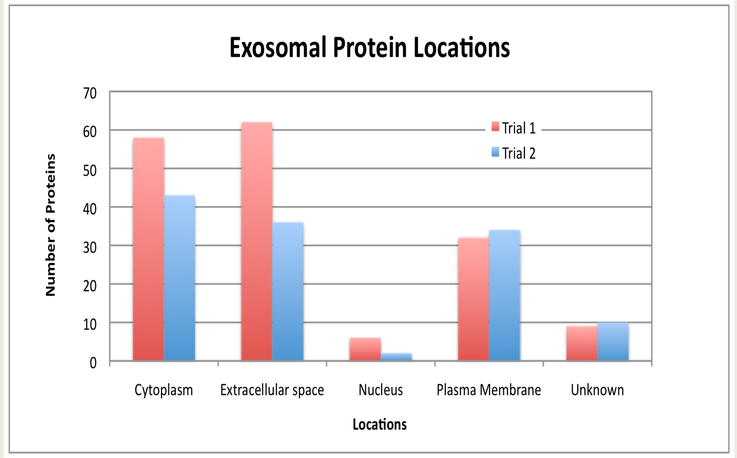
Whole Saliva

Mass Spectrometer

Ultracentrifuge

RESULTS

Mass Spectrometry

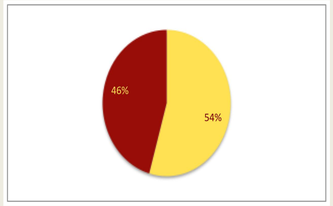


Besides the control, trial 1 included samples treated with 800µl 1% acetic acid and trial 2 included samples treated with 400µl and 800µl of 1% acetic acid. The vast majority of proteins found in our samples were located in the cytoplasm and extracellular space.

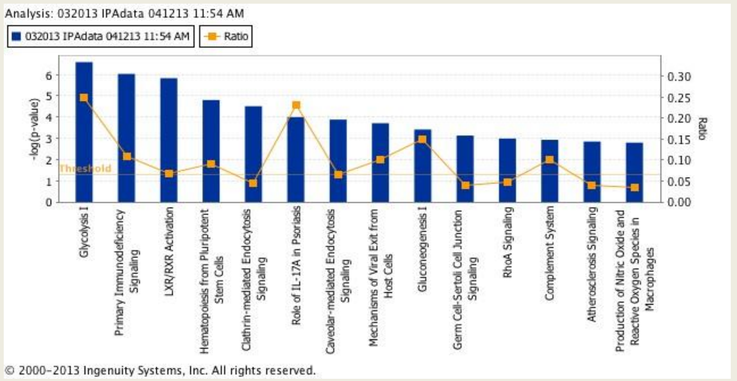
Protein Markers	Trial 1	Trial 2
Programmed cell death 6-interacting protein	YES	YES
Tumor susceptibility gene 101 protein	YES	YES
CD9 antigen	YES	YES
CD81 antigen	YES	NO
CD63 antigen	NO	NO
Heat-shock protein 70	NO	NO

Some characteristic exosomal proteins used to identify the presence of exosomes (Mathivanan, S., et al., 2010).

Comparison to Published Exosome Proteomes



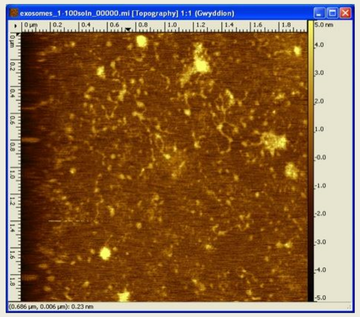
Proteins identified via MS/MS were matched to published works such as the article by Ogawa, et al., 2011). The gold portion represents percent of protein matched , while the maroon portion represents percent of protein not matched.



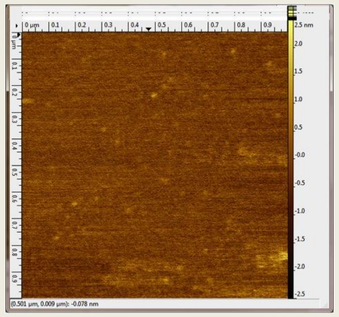
Ingenuity Pathway Analysis (IPA) showed the different pathways associated with exosomal proteins isolated from our saliva sample.

RESULTS CONTINUED...

Atomic Force Microscopy (AFM)



2µmx2µm topography image of 1:100 dilution of exosome sample. These molecules were about 30nm in diameter and 2.5nm in height.



1µmx1µm topography image of PBS (control). Much less molecules were observed. Molecules were also about 25nm wide and 2nm high.

CONCLUSIONS

•From our results, we were able to successfully isolate exosomes from whole saliva because characteristic exosomal proteins were identified in our samples via MS/MS.

•In our samples, the majority of the proteins were found to be located in the cytoplasm and extracellular space. This was expected as exosomes are secreted by cells and are known to be involved in intercellular communications (Mathivanan, S., et al., 2010).

•In addition, we have also found the different pathways, such as glycolysis, that our exosomal proteins are involved in using IPA.

•Lastly, although not 100% certain, AFM was able to provide us with a general sense of the salivary exosome morphology.

•The next step in this study will be to isolate exosomes from healthy and cancerous patients and compare their exosomal proteomes.

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