Beckman Coulter Webinar Series
Proteome Profiling of the Tumor Microenvironment: Role of
Human Primary Fibroblasts Derived Exosomes in Oral Cancer Progression
View the On-Demand Recording Here

Presented by: Simona Principe, PhD
Post-Doctoral Fellow,
Princess Margaret Cancer Center, University Health Network
October 8th, 2015
Q&A Session

Question: How did you isolate and perform the proteomics of the membrane proteins against the high cell lysis protein load?
Answer: *Plasma membrane proteins have been isolated according to a protocol optimized in my lab and published few years ago: Use of colloidal silica-beads for the isolation of cell-surface proteins for mass spectrometry-based proteomics. Methods Mol Biol (2011).*

Question: Did you do any proteomics on the resected tumors to assay the exosomes?
Answer: *Proteomics on the resected tumors has not been performed. This type of analysis would not add any information on the exosomal fraction since exosomes are secreted vesicles.*

Question: 1/ How many cells (Cafs) did you use to obtain enough exosomes to see migration/growth effects on cancer cells? 2/ How did you measure the exosome concentration? 3/ you used 120000g to isolate your exosomes, did you use ''pre centrifugation'' to remove microparticles? If not, it is not better to speak about extracellular vesicles than exosomes? Thank you for your answers.
Answer: *1/ I seed 30-40 million CAFs to obtain roughly 30-40ug of exosomes and I treat cancer cells with 10ng/mL to see migration/proliferation effects. 2/ Exosomes concentration is determined by BCA assay on the exosomal pellet, resuspended in PBS. 3/ Pre-centrifugation steps are included in the exosomes isolation according to Théry C et al protocol [PMID: 18228490].*

Question: The last slide before conclusion was not long enough on the screen. Can you show that again and explain the top right graph?
Answer: *The 3D plot shows in red a cluster of 422 media-enriched proteins (2-fold change 0.05 p-value). This is a 3D visualization of the dataset since the enrichment is calculated versus two other fractions (exosomes and whole cell lysate)*

Question: What was the protocol for isolation of exosomes? How did you make sure that proteins isolated came straight from exosomes, not from other extracellular fractions?
Answer: *The protocol used for exosomes isolation has been adapted from Théry C et al [PMID: 18228490]. Characterization of the exosomes has been confirmed by EM (size and integrity) and by WB (known exosomal markers). The microvesicles fraction (10,000g pellet) has also been tested in the functional assays and had shown no effect on the migration of cancer cells.*

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Question: Has lipidomics being used to characterize the phospholipid/lipid composition of these exosomes?
Answer: Yes, there are few papers elucidating the lipid composition of exosomes (for example from Prostate Cancer cells) but this field has not been investigated in-depth yet.

Question: What is shotgun proteomics?
Answer: *Shotgun proteomics or bottom-up proteomics is a very well-known and largely applied method for the identification of proteins in complex mixtures. Briefly, the protein mixture is proteolytically digested, peptides are separated by high pressure liquid chromatography (LC) and then analyzed by a mass spectrometer.*

Question: What marker is sued to characterize the Af and CAFs?
Answer: *The most common makers used to identify a CAF-like state are: α-SMA, SDF-1, FAP and also secreted markers such as Collagen typeI, SPARC and ECM components.*

Question: Is there any influence of immune associated molecules like chemokines on the tumor milieu on the exosomes of CAf?
Answer: *immune associated molecules in the tumor milieu play an important role in the crosstalk between fibroblasts and cancer cells as well as in the activation of the fibroblasts to a CAF-like state. To the best of my knowledge, the role of chemokines on the exosomes of CAF has not been investigated.*