

“Enabling Technologies for Bulk and In-situ Molecular Analysis of Extracellular Vesicles from Cancer Patient Biofluids”

Liquid biopsies consist of sampling and analysis of tumor-derived content from a blood draw, and they are rapidly gaining interest in the laboratory and clinic due to significant advantages over traditional tissue analysis (e.g., frequent sampling, less invasive). One “candidate” to develop liquid biopsy assays are tumor extracellular vesicles (tEVs). tEVs are shed continuously from tumor cells as tiny lipid nanoparticles and have been identified in different biofluids. tEVs can be used as a surrogate for tumor cells since they carry similar proteins, mRNAs, miRNAs, DNAs. While the EV field has expanded in the last few years, it is still limited to the lack of methods that can specifically isolate tEVs from tumors. All cells in the human body release EVs; thus isolation methods that are size-based or utilize generic markers against EVs result in high levels of background prohibiting robust downstream molecular characterization. Our laboratory and others have developed different technologies that enable the isolation and molecular quantification of tEVs. In this talk, we will describe a novel EV purification method based on ultrafiltration and immunoaffinity selectivity (“iSUF”) to enrich tEVs from different biofluids at small or large volumes, and with 99 % removal of contaminant proteins. Second, we will present a microfluidic approach (“^{EV}HB-Chip”) with biodegradable surface chemistry engineered at the nanoscale to maximize tEV isolation. These technical gains are reflected in our ability to detect tumor mutations and perform next-generation RNA sequencing of the tEVs (bulk molecular analysis). Third, we will show an ultrasensitive detection method for in-situ simultaneous protein and RNA profiling of tEVs at the single vesicle level (^{Au}SERP) using total internal reflection fluorescence (TIRF) microscopy that provides better selectivity for immunotherapy response over standard tissue immunohistochemistry methods. The different technologies' translational potential will be shown through proof of concept experiments performed with clinical samples from glioblastoma (GBM), breast, and lung cancer patients.

Bio

Dr. Eduardo Reátegui is an assistant professor in the Department of Chemical and Biomolecular Engineering at The Ohio State University. He is also a member of the Molecular Biology and Cancer Genetics Program at the James Comprehensive Cancer Center. He obtained his Ph.D. at the University of Minnesota in 2012. His doctoral thesis focused on developing inorganic biomaterials and polymeric nanointerfaces with tunable properties for reversible encapsulation of proteins and cells. After graduation, he was a postdoctoral research fellow at Harvard Medical School and the Massachusetts General Hospital Cancer Center in Boston. His postdoctoral work involved the design and integration of biodegradable nanofilms for microfluidic devices that enabled the selective recovery of circulating tumor biomarkers, including circulating tumor cells (CTCs) and tumor extracellular vesicles (tEVs) for downstream molecular characterization. Moreover, he also invented the neutrophil swarming on a chip platform that enabled high throughput characterization of soluble factors, and inflammation EVs (iEVs) produced from neutrophils during the onset of inflammation. Dr. Reátegui's research at Ohio State integrates microtechnologies, biomaterials, and molecular imaging strategies for high-throughput sorting and molecular profiling of circulating cancer biomarkers and the development of infection/inflammation on-a-chip devices for the biophysical and molecular characterization of immune cell interactions with pathogens and cancer cells.