Thesis Defense - Emna El Gazzah, MS Biology
April 14th, 2021 , 1:00 - 3:00 PM
VIEW EVENT
All are invited to attend the defense. For more information please contact Graduate Coordinator at kharrism@gmu.edu

Candidate: Emna El Gazzah
Program: Biology, MS

Date: April 14, 2021
Time: 1:00 pm

Place: Join Zoom Meeting https://gmu.zoom.us/j/91883741501?pwd=TDJDQnVCa0pCNHV0WWltRDNVQVdWZz09

Title: Exploring differences in KRAS oncogenic signal transduction in isogenic Mouse Embryonic Fibroblasts (MEF) expressing different mutant and wild-type allelic variances

Committee Chair: Dr. Mariaelena Pierobon
Committee Members: Dr. Ancha Baranova, Dr. Emanuel F Petricoin, Dr. Maria Emelianenko

ABSTRACT:
RAS proteins are a family of small GTPases, they are central to transduction of mitogenic signals that control cell growth, proliferation, survival and metabolism. A gain of function mutation in this family of proteins, either by increased expression or activation state, provides the cell with sustained proliferative signalling, one of cancers basic eight hallmarks. For that reason, RAS proteins are one of the most frequently mutated oncogenes, mutated in up to 85% of all human cancers. Of the three RAS isoforms: HRAS, NRAS and KRAS, KRAS astoundingly makes up to 85% of all RAS mutations. 99.2% of KRAS mutations occur at three distinct codon hotspots: 12, 13 and 61, with mutations affecting the G12 residue making up 90% of these. Moreover, compiling evidence points towards the notion that not all KRAS mutations act equally. There is a clear imbalance in the frequency of KRAS mutations that appear not only across different cancer types, but among the same type too. There is a strong correlation between the appearance of a distinct mutation and cancer type, histology and carcinogen. These context specific trends really reflect the underlying complexity behind the different mutations.

To study KRAS mutant specific effect on cell signalling networks, we used isogenic Mouse Embryonic

Fibroblasts (MEFs) engineered to selectively harbour and express 6 KRAS oncogenic variants. We first used a multiplex, high throughput immunoassay, Reverse Phase Protein Microarray (RPPA), to capture broad signalling changes across our cell lines. To further explore the effect of KRAS mutations on downstream signalling events, we characterized and compared functional scaffold-kinase interactions across models harbouring mutations affecting codon 12. Scaffold-kinase interactions were analysed using the newly developed Multi-nodal Protein Interactome Network Array (MPINA) which combines serial Co-immunoprecipitation (Co-IP) with RPPA to selectively isolate protein complexes and identify the activation status of their constituents.

Broad signalling profile of the 6 models has shown heterogeneous and mutation-specific signalling activity. Overall, mutations at codon 12 cluster together exhibiting higher signalling activity compared to all models and presented with the least intricate interconnection network. On the contrary, mutations of codon 61 presented with the least active signalling dynamics and interestingly interconnection network with the highest complexity. Remarkably, we observe the highest difference in SKI dynamics among the two cell lines with highest signalling activities. G12C mutations presented with the most active SKI events while the G12D presented with significantly reduced SKI action.

Taken together our data indicate that not all KRAS mutations are equal as downstream signalling nodes are differentially activated based on the type of mutation. Exploring SKI of KRAS downstream signalling molecules may provide additional information of how signalling networks are rearranged in the presence of a KRAS mutation.