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"In Vitro Characterization of Proteins Associated with Cell Migration and Invasiveness in a Murine Model of Cancerous Progression"

Tumorigenesis, the process by which cancer progresses and metastasizes, is of critical importance in cancer research and medicine, as the vast majority of cancer deaths are caused by advanced, metastatic stages of the disease. Therefore, there is a great need to have an ideal model system to study the complex development of tumorigenesis. The murine model of cancerous progression used in these experiments is comprised of seven cell lines and was developed to better research the transformation of cancer from a non-tumorigenic cellular phenotype to a highly aggressive metastatic phenotype. Each cell line represents a different stage of tumorigenesis, and our lab recently characterized all cell lines measuring cell motility and invasiveness. The studies concluded that the behavior of the cell lines matched the expected phenotype based on the stage of the tumor from which they were derived. Cell Lines derived from advanced distant metastatic tumors had higher levels of motility and cell invasiveness when compared to cell lines derived from localized early stage tumors. In addition, a large-scale proteomic analysis was performed on each cell line that identified novel targets, such as semaphorin 7A, for further investigation of proteins associated with metastatic progression. However, the proteomic analysis was not able to provide expression data on our lab's primary proteins of interest, the voltage gated sodium channels (VGSCs), which are known to be upregulated in highly metastatic carcinomas. Based on our laboratory's experimental data, we predicted that immunocytofluorescent analysis would show that the metastatic cell lines have an upregulation of VGSC expression and that the expression patterns of semaphorin 7A would qualitatively correlate with the lab's proteomic data. Cell lines were seeded on coverslips in a six well plate and fix for labeling with primary antibodies, anti-Pan-VGSC (1:200 dilution) and anti-semaphorin 7A (1:100 dilution), with the secondary antibody Alexa 488 (1:1200 dilution), and with DRAQ5 nuclear counter stain (1:600 dilution). Slides were imaged on Leica DMi8 confocal microscope. Our results show that the cell lines in this murine model of tumorigenesis do express VGSCs, but we cannot say that we see an increase in VGSC expression as the cell lines become more metastatic. However the expression of semaphorin 7A correlates well to our proteomic data.