Expression of the INK4AB/ARF Tumor Suppressor Transcription Factor MSK1 in Canine Breast Cancer: Quantification through qPCR and Correlation with Established Phenotypes

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MSK1 is a regulator of a protein that encodes for INK4A/p16, a vital tumor suppressor gene that is found defective in both human and canine cancers. While the cause of many of these defects are known and lie within mutations of the genetic coding for INK4A/16, the other causes are most likely due to upstream mutations, possibly with MSK1. Canine mammary tumors cells are used in this study since dogs are excellent models for human cancer and exhibit comparable molecular targets. Prior research exhibited heightened expression of MSK1 within tumor cell tissue after traditional polymerase chain reaction (PCR) amplification and gel electrophoresis. To quantify the levels of this expression, quantitative-PCR (qPCR) was utilized using a SYBR Green fluorophore, which preferentially binds to double-stranded DNA during amplification. Therefore, the levels of fluorescence correlate directly with the amount of product as it increases exponentially. Following analysis, MSK1 was confirmed to have more starting product within tumor cell lines than the normal epithelial cells, albeit varying degrees among the six lines used. Future work will compare the data to the known phenotypes of the respective cells. Additionally, further tests exploring downstream protein kinases of INK4A/p16 and CDKs 1 and 2 with flow cytometry and fluorescence microscopy will further verify expression and location of the transcription factor during the rapid growth of tumor cells. With these tools, description of MSK1 profiles among the different canine mammary cell lines and levels of expression will lead to evidence about defects within the mechanism of the INK4A/p16 tumor suppressor and changes in cellular location. Ultimately, this knowledge of the faulty cellular mechanism within canine cancer will allow for potential gene therapy treatments to correct the known defects in both dogs and humans.

Statement of Research Advisor:

Jonathan helped develop a research strategy to first determine if the canine breast cancer cells expressed this newly described transcription factor thought to regulate cell proliferation and then designed a qPCR assay to assess the levels of expression. Though it took most of the summer to first develop and then optimize the assay, he was able to detect this important regulator of gene expression and to quantify its expression levels in cancer cells he grew in culture. This technology will allow the further investigation of this important transcription factor and its contribution to cancer cell proliferation.

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