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13. ABSTRACT (Maximum 200 Words)

A significant fraction of breast cancer cell lines and primary tumors exhibit elevated Src tyrosine kinase activity. The mechanism(s) by which Src kinases become activated is not well understood. In some cases, these enzymes form complexes with various growth factor receptors, leading to their activation. Conceivably other gene products may act in a similar manner. We have cloned a novel adapter-like signaling molecule from epithelial cells that we call SRCASM, for SRC Activating and Signaling Molecule. We hypothesize that elevated expression of SRCASM in mammary epithelia may result in increased Src activation, leading to hyperplasia or transformation. This will be studied by: (1) generation of transgenic mice expressing Srcasm in mammary epithelia. Mice will be monitored for changes in mammary gland morphogenesis as well as tumor development; (2) analyze mammary carcinoma cell lines and primary tumor samples to determine whether specific subset of tumors have elevated levels of Srcasm. The relative expression levels will be correlated with patient outcome or metastatic phenotype to determine whether monitoring Srcasm expression has any predictive value. Together these studies should provide insight into the function of Srcasm in mammary gland biology.

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INTRODUCTION: A majority of breast cancer cell lines and primary tumors exhibit elevated tyrosine kinase activity, and a significant fraction of the kinase activity can be ascribed to increases in the specific activity of Src family kinases. The mechanism(s) by which Src kinases become activated is known for only a small percentage of tumors. In some cases, these enzymes form complexes with various growth factor receptors, leading to Src activation. This suggests that other gene products may interact with Src to promote its activation. We have cloned a novel adapter-like signaling molecule from epithelial cells that we call SRCASM, for SRC Activating and Signaling Molecule. Because of its unique biochemical properties, we hypothesize that elevated expression of SRCASM in mammary epithelia may result in increased Src activation and subsequent induction of hyperplasia or overt transformation. To investigate this further transgenic mouse that overexpress Srcasm in mammary tissue will be generated and analyzed to determine whether the cells develop a neoplastic fate. In addition, both human mammary cell lines and primary mammary tumor samples will be analyzed to determine if there is a correlation between tumor subtype and Srcasm expression.

BODY: Much of the first year was devoted to "reagent making". To this end, we have generated transgenic mice (Task I) designed to express SRCASM in mammary epithelia. Because we do not have antibodies that recognize the endogenous Srcasm protein, we cloned an epitope tagged version of SRCASM into a vector that drives expression from the MMTV LTR (promoter/enhancer). This construct has been used successfully to target expression of cDNAs to mammary tissue. At present we have seven transgenic founder lines. We are currently breeding them to determine which founders transmit the transgene to progeny and if they express the tagged protein.

We have also begun analyzing mammary cell lines and primary tissue for Srcasm expression (Task 3). As outlined it the original proposal we were going to perform in situ hybridization on archived breast cancer tissue. This procedure can be highly variable because much of the RNA may not be intact due to poor handling of tissue prior to embedment. To mitigate this problem as much as possible, we have altered the approach to focus more on measurements using quantitative RT-PCR. We have developed a sensitive assay and started analyzing samples. Of the cell lines tested, MCF-7 appears to express Srcasm at relatively high levels. To expand on the number of primary samples available we are also obtaining specimens from the Cooperative Human Tissue Network (CHTN). To date, we have analyzed approximately 20 normal and 20 cancerous samples. At present, no clear pattern has emerged. However, we are also trying to develop better, quicker assays. For example, the throughput can be increased if we have antibodies that specifically recognize endogenous Srcasm and work for immunohistochemistry. We now have two candidate rabbit polyclonal antibodies that are being characterized. In addition, we started making monoclonal antibodies. The hybridomas from the first fusion did not yield any antibodies that would work on Western blots (and therefore probably not be good candidates to further testing by immunohistochemistry). We then prepared a second fusion, which produced 5 candidate hybridomas. An initial screen of the new hybridomas showed that 2/5 recognized Srcasm by Western Blot. We are now characterizing the new antibodies in more detail to determine if they will be suitable as reagents for immunohistochemistry.

KEY RESEARCH ACCOMPLISHMENTS:

- Generated transgenic mice expressing Srcasm in mammary epithelia
- Begun characterizing relative SRCASM RNA levels in primary human tissue and cell lines using quantitative RT-PCR.
- Started making both rabbit polyclonal and mouse monoclonal antibodies to Srcasm to aid in the analysis of its expression in vivo.

REPORTABLE OUTCOMES:

None

CONCLUSIONS:

This work is designed to explore the function of Srcasm in the mammary gland. Specifically, we are interested in determining whether it can play a role in inducing mammary neoplasia. This is to be evaluated two ways. First, transgenic mice will be analyzed for development of mammary carcinoma. Second, primary human breast tissue and cell lines will be analyzed for Srcasm expression to determine if there is a correlation between tumor types and altered expression. We are trying to improve on the original approach be generating high quality antibodies that will recognize both endogenous and transgenically supplied Srcasm. If successful, we envision that the antibodies will enable us to rapidly screen large numbers of tissue sections for Srcasm expression.

REFERENCES:

N/A