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14. ABSTRACT

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 hypothesized that elevated expression of SRCASM in mammary epithelia may result in increased Src activation, leading to hyperplasia or transformation. This was explored further by: (1) generation of transgenic mice expressing Srcasm in mammary epithelia. Mice were 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 were correlated with patient outcome or metastatic phenotype to determine whether monitoring Srcasm expression has any predictive value. We found no correlation between Srcasm expression and either patient outcome or metastatic phenotype. Furthermore the transgenic mice proved uninformative. There was no evidence of accelerated tumor development. This may be due to relative low expression of the transgene. Based on the data accumulated there does not appear to be a significant importance of Srcasm in breast cancer. However, this does not rule out a role in other epithelial cancers. Subsequent reports suggest that Srcasm may be upregulated in cutaneous dysplasias, which may implicate a role for this protein in cutaneous disorders.

15. SUBJECT TERMS Src, breast cancer, Srcasm, Src-family kinases

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1. 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 hypothesized 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 were generated and analyzed to determine whether the cells develop a neoplastic fate. In addition, both human mammary cell lines and primary mammary tumor samples were analyzed to determine if there is a correlation between tumor subtype and Srcasm expression.

2. KEYWORDS: Src, breast cancer, Srcasm, Src-family kinases

3. OVERALL PROJECT SUMMARY:

Transgenic Mice. During the first funding period, we made transgenic mice expressing Srcasm under control of the MMTV promoter. This would allow us to target expression to mammary epithelia primarily. This was designed to test the hypothesis that overexpression of Srcasm would predispose female mice to breast cancer. We established four transgenic lines on the FVB genetic background, each of which had different levels of transgene insertions. While expression could be detected, it was not robust. We also let female mice age to determine if there was a increased onset of mammary tumors. After 9-12 months, we did not detect tumors in either nulliparous or multiparous mice. This may be a reflection of the relatively low level of expression of the transgene.

Srcasm –specific antibodies. Another goal was to generate high affinity antibodies to detect Srcasm. We were successful in producing both monoclonal and polyclonal antisera. Despite immunoaffinity purification the antibodies could detect overexpressed protein but had difficulty detecting endogenous Srcasm. This significantly limited our ability to perform immunohistochemistry on primary tissue samples. Nevertheless, the antisera was successfully used in the following publication: J. Biol. Chem. 280:6038-46.

Adenovirus vector: an adenovirus vector for ectopic expression of Srcasm was developed so that epithelial cells could be infected and yield high level expression of Srcasm. This was described in the following publication: J. Biol. Chem. 280:6038-46

Expression in cell lines and primary tissue. As described in the first progress report, there was a large variation in expression between cell lines and primary samples. We could not discern a clear pattern that would correlate with aggressiveness of the tumor type.

4. KEY RESEARCH ACCOMPLISHMENTS:

- Analysed transgenic mice expression Srcasm in mammary epithelia. We were unable to detect robust expression, nor did the mice develop tumors
- Completed generating rabbit polyclonal and mouse monoclonal antibodies. These antibodies detected endogenous Srcasm only poorly, making it difficult to use for histology. However the antibodies were used successfully in a publication.
- Prepared srcasm expressing adenovirus. This was described in a publication.
- Analysed Srcasm expression in mammary cell lines and primary tissue biopsy by qRT-PCR. Found variable expression that did not correlate with tumor phenotypes.

5. CONCLUSION:

This work was designed to explore the function of Srcasm in the mammary gland. Specifically, we were interested in determining whether it can play a role in inducing mammary neoplasia. Transgenic mice were analyzed for development of mammary carcinoma. Within the constraints of the current systems, we were unable to assign a role for Srcasm in mammary tumorigenesis. This may be due to poor expression of the transgene. Antibodies were also raised against Srcasm with mixed success. While they could detect expression in cell lines that overexpress the protein, it was difficult to obtain a reliable signal when analyzing endogeonous Srcasm. These results significantly limited our ability to drive this project forward.

Primary human breast tissue and cell lines were analyzed for Srcasm expression to determine if there is a correlation between tumor types and altered expression. We found variable levels of expression at the RNA level, which suggested that there is no correlation between Srcasm expression and tumor stage or aggressiveness. While some of the reagents generated in these studies have been used to study Srcasm in cutaneous epithelia, Srcasm is unlikely to be a major predictor of mammary carcinoma or stratifier for staging.

6. PUBLICATIONS, ABSTRACTS, AND PRESENTATIONS:

The following are relevant publications:

1) Seykora, J.T., Mei, L., Dotto, G.P., and Stein, P.L. (2002) “Srcasm: a novel Src activating and signaling molecule”. J. Biol. Chem 277:2812-2822. PMID: 11711534.
2) Li, W., Marshall, C., Mei, L., Dzubow, L., Schmults, C., Dans, M., and Seykora, J. (2005) “Srcasm modulates EGF and Src-kinase signaling in keratinocytes”. J. Biol. Chem. 280:6036-6046. PMID: 15579470.

The following are relevant abstracts:

1) Srcasm: An activator of Src-family tyrosine kinases expressed in differentiating keratinocytes. Society of Investigative Dermatology (2002). J. Invest. Derm. 119: 278. Abstract 425.

7. INVENTIONS, PATENTS, AND LICENSES:

Nothing to report

8. **REPORTABLE OUTCOMES**:
   Nothing to report

9. **OTHER ACHIEVEMENTS**:
   Nothing to report

10. **REFERENCES:**
    None

11. **APPENDICES:**
    None