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TITLE: The Role of Smad4 on Tumorigenesis, ERK Pathway, and SERM Resistance in Estrogen Receptor Alpha-Positive Breast Carcinoma Cells

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14. ABSTRACT This pre-doctoral training award was for Robert Nichols, a Ph.D. graduate student, to determine the interaction among signaling pathways of TGF-beta/Smad4, ERK, and estrogen in the regulation of breast carcinogenesis and resistance to selective estrogen receptor modulators. During the past year, the PI established various breast cancer cell lines with modulated expression of Smad4 and performed some proposed experiments using the established cell lines. However, the progress was significantly hindered after the PI was ordered to join National Guard for combat training two months after the award and was eventually deployed in Iraq in May 2007. The mentor, Dr. LuZhe Sun, was asked by the DOD Program to prepare this interim progress report in lieu of an annual report.					
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Introduction

It has been reported that 23% of breast tumors were smad4 negative, and 41% expressed low levels of smad4. Smad4 negative tumors had a longer disease free survival and better clinical outcome. Overexpression of Smad4 in estrogen receptor alpha positive breast carcinoma cells induces apoptosis via bim and bax intrinsic pathway. However, in estrogen receptor alpha negative breast carcinoma cells, Smad4 promotes epithelial-to-mesenchymal transdifferentiation and metastasis. For this predoctoral training application, we proposed to test the hypothesis that TGF-beta signaling mediated by smad4 positively regulates the ERK pathway, and negatively regulates estrogen receptor alpha, which inhibits the progression of tumorigenesis but promotes resistance to selective estrogen receptor modulators in MCF-7 and ZR-75-1 estrogen receptor alpha breast carcinoma cells.

Body

There were two tasks proposed for the first year of the award. In Task 1a, we will perform various assays, like MTT growth assay, Cell Cycle Analysis, Soft Agarose assay, migration and invasion assays, Apoptosis ELISA and Western Blot Analysis to determine the functional role of Smad4 on tumorigenesis. In Task 1b, we will orthotopically inject 5-6 week old female athymic nude mice and determine tumor burden and incidence, and euthanize mice at 10 weeks to determine the histological classification, distal metastasis, apoptosis, and perform immunohistochemistry. Task 1a was partially accomplished and Task 1b has not been started. The PI was successful in stably overexpressing a tetracycline-regulatable Smad4 in ZR-75-1 breast cancer cells that express little Smad4 and in stably knocking down the expression of Smad4 in MCF-7 breast cancer cells with a RNA interference approach. Knockdown of Smad4 in MCF-7 breast carcinoma cells increased the protein and mRNA levels of estrogen receptor alpha (ER α). Sensitivity to estrogen and raloxifene was also enhanced with the knockdown of Smad4. Overexpression of Smad4 in ZR-75-1 cells decreased the protein and mRNA levels of ER α . Sensitivity to estrogen and raloxifene was also decreased when Smad4 was overexpressed. Smad4 also induced ERK1/2 phosphorylation and apoptosis and senescence, which could be inhibited when treated with an ERK inhibitor, PD98059. The research was halted after the PI was ordered to join National Guard for combat training two months after the award and was eventually deployed in Iraq in May 2007.

KEY RESEARCH ACCOMPLISHMENTS

1. Generated Smad4-overexpressing stable ZR-75-1 cells.
2. Generated MCF-7 cells with reduced Smad4 expression by a Smad4 shRNA.
3. Demonstrated the regulation of ERa expression by Smad4 pathway.

REPORTABLE OUTCOMES

Cell lines: Smad4-overexpressing ZR-75-1 cells and Smad4-underexpressing MCF-7 cells

CONCLUSIONS

Smad4 is a major intracellular mediator of TGF-beta signaling. Our study indicates that Smad4 negatively regulates ERa. This is consistent with the observations that many ERa positive breast cancer cells are resistant to TGF-beta suggesting that loss or attenuation of TGF-beta signaling may be a major mechanism for the ERa-mediated breast carcinogenesis. These breast tumors are likely to express higher levels of ERa and more responsive to anti-estrogen therapy. On the other hand, some ERa positive breast cancer that retains TGF-beta sensitive may express a relatively low level of ERa and thus be more resistant to anti-estrogen therapy resulting in a poorer outcome. Therefore, our study may yield novel insights that will explain Smad4-associated poor clinical outcome and decreased disease-free survival of breast cancer patients.