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Table of Contents

Cover.....i

SF 298.....ii

Table of Contents.....iii

Introduction.....1

Body.....1

Key Research Accomplishments.....1

Summary 2

Annual Summary Report for DAMD17-02-1-0487
George Theodore

TRAINING
Took and passed with A grade the Tumor Biology course in the Department of Cell Biology and Anatomy
Took and passed the Seminar and Journal Club course in the Department of Cell Biology and Anatomy

RESEARCH ACCOMPLISHMENTS
Introduction
Muc4/sialomucin complex (SMC) is a high M_r heterodimeric glycoprotein complex which was originally observed at the cell surfaces of 13762 rat mammary adenocarcinoma cells and has been more recently found in many accessible and vulnerable epithelia. It is composed of a mucin subunit ASGP-1 and a transmembrane subunit ASGP-2. The latter has two EGF-like domains and can form intramembrane ligand-receptor-type complexes with the receptor tyrosine kinase ErbB2. An important aspect of SMC/Muc4 is its ability to repress apoptosis when transfected into tumor cells. Our hypothesis is that SMC/Muc4 is multifunctional. It acts as an epithelial protective agent by forming a steric barrier at epithelial apical surfaces and by contributing to signaling through ErbB2 involved in epithelial differentiation and repression of apoptosis. Both of these functions may contribute to tumor progression when Muc4/SMC is inappropriately overexpressed.

HER-2 (ErbB-2, neu) is a member of the family of epithelial growth factors (EGF), and appears to overexpressed in 25-30 % of human breast carcinomas. Its presence is associated with a poor prognosis. No soluble ligand for HER-2 has been discovered so far, but our research group has shown that the ASGP-2 subunit of Muc4 binds to HER-2 (ErbB-2, neu), and affects its phosphorylation. Muc4 has also shown to suppress apoptosis in A375 human melanoma cells and to upregulate an apoptosis associated protein $p27^{kip1}$. High levels of expression of $p27^{kip}$ have been seen in some highly proliferative human breast cancer cells. Thus the study of HER-2-Muc4 complex in regards to its suppression of apoptosis will provide us with a more complete understanding of the mechanisms by which these molecules participate in breast cancer; this in turn we hope, will ultimately contribute to the refinement of breast cancer therapies.

Task 1. To detect the interaction of HER-2 (ErbB-2) and Muc4 with endogenous cellular proteins in cultures cells
The key to understanding the downstream signaling pathways from the Muc4/ErbB2 complex is the phosphorylation of the ErbB2. We previously showed that Tyr 1248 is phosphorylated in this complex. We are not able to investigate the phosphorylation of other residues at the time, because of the difficulty of the procedures involved. Thus, we did not propose such studies. Recently, antibodies have become available which permit analyses of the phosphorylation of other ErbB2 tyrosines. Our expectation is that possibly only one Tyr was phosphorylated during formation of the Muc4/ErbB2 complex. However, the situation appears more complex than that.

Our preliminary data suggest that phosphorylation of one residue other than 1248 is upregulated, one is downregulated and the others are unchanged. These studies will provide us the preliminary information to interpret the association of addtional components with the Muc4/ErbB2 complex in the initiation of signaling pathways.

Task 2. To investigate the hypothetical link between HER-2 (ErbB-2)-Muc4 complex formation and suppression of apoptosis by Muc4

Our attempts to determine the link between ErbB2 and apoptosis have not been successful, as the methods that we have used for blocking ErbB2 expression have direct effects on apoptosis. We have put this aim aside at present to work on other aspects of the project. We plan later to investigate methods for blocking ErbB2 activity without blocking its expression.

Task 3. To determine which pathway of caspase activation is inhibited by Muc4-HER-2 (ErbB-2) complex

The repression of apoptosis by Muc4 raises the question of the mechanism by which Muc4 acts. To begin to address this question, we analyzed caspases associated with the two primary pathways linked to apoptosis. Caspase 9 and caspase 8 are key elements of the intrinsic and extrinsic pathways, respectively. As shown in Fig. 1, caspase 9 activation was strongly inhibited by expression of Muc4 when cells were treated with the apoptotic agent actinomycin D. In contrast, Muc4 did not repress activation of caspase 8 when the extrinsic pathway was induced (data not shown).

These results clearly show that Muc4 blocks the intrinsic (mitochondrial) pathway. We are now seeking to identify elements further upstream in this pathway which are susceptible to Muc4 expression. This effort will now become a major part of the work pursued in this research.

Task 4. To investigate the contribution of $p27^{kip}$ in suppression of apoptosis by Muc4-HER-2 (ErbB-2) complex

One mechanism for Muc4 repression of apoptosis is via $p27^{kip}$, which has been shown to be involved in inhibition of apoptosis in some systems. However, blockage of $p27^{kip}$ expression using an antisense approach did not inhibit the ability of Muc4 to repress apoptosis. Thus, $p27^{kip}$ does not appear to be involved in the Muc4 repression of apoptosis. This result completes this task with a negative conclusion and ends research on this aim.

Task 5. Investigate the regulation of $p27^{kip}$ expression by Muc4

Work on this task has not yet begun.

Summary. The work during this period has clearly shown that the intrinsic pathway is the site of Muc4 repression of apoptosis, and that $p27^{kip}$ is not involved. These studies now allow us to focus the work on the steps involved between Muc4-ErbB2 complex formation and inhibition of caspase 9. If this work goes well, we may defer work on task 5, since it now is less relevant to the primary goals of this work.

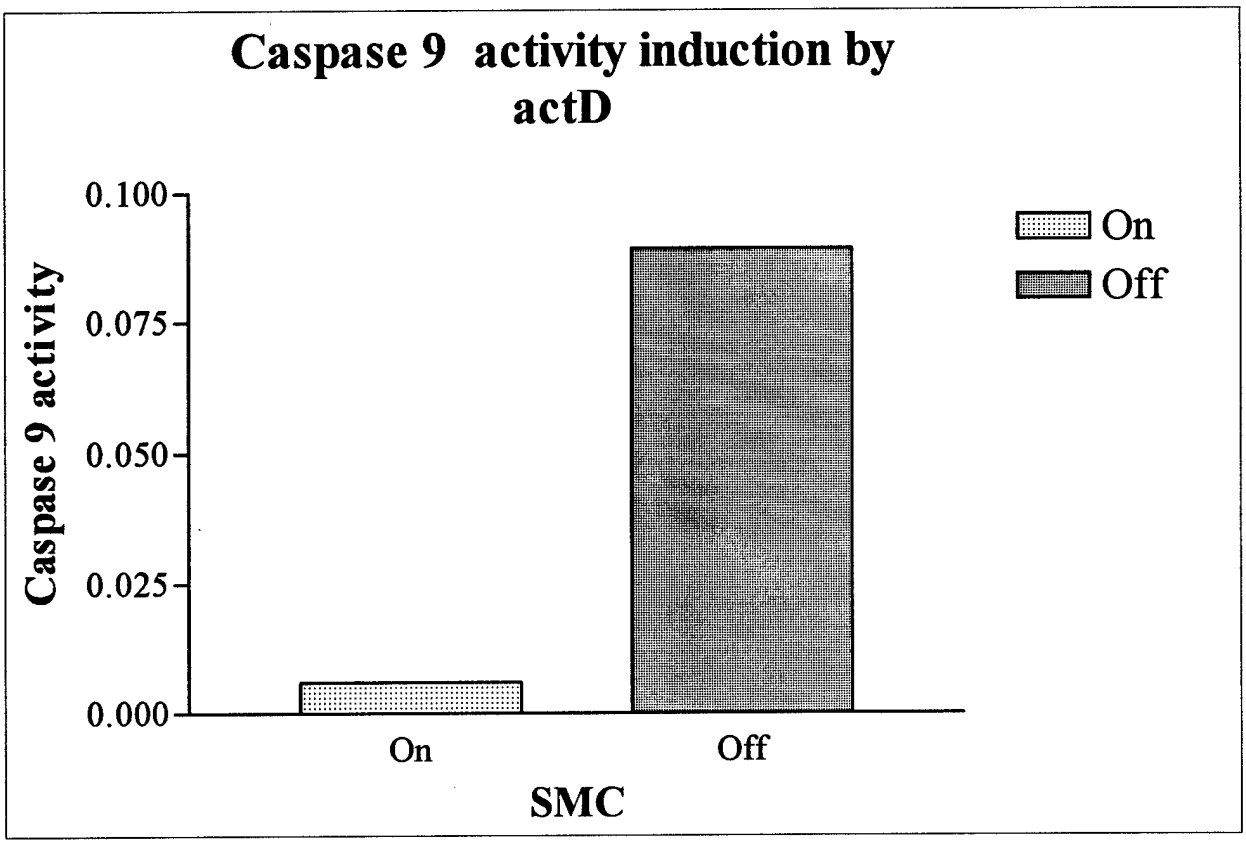

Figure 1. *Inhibition of caspase 9 activity by Muc4/SMC.*