

3. MEMBRANE MUCINS AND BREAST CANCER

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Membrane mucins have been implicated in a number of carcinomas. The prototype MUC1 is overexpressed in most breast cancers and is the target for both diagnostic assays and immunotherapy.¹ Two attributes of membrane mucins contribute to the interest in their expression in tumors.² First, their rigid structures provide an antirecognition function that alters cell adhesiveness and blocks killing by cells of the immune system. Second, they are proposed to be involved in cellular signaling processes that regulate cell behavior. These properties contribute to two of the most important aspects of tumor cell progression.

The second membrane mucin MUC4 has been less studied but has been shown to be overexpressed in a substantial fraction of aggressive breast carcinomas from patient effusions. In addition to its antirecognition functions, MUC4 (also called sialomucin complex) acts as a ligand for the receptor tyrosine kinase ErbB2/HER2/neu, which has been strongly implicated in breast cancer as an indicator of a poor prognosis. Thus, MUC4 has the potential to act as a tumor progressor factor and to serve as a target for novel therapies in tumors in which it is expressed.

Sialomucin complex (SMC, rat MUC4) is a heterodimeric glycoprotein isolated from metastatic 13762 rat mammary adenocarcinoma ascites cells. It is composed of a high M_r , highly glycosylated

mucin subunit (ascites sialoglycoprotein [ASGP]-1), and a 120-kDa transmembrane N-glycosylated component (ASGP-2) (Fig 1).² The complex is present at very high levels in the 13762 ascites cells ($>10^6$ copies/cell), and the sialomucin has been implicated in the metastatic potential of 13762 cells and their resistance to killing by natural killer cells.

Biosynthesis studies have shown that the complex is synthesized as a 300 kDa N-glycosylated precursor and cleaved to the two subunits early in its transit to the cell surface.³ Molecular cloning and sequencing and Northern blots indicate that the precursor is made from a 9-kb transcript.⁴ ASGP-1 contains predominantly mucin sequence,⁵ but ASGP-2 has 7 domains: 2 hydrophilic N-glycosylated domains, 2 epidermal growth factor (EGF)-like domains, a non-EGF cysteine-rich domain, a transmembrane domain, and a short cytoplasmic domain.⁴ Recent studies have demonstrated that SMC is the rat homolog of human mucin MUC4.⁶ The EGF-like domains of ASGP-2 have all of the consensus amino acid residues required for growth factor activity. Moreover, transfection and co-immunoprecipitation studies indicate that ASGP-2 can form an intramembrane complex with ErbB2 but not other ErbB receptors, which can potentiate neuregulin-induced phosphorylation of ErbB2 and ErbB3.⁷ These

results indicate that SMC may act as a special type of autocrine growth factor. SMC has also been shown to be a potent antiadhesive molecule at cell surfaces, acting via steric effects of its mucin subunit.⁸ Thus, the complex is at least bifunctional as well as heterodimeric.

SMC is expressed in the normal rat mammary gland, upregulated at midpregnancy and secreted into milk as one of two milk membrane mucins, MUC1 and MUC4, both of which are also present in soluble form.⁹ SMC/MUC4 levels in the rat

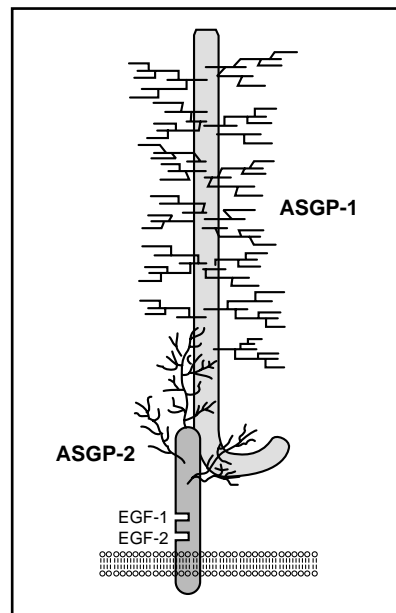


Fig 1. — Model of sialomucin complex/MUC4. ASGP = ascites sialoglycoprotein, EGF = epidermal growth factor. From Corfeld AP, ed. *Glycoprotein Methods and Protocols: The Mucins*. (Series: *Methods in Molecular Biology*, #135). Totowa, NJ: Humana Press, Inc. In print (November 1999). Reprinted with permission.

ascites tumor are approximately 100-fold greater than in the lactating gland, which are approximately 100-fold greater than in the virgin gland. Experiments in primary mammary epithelial cells (MECs) have demonstrated three different mechanisms for regulating those levels (Fig 2). First, SMC transcript levels are upregulated in mammary gland in the virgin animal and remain unchanged between the virgin and lactating animals.¹⁰ SMC transcript levels are upregulated in MECs by serum, an effect that can be partly reproduced by insulin or insulin-like growth factor (IGF). Inhibitor and transfection experiments indicate that this regulation of transcript levels requires participation of the mitogen-activated protein (MAP) kinase pathway. Second, in contrast

to transcript levels, SMC protein production is repressed in the virgin gland by epithelial cell interactions with its extracellular environment. This posttranscriptional effect is mimicked in culture by Matrigel and appears to be due to inhibition of translation.¹⁰ Third, SMC expression is inhibited by transforming growth factor-beta (TGF- β) by a posttranslational mechanism that retards biosynthetic processing of the precursor to yield the two subunits.

All three of these regulatory mechanisms have been altered in the ascites tumors. Furthermore, SMC transfection into human A375 melanoma cells has demonstrated that SMC overexpression can promote both primary tumor growth and metastasis as xenografts in

nude mice. These results suggest that SMC overexpression in the mammary gland can promote tumor progression, with postulated roles for both the mucin and transmembrane subunits.

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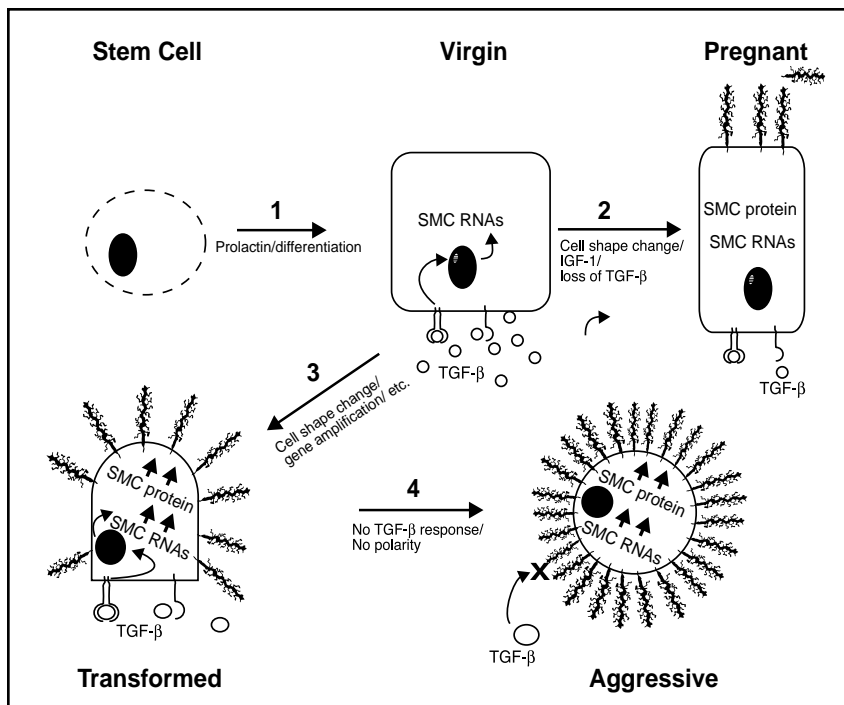


Fig 2. — Model for regulation of sialomucin complex (SMC) in mammary gland and misregulation in mammary tumors. IGF-1 = insulin-like growth factor-1, TGF- β = transforming growth factor-beta.