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SIALOMUCIN COMPLEX IN TUMORS AND TISSUES

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1. ABSTRACT

Sialomucin complex (SMC) is a high M_r glycoprotein heterodimer, originally discovered on the cell surfaces of ascites sublines of the highly metastatic 13762 rat mammary adenocarcinoma, and composed of mucin (ASGP-1) and transmembrane (ASGP-2) subunits. SMC is encoded by a single gene and synthesized as a large precursor protein which is cleaved into its subunits early in its transit to the cell surface. SMC exhibits behavior typical of both membrane and secreted mucins. In the ascites cells, it is found only in the membrane form, creating a protective barrier at the cell surface to reduce cell adhesiveness and protect the tumor cell from immune killing. Normal tissues express both the membrane formand a non-membrane form, which may be secreted by either constitutive or regulated, secretory granule mechanisms. This soluble form is proposed to contribute to multilayer mucus gels which protect epithelia, though it may also play other roles. ASGP-2 contains two EGF-like domains, one of which binds the receptor tyrosine kinase ErbB-2. Thus, SMC may be a bifunctional protein, the mucin serving a protective function and the transmembrane domain possibly playing a role in the proliferation of metastatic tumor cells or repair processes necessary for the maintenance of damaged epithelia.

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2. INTRODUCTION.

Interest in mucins has grown recently as a result of cloning studies which have served to broaden and diversify the scope of our understanding of their structure and function. Unfortunately, a standardized system of nomenclature is lacking for mucins and mucin domain-containing proteins, and this has led to some confusion. For this reason, the term mucin will be used to refer to cell surface sialomucins as well as the secreted protein products. Mucins can be subdivided into membrane-associated and secretory classes (Table 1), which differ in structure and potential function (1). The former includes those cell surface molecules also known as nonepithelial mucins (1) and those proteins with mucin domains that constitute only a fraction of the protein structure (2). Almost all true mucins have in common the presence of multiple tandem repeat domains rich in serine and/or threonine that provide extensive numbers of sites for the addition of O-linked oligosaccharides (3). The glycosylation of these repeats (mucin domain) results in a large glycoprotein with an extended, rigid conformation (4).

Mucin domains can be variably glycosylated and are thought to confer upon proteins a variety of functions, ranging from anti-recognition, protection and lubrication (5-7) to senescence antigens on erythrocytes and platelets (8) and involvement in lymphocyte trafficking (9). For example, antibodies to the mucin domain of CD45, the lymphocyte common antigen, are able to modulate lymphocyte activation, suggesting a recognition function (10). In the low density lipoprotein receptor the mucin domain is thought to act as a

Table 1. Some mucins and mucin-like glycoproteins

NAME	MAJOR SOURCE	MEMBRANE/SOLUBLE	REFERENCE
Components A/B/C	Human milk	M	66
Glycocalicin	Platelets	M	67
Glycophorin	Erythrocytes	M	68
Sialophorin	Leukocytes	M	69
Leukosialin	Leukocytes	M	70
Low density lipoprotein receptor	Liver	M	71
ZP3	Oocyte	M	72
Epiglycanin	TA3 carcinoma	M	11
Sialomucin complex	13762 carcinoma	M/S	3, 33, 35
MUC1/episialin	Epithelia, carcinomas	M/S	1, 24
MUC2	Intestine	S	73
MUC4	Tracheobronchial	S	5

stalk or spacer to free the ligand binding site from steric hindrances at the cell surface (4). Carcinoma sialomucins utilize the mucin domain to effect an anti-recognition function. The presence of the mucin epiglycanin on sublines of the TA3 mouse mammary adenocarcinoma is correlated with allotransplantability and a decrease in binding of anti-H2 antibody (11). Epiglycanin, because of its highly elongated structure (12), is thought to sterically hinder the approach of antibody to its antigen.

The best known examples of membrane-associated mucins are MUC1 and tumor sialomucin complex (SMC) (Table 1), the latter being the focus of this paper. Rat Muc3 also appears to be a membrane mucin, but it is less well characterized (13). MUC1, a transmembrane protein (14), has a deduced protein sequence comprising a large extracellular domain consisting of a variable number of 20 amino acid repeats, a transmembrane domain and a 69 amino acid cytoplasmic domain which has been proposed to interact with the actin cytoskeleton (15). MUC1 is expressed on the cell surface as a heterodimeric complex, both subunits originating from a single polypeptide precursor (16). A soluble form of MUC1 has been reported to be present in cell culture media and body fluids (17, 18). Although alternative splicing has been suggested to explain the generation of a soluble form of the protein (19), the action of a protease has been implicated in other cases for releasing the mucin from the cell surface (17). Another proposed secretion mechanism involves the recycling of MUC1 from the cell surface back to intracellular compartments where a cleavage event occurs which removes the cytoplasmic and transmembrane domains of the mucin (16, 20). MUC1 is aberrantly expressed by numerous carcinomas (21) and is an important marker in malignancy. Its expression is developmentally regulated in epithelial tissues in the mouse (22) where, like epiglycanin (23), it may play a role in epithelial morphogenesis (22, 24).

The soluble or secreted mucins, which include human MUCs 2-7 (1), differ from MUC1 in the number and composition of tandem repeats. Some of these contain cysteine-rich domains that are homologous to domains of prepro-von Willebrand factor (vWF) (25, 26). These cysteine-rich domains may be responsible for the dimerization of secreted mucin molecules. The best characterized secreted mucin is MUC2, which is present in the intestine and airway (27). It has two tandem repeat domains, one with ≈100 repeats (23 amino acid) and a second with an irregular Thr/Ser/Pro-rich repeat (28, 29). Together these two regions can be glycosylated with up to 1000 O-linked oligosaccharide chains (30). Hence, MUC2 and some of the other secreted mucins are large glycoproteins capable of disulfide-linked multimerization to form viscous gels which, among other potential functions (25), protect and lubricate epithelial surfaces. In accessible tissues such as the airway, these mucins provide protection by binding to exogenous environmental agents, thus preventing their binding to the epithelium and aiding in their subsequent expectoration (5).

The purpose of this paper is to review current knowledge of the structure, biosynthesis and function of SMC, particularly its expression and role in normal tissues. These studies offer for the first time a glimpse of the potential functions of this multi-functional mucin in normal processes.

3. SIALOMUCIN COMPLEX

3.1. Tumor Sialomucin Complex

3.1.1. Tumor Sialomucin Complex Structure

Asites sialoglycoprotein-1 (ASGP-1) is a 600 kDa cell surface sialomucin consisting of a polypeptide of about 220 kDa which is extensively O-glycosylated (70% carbohydrate) (31). Elucidation of the cDNA sequence of SMC (32) has revealed that ASGP-1 possesses 1) a 5' unique region consisting of 80 amino acids, the first 30 being the signal sequence; 2) a repeat domain of 1513 amino acids in 12 repeats with some intervening sequences; and 3) a large 3' non-repeat unique region containing 609 amino acids (Figure **1A**). ASGP-1 is a peripheral membrane protein which is bound to the cell membrane through a stable non-covalent association with ASGP-2 (33, 34), the transmembrane subunit of tumor SMC. Sequence data for ASGP-2 (35) predict it to be an 80 kDa protein consisting of seven domains: two N-glycosylated

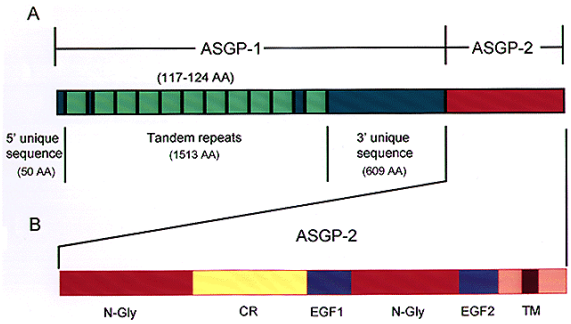

Figure 1: Domain structure of SMC and ASGP-2.

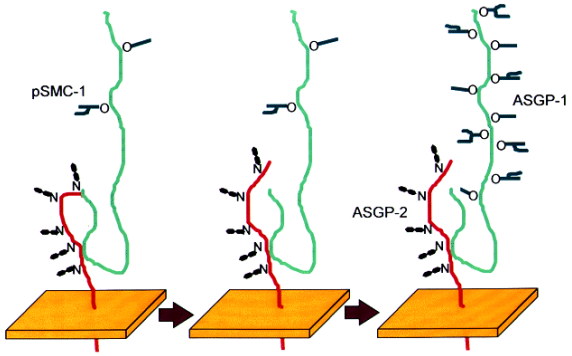

Figure 2: Biosynthesis of ASGP-1 and ASGP-2.

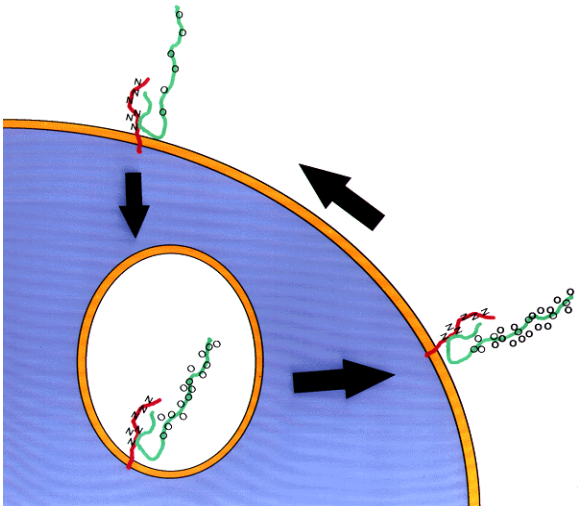

Figure 3: Recycling/glycosylation mechanism for maturation of ASGP-1.

hydrophilic domains (N-Gly1 and N-Gly2), two domains which are similar to the EGF-like sequences found in many proteins (EGF1 and EGF2), a separate cysteine-rich (CR) domain which is unrelated to the EGF-like regions, a hydrophobic transmembrane (TM) domain and a cytoplasmic domain (Figure 1B). There are 24 potential N-linked glycosylation sites on the subunit, about 17 of which are N-glycosylated (36). ASGP-2 is particularly interesting because its two EGF-like domains contain all of the consensus residues present in active EGF-like proteins (35). This observation suggests that tumor SMC may be an active growth modulator, and that it participates in the malignant process of the tumor cells from which it was characterized (see 3.2.).

3.1.2. Tumor Sialomucin Complex Biosynthesis

ASGP-1 and ASGP-2 are stably associated in a noncovalent manner which has yet to be characterized. This association was demonstrated by detergent extraction and fractionation (33) and by observations of the redistribution of specifically bound fluorescent lectins (34). Labeling studies showed that both are derived from a precursor protein which could be immunoprecipitated by both anti-ASGP-1 and anti-ASGP-2 antibodies (37). Subsequent pulse-chase experiments, mannose labeling, Endo H treatments and peptide mapping established a precursor-product relationship between the high M_r precursor pSMC-1 and both ASGP-1 and ASGP-2. To determine when the cleavage of pSMC-1 occurs during biosynthesis, ascites cells were labeled with mannose, treated with Endo H to release high mannose oligosaccharides and immunoprecipitated with anti-ASGP-2. No mannose was detected in the precipitates, indicating that the cleavage event occurs before the conversion of high mannose to complex oligosaccharides (37).

As shown in Figure 2, it was proposed that pSMC-1 is synthesized and N-glycosylated in the endoplasmic reticulum. Intramolecular interactions which will become the site(s) of interaction between ASGP-1 and ASGP-2 are formed as the polypeptide folds. Before reaching the medial Golgi, the precursor is cleaved to yield the complex. The bulk of O-glycosylation occurs either concomitantly or rapidly following this event (37). Elongation of the O-linked oligosaccharides occurs as the complex transits through the Golgi.

Interestingly, ASGP-1 of the complex reaches the cell surface with only about 40% of its serines and threonines glycosylated (38). Furthermore, glucosamine labeling of GalNAc of cell surface biotinylated ASGP-1 confirmed that new oligosaccharides could be added after the mucin reaches the cell surface. It was proposed that this addition occurred during recycling of SMC (Figure 3). Thus, two discrete stages were proposed for complex biosynthesis. In the first stage, ASGP-1 polypeptide is incompletely O-glycosylated as it transits from its site of synthesis in the endoplasmic reticulum to the plasma membrane, which occurs within about 2 hours. SMC maturation occurs with additional O-glycosylation of the polypeptide during the second or recycling stage, which continues for several hours. When plasma membrane is recycled, SMC is transferred to intracellular compartments where additional GalNAc residues are added to the unoccupied sites for O-glycosylation. During this recycling additional sugars are also added to the oligosaccharides so that the complex acquires mature oligosaccharides. This model has significant implications in the use of tumor mucins for cancer diagnosis and treatment (2).

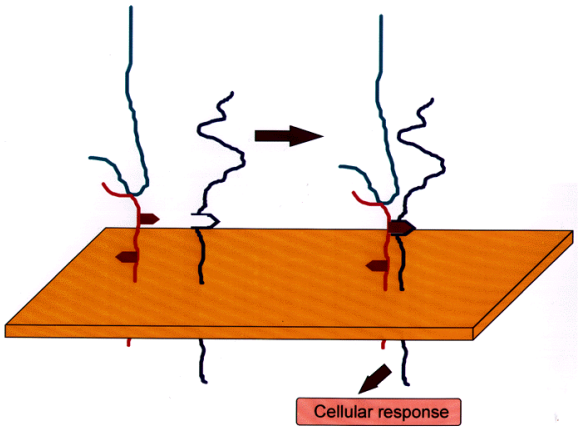

Figure 4: Intramembrane mechanism for interaction of ASGP-2 of SMC with receptor.

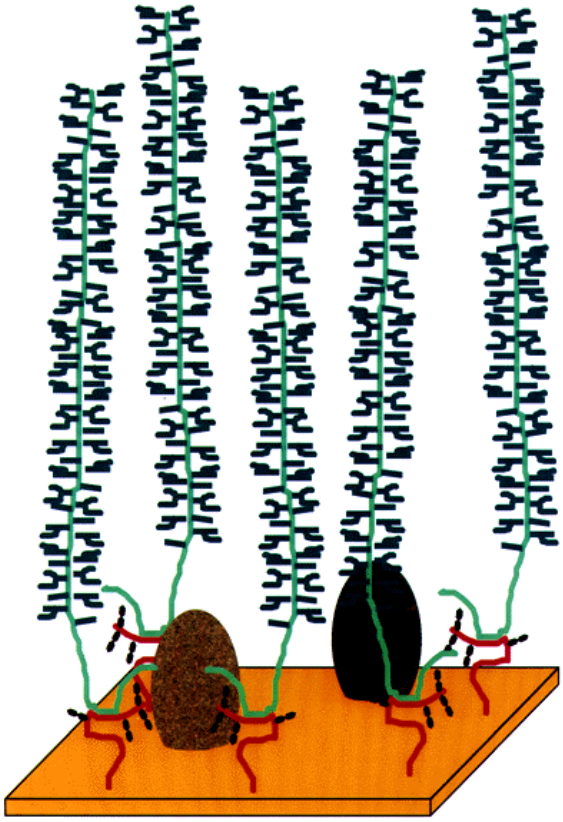

Figure 5: Model for SMC in the protection of cell surfaces.

3.2. Role of Sialomucin Complex in Tumor Cells

Tumor progression results in cells which are capable of autonomous growth, invasiveness, and metastasis. Substantial information is available concerning the early stages of neoplastic transformation (39), a key event being the loss of control of cell proliferation. Polypeptide growth factors are often instrumental in the regulation of normal control of cell proliferation. Thus, the discovery of growth factor-like domains in SMC from the highly metastatic 13762 mammary adenocarcinoma was of considerable interest. Analyses of these cells showed that they contain the receptor tyrosine kinase $p185^{neu}$/ErbB2 but not the EGF receptor, the prototype of this family (40). The $p185^{neu}$ protein structure shows considerable homology to the EGF receptor, with 40% and 82% of the amino acids being identical in the extracellular and cytoplamic domains, respectively (41). In addition, similar autophosphorylation sites exist in the tyrosine kinase domain (41). However, $p185^{neu}$ does not bind EGF, and its biological ligand has not been reported. Instead, it is activated through formation of heterodimeric receptor complexes with other members of the EGF receptor family (42, 43).

Traditional growth factors usually act on receptors by an endocrine or paracrine mechanism (44), but other mechanisms have been proposed to occur in malignant cells. An autocrine mechanism (45), exemplified by tumor cells which express membrane-bound epidermal growth factor receptor (EGFR) and secrete soluble tumor growth factor alpha (TGF-alpha) (46), may establish an auto-stimulatory loop. A juxtacrine mechanism has also been proposed (47), in which membrane-associated growth factors (48) on one cell can stimulate a receptor on the surface of a second cell. An intracrine stimulatory mechanism may occur in some cells (49). Transformation of cells by the *v-sis* oncogene containing a KDEL endoplasmic reticulum localization signal leads to auto-stimulation (49), even though there is no secreted product. A general mechanism in which organelle- or membrane-associated growth factors enhance proliferation and malignancy can be proposed, but presently, little evidence of such membrane complexes in tumors has been found.

Two important observations suggest that SMC forms a specific complex with $p185^{neu}$ by a unique autocrine mechanism (43). First, ASGP-2 and $p185^{neu}$ are co-immunoprecipitable from cell surface fractions prepared from 13762 ascites tumor cells. Second, a complex of ASGP-2 and $p185^{neu}$ extracellular domains (ECDs) is formed and secreted from insect cells when the two are co-infected into the same cell population. Interestingly, in insect cells, ASGP-2 and $p185^{neu}$ associate only when they are co-expressed in the same cell; when $p185^{neu}$ extracellular domain and ASGP-2 ECD were expressed in separate cells and the conditioned media mixed together, no complex was observed. Experiments using deletion mutants of ASGP-2 indicate that the binding of ASGP-2 occurs through the EGF1 domain. Since ASGP-2 and $p185^{neu}$ are transmembrane proteins in the ascites cells, it was proposed that they are a part of an autocrine mechanism for modulation of receptor signaling and proliferation in cells in which the two proteins are expressed in the same membrane (intramembrane mechanism) (43) (Figure 4). Therefore, ASGP-2 may play a role in those processes of malignancy responsible for loss of growth control.

Table 2. Tissue expression levels of SMC

TISSUE	EXPRESSION LEVEL	MEMBRANE/SOLUBLE	REGULATION	POTENTIAL FUNCTION
Mammary gland/milk	++++	M/S	Post-transcriptional	Ligand/protection
Intestine	+++	S	Constitutive?	Ligand/lubrication/ protection
Airway	++	M/S	Constitutive?	Protection
Uterus	+++	M/S	Transcript level	Protection/anti-implantation

Later stages in tumor progression, such as invasiveness and metastasis, are not well understood. Changes at the tumor cell surface are thought to result in decreased adhesiveness, increased degradative capabilities, altered motility and altered antigenicity (50). This complex assortment of cell surface effects commonly results from the altered expression of cell surface glycoconjugates, such as the increased size of N-linked oligosaccharides that occurs with transformation (51), expression of blood group antigens (52), and expression of truncated or incomplete mucin-type oligosaccharides (53). It has been suggested that SMC along with MUC1 and epiglycanin have anti-recognition functions (24, 54). SMC has been implicated in the metastasis (55) and resistance to killing by natural killer (NK) cells in 13762 ascites cells (56), presumably by masking antigens or proteins at the tumor cell surface (Figure 5). Hence, in addition to influencing signaling and proliferation processes of malignancy via the transmembrane subunit ASGP-2, SMC may aid in later processes affecting the metastatic state via the mucin subunit ASGP-1. In support of this hypothesis SMC has been shown to modulate cell adhesion. A375 melanoma and MCF7 breast carcinoma cells were transfected with SMC cDNA constructs under the control of an inducible promoter. When expression of SMC was blocked, the cells attached to their culture substratum and to each other. However, when SMC was expressed, the cells became rounded and released both cell-matrix and cell-cell attachments, a process which was completely reversible with inhibition of expression (M. Komatsu, unpublished observations). One of the first events to occur in metastasis is a decreased adhesiveness, which may occur in 13762 cells by the overexpression of SMC. We have proposed that during migration of the cells during metastasis, the presence of SMC on the cell surface protects the tumor cells from the immune surveillance (54). This protection was demonstrated by the induction of resistance to NK cell killing when SMC was expressed under the inducible promoter (M. Komatsu, unpublished observations).

3.3. Sialomucin Complex in Normal Tissues

Recently, SMC expression has been investigated in a number of epithelial tissues (57). These studies have allowed comparisons of expression levels, structure and regulation of expression between tumor and normal forms of the complex (Table 2), permitting for the first time the discussion of a hypothesis about SMC function in normal tissue.

3.3.1. Sialomucin Complex in Mammary Gland

SMC is expressed in lactating mammary gland at a level 100-fold less than that in the ascites tumor (57). It is localized to the apical portion of secretory epithelial cells of the gland. SMC is barely detectable in virgin and early pregnant mammary gland, but its expression increases dramatically around mid-pregnancy, with maximal expression occurring in late pregnancy and in post-partum mammary gland. This temporal pattern is similar to that of beta-casein (see 3.4.2). Interestingly, maximal expression of SMC and $p185^{neu}$ occur at similar times. SMC is present in rat milk as both soluble and membrane forms. An antibody to the cytoplasmic domain of ASGP-2 was raised to analyze the mechanism of secretion. Serial immunoprecipitation analysis with this antibody and subcellular fractionation experiments showed that SMC is expressed as two isoforms in the mammary gland and milk, a membrane (75%) and a soluble or secreted (25%) form (57). Since no evidence of alternative splicing was observed, a proteolytic cleavage event was suggested to be responsible for the generation of the soluble form. Milk mucins have been observed to bind microorganisms (58, 59) and thus are proposed to be involved in the protection of newborns from these agents. Previously, two different milk mucins have been described in a number of species (58), MUC1 and Component A (MUCX), the latter being poorly characterized (57). The presence of ASGP-1 in both rat (57) and human milks suggests that it is a good candidate for MUCX. Milk plays a major role in the development of the intestine in the neonate; one of the active factors is EGF. Thus, we have suggested that SMC may contribute to intestinal development through its ability to act as a ligand for ErbB-2, though the mechanism of action is unclear (57, also see 3.3.2.). Surprisingly, the SMC molar concentration in rat milk is an order of magnitude higher than that of EGF (60). Interestingly, the EGF domains of ASGP-2 have two extra cysteine residues (35), which probably form an additional disulfide to increase the stability of the domain and improve the chances of active protein reaching the intestine. SMC can be co-immunoprecipitated with $p185^{neu}$ from lysates of lactating gland, although no function can be presently ascribed to SMC in mammary gland.

3.3.2. Sialomucin Complex in the Developing and Adult Digestive System

In the embryonic rat, SMC is first detected in the nasopharynx at embryonic (E) day 14.5 (E14.5). By E16.5, the salivary glands express SMC and the complex is localized to the surface of the buccal and nasal epithelia. Expression of SMC in the intestine first occurs on day E16 at the gastroduodenal junction, where it is expressed on the apical surface of the stratified squamous epithelium, which has yet to undergo

morphological differentiation. From E16 to birth, onset of expression proceeds distally toward the colon. In postnatal intestine, SMC is present in cells at the base of the crypts. In the small intestine, this pattern of expression remains unchanged into adulthood (see below). However, in the colon at about the time of weaning, SMC expression switches to the adult pattern, predominantly in the goblet cells (57). SMC is found throughout the lower adult gastrointestinal tract, with expression levels increasing distally along the duodenal-colonic axis. In the small intestine, SMC is localized to the cells present at the base of the crypts, which are known as Paneth cells. Paneth cells secrete anti-microbial agents (61) and EGF (62), and interestingly, SMC appears to be localized to the secretion granules of these cells. No other cells, including the goblet cells, in the small intestine express detectable SMC. However, in the colon, SMC is present predominantly in the secretion granules of the goblet cells. Biochemical and immunologic methods showed that the predominant form of SMC in both developing and adult small intestine and colon is the soluble isoform (57).

A model for SMC participation in intestinal development and physiology can be proposed. Starting on E16.5, SMC is synthesized by the salivary glands. Swallowing of salivary secretions or of amniotic fluid, in which SMC is also present, would deliver SMC to the intestine at the time of its intense period of cytodifferentiation (63). Once the newborn starts to suckle, SMC present in the milk is ingested. Hence, potentially there is a constant supply of SMC from late embryo to weaning stages of intestinal development. It is intriguing that weaning, which occurs around the fourth postnatal week, temporally correlates with the onset of the adult pattern of expression of SMC in the colon. This timing may reflect a function of SMC as a classical mucin secreted from goblet cells for the purpose of protection and lubrication. In the small intestine, SMC secretion from Paneth cells could serve as an antimicrobial factor or to supply growth factor activity for maintenance purposes. In developing intestine, there are numerous factors involved in directing cytodifferentiation and morphological changes (63) which are needed for maturation of the tissue. Once mature, homeostasis must be maintained so that the major cell types are renewed, properly differentiated and moved to their appropriate positions along the crypts. So far there is circumstantial evidence that SMC can play a role in these processes as a growth factor and traditional mucin. Further investigation is needed in this area to delineate specific functions.

3.3.3. Sialomucin Complex in the Airways

The respiratory airway is partially protected from noxious environmental agents (e.g. smoke, bacteria and viruses) by a viscous layer of mucus which coats the surface epithelium. SMC was detected in tracheal homogenate (57) and shown to be apically expressed on the airway epithelium. Since SMC can be expressed in either membrane or soluble form, two possibilities can be envisioned for its function in the airways. 1) SMC in the membrane form could provide a protective barrier at the cell surface. 2) SMC in a soluble form could be secreted at the luminal surface to form part of the mucociliary blanket. Subsequent characterization of airway SMC revealed that both are true. SMC is present in trachea as membrane (30%) and soluble (70%) forms, both of which are present at the apical cell surfaces. The latter can be removed from the tracheal surface by rinsing under mild conditions. A model has been suggested based on these results in which membrane SMC is localized to the apical surface of epithelial cells where it can aid in the protection against exogenous agents. The soluble form is postulated to be secreted by the luminal cells of the trachea into the pericellular fluid layer which provides a boundary between the cell surface and the mucociliary gel composed of disulfide-crosslinked mucins.

3.3.4. Sialomucin Complex in the Uterus

The protective function of apical cell surface SMC presents a dilemma in the uterus, since the presence of mucins inhibits cell-cell interactions necessary for blastocyst implantation (64). In this tissue, SMC is modestly expressed on the apical surface of the luminal epithelial cells. Sequential immunoprecipitation experiments using anti-cytoplasmic domain antibody showed that 60% of uterine SMC is the membrane isoform. SMC expression is estrous cycle-dependent, the estrous uterus exhibiting about an order of magnitude more SMC than the anestrous uterus. Since SMC can block cell-cell interactions, it must be removed from the uterine luminal surface for blastocyst implantation to occur. During pregnancy, SMC expression diminishes during the first two days post-coitus (pc) but remains expressed until the early part of pc 5. Expression abruptly disappears at a time which coincides with the beginning of the receptive state for blastocyst implantation. Furthermore, administration of mifepristone (RU486), which inhibits receptivity, showed an inverse correlation between implantation and SMC expression. These results suggested that SMC expression is regulated by ovarian hormones (see 3.4.), and more importantly, that SMC may play a role in preparing the uterus for receptivity. Blastocyst implantation requires as a first step the interaction between the trophoblast and uterine epithelial cells. Therefore, one function of SMC may be to prevent untimely or inappropriate implantation up to the time of receptivity. The other mucin present in uterus is MUC1. MUC1 has a similar time course of expression to SMC and has also been proposed to play a role in receptivity. However, SMC differs from MUC1 in that it is expressed as both a membrane and a soluble isoform. The soluble SMC may play a similar role at the endometrial surface to that proposed for the airway. It remains to be seen whether the two membrane mucins have overlapping and/or complementary functions.

3.4. Regulation of SMC Expression in Normal Tissues and Ascites Tumor Cells

Insight into how a protein functions in a tissue can

Table 3. Expression and regulation of SMC in tumor and tissues

	EXPRESSION LEVELS *IN VIVO*				REGULATORY FACTORS		
SMC Source	Virgin	Implant receptive	Late pregnant	Involuting	Positive	Negative	No effect
Mammary cells	-/+	+	++	-/+	Lactogenic hormones	Matrigel	Estradiol Progesterone
Uterus					Estradiol	Progesterone	
Estrous	++	-	++	++			
Anestrous	+			+			
Ascites tumor cells	++++	++++	++++	++++	Transcript level?	Unknown	

often be obtained from an understanding of the regulation of its expression, including identification of factors involved in the regulation. SMC is overexpressed on the surface of 13762 ascites tumor cells. Thus, regulation (or lack of regulation) of SMC expression in the tumor cells appears to differ from that in normal tissues. Moreover, since SMC is potentially bifunctional and is expressed in tissues with very different functions, each tissue may have different regulatory mechanisms. Preliminary investigations have indicated that this is true in mammary gland and uterus, and probably applies to other tissues as well (Table 3).

3.4.1. Regulation of SMC in the Uterus

The behavior of SMC in the uterus during pregnancy suggests that it is regulated by the ovarian hormones, estradiol (E2) and progesterone (P). To test this hypothesis, rats were ovariectomized and injected with E2, P, both or neither. Immunoblotting of uterine tissue samples from these animals indicated that SMC expression is induced by E2, and that this induction is inhibited by P. This regulation by ovarian hormones is consistent with the pattern of expression during early pregnancy (see 3.3.4). 1) E2, which binds to the uterus from coitus through pc 4, maintains expression of SMC. 2) P, which binds from pc 2 to pc 4, accounts for the decreased expression during this time (McNeer *et al.*, manuscript submitted). However, it is not clear why SMC so completely and abruptly disappears at receptivity. Attainment of the receptive state requires P and a nidatory pulse of E2 on pc 4, which occurs a day before receptivity begins and SMC disappears. Clearly, further studies are needed to investigate additional factors responsible for regulation of SMC expression during early pregnancy.

3.4.2. Regulation of SMC in Mammary Gland

The temporal pattern of SMC expression in the mammary gland is different from that in the uterus (see 3.3.1, 3.3.4 and Table 3), but resembles the expression pattern of the milk protein beta-casein (57). Primary cell culture systems have shown that beta-casein expression is modulated by the extracellular matrix and prolactin (65). Primary mammary epithelial cell cultures were investigated to determine whether SMC is regulated in a similar way. Studies on cultures from mid-pregnant rats demonstrated that matrix has a negative effect on SMC expression, while lactogenic hormones (prolactin, hydrocortisone and insulin) have a slight positive effect (S. Price-Schiavi, unpublished observations). These results are in contrast to results obtained for beta-casein (65), indicating that the two milk proteins are regulated differently. The ovarian hormones E2 and P have little effect on SMC expression in the mammary cell cultures. Furthermore, matrix has no effect on SMC expression in 13762 ascites tumor cells. Thus, SMC regulation is different in the tumor cells, mammary gland and uterus.

4. PERSPECTIVE

The last decade has seen an explosion of information concerning mucins and mucin-like proteins. Cloning and expression studies have provided insights into structural features and glycosylation patterns of mucins. For some mucins the incorporation of these data into plausible hypotheses of function has been facilitated by the study of the mucins in normal and malignant tissues. Tumor SMC was discovered and characterized on the surface of a highly metastatic adenocarcinoma, where it is thought to play at least a bifunctional role in tumor progression. The classical role is as a protective agent at the cell surface to prevent killing of the tumor cells. A second role is as a modulator of signaling through the EGF receptor family of tyrosine kinases as a ligand for ErbB-2, the key receptor in forming heterodimeric signaling complexes (43). The recent discovery and characterization of the SMC in several normal tissues have presented evidence for other possible functions, including a role in regulating blastocyst implantation. However, an interesting observation in normal tissues is the presence of a soluble form of SMC. Its cell-specific expression in different tissues suggests that it may play a different role in protective mucin functions from that of the secreted, gel-forming mucins. The nature of that role is still unclear. Also not understood is how the EGF-like domains of the transmembrane subunit ASGP-2 contribute to cellular behavior in epithelia. Complexes of SMC with the receptor tyrosine kinase ErbB-2 have been demonstrated in both mammary ascites tumor cells and mammary epithelial cells. Although it is reasonable to expect that such complexes will act as modulators of receptor signaling, how that modulation in cell behavior is accomplished is uncertain. However, investigations in progress should aid in answering some of these questions.

5. ACKNOWLEDGEMENTS

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