Emerging Roles of MUC4 in Cancer: A Novel Target for Diagnosis and Therapy

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Abstract
The MUC4 mucin is a transmembrane glycoprotein that is implicated in the pathogenesis of pancreatic cancer and is aberrantly expressed in many other epithelial carcinomas. Recent studies suggest its significant potential as a clinical tool for cancer diagnosis and prognosis. MUC4 modulates HER2/ErbB2 signaling and is a determinant of therapeutic outcome of Herceptin-based therapy, which further indicates its prospective usefulness in cancer therapy and treatment planning. [Cancer Res 2007;67(2):433–6]

Introduction
Mucins, in general, are perceived as the biomolecules implicated in the protection and lubrication of epithelial surfaces. However, the recent realization that mucins can also function as signaling modulators and affect tumor cell phenotype has increased our interest in exploring their potential clinical usefulness. MUC4, a transmembrane mucin, has gained significant biological importance in the past few years. The gene encoding for the MUC4 mucin was first identified in 1991 from a tracheobronchial cDNA library (1). However, due to its large size (≈100 kb) and complex structure, its complete genomic organization could not be established until 2000 (2, 3). Despite this initial slow progress, the recognition of altered MUC4 expression in a variety of carcinomas and parallel studies on its rat homologue [sialomucin complex (SM/rat Muc-1)] have renewed the interest in examining its biological functions in cancer pathogenesis and in discovering potential clinical applications. Current research has indicated multiple prospective uses of MUC4 in cancer diagnosis and prognosis and as a therapeutic target.

MUC4 in Cancer Diagnosis and Prognosis
A key area of cancer research is to investigate the genetic and epigenetic alterations occurring during the cancer development to serve as clinical tools for disease diagnosis and prognosis. Efforts are being made to identify genes that will not only allow discrimination between normal and cancer cells but will also enable differentiation of a less aggressive cancer from its highly metastatic form. Such knowledge is imperative from a clinical standpoint to develop novel assays for early diagnosis and to understand the clinical course of cancer progression for effective disease management. MUC4, in this regard, has shown great promise. Studies on the MUC4 expression in cohorts of normal and tumor tissues of varying stages and types have revealed its potential for clinical use in the diagnosis and/or management of pancreatic, lung, breast, gall bladder, salivary gland, prostate, and ovarian cancers. Whereas, in some cases, overexpression of MUC4 was associated with a better prognosis (such as mucosidermoid carcinomas of salivary gland; ref. 4), in others (invasive ductal carcinoma of the pancreas, intrahepatic cholangiocarcinoma, and extrahepatic bile duct carcinoma), it was related to a poor clinical outcome (5–7). Another important aspect of MUC4 was recognized in distinguishing epithelial mesothelioma from invading lung adenocarcinoma (8). The distinction between the two malignancies is complicated due to the wide range of growth pattern of mesothelioma, a mesenchymally derived cancer with epithelial, fibrous, or mixed morphologic features. Immunohistochemical analysis showed that MUC4 is overexpressed in lung adenocarcinoma and is useful in distinguishing lung adenocarcinoma from malignant mesothelioma, with 91.4% sensitivity and 100% specificity (8).

Previous studies from our laboratory and others have raised future hopes that MUC4 can be a specific early tumor marker for pancreatic malignancy (9, 10). Pancreatic cancer, often referred as the “silent” killer due to its cryptic course of progression, is the fourth leading cause of cancer-related deaths in the United States (11). No recognizable symptoms are apparent until the disease has advanced and metastasized; therefore, there is an urgent need for reliable early diagnostic markers. MUC4 was identified among the most differentially expressed genes in pancreatic adenocarcinoma with an undetectable expression in the normal pancreas (9). Moreover, neoexpression of MUC4 was observed early in precancerous pancreatic intraepithelial neoplastic lesions, exhibiting a progressive increase with the disease advancement (10). Consistent with these observations, a recent study has shown that MUC4 is a good candidate marker for early diagnosis of pancreatic cancer in fine-needle aspirates, exhibiting 91% sensitivity and 100% specificity (12). All these research findings clearly indicate that MUC4 can be exploited clinically as an early diagnostic tool and for monitoring patients with pancreatic cancer.

Ovarian cancer is another occult disease that presents no detectable signs and hence eludes early detection. CA125, a mucin (MUC16) being used as tumor biomarker in clinics for the diagnosis of epithelial ovarian cancer (13), has proved to be sensitive; however, its usefulness is limited due to its elevation in benign conditions such as endometriosis, pelvic inflammatory disease, and pregnancy. A recent study from our laboratory (13) has shown a 100% incidence of the overexpression of MUC4 in early-stage ovarian cancer, whereas only a faint staining was observed in some cases of nonneoplastic ovary. Moreover, our data also suggested that MUC4 could be used in combination with MUC16 to achieve greater sensitivity for the detection of late-stage tumors. The potential usefulness of MUC4 as a combination marker can...
also be explored in other malignancies and may prove useful in improving the sensitivity and specificity of the current diagnostic assays.

**MUC4 as a Modulator of Cell Signaling**

MUC4 is a high molecular weight glycoprotein with multidomain organization. The deduced full-length amino acid sequence of MUC4 apoprotein shows the presence of a leader peptide, serine and threonine rich non–tandem repeat region, central large tandem repeat domain containing 16-amino-acid repetitive units, regions rich in potential N-glycosylation sites, two cysteine-rich domains, a putative GDPase proteolytic cleavage site, three epidermal growth factor (EGF)-like domains, a hydrophobic transmembrane domain, and a short cytoplasmic tail. Initial recognition that MUC4 can putatively function as a signaling modulator came from studies on its rat homologue, SMC. SMC/rMuc4 exhibits extensive similarities in the Nt and Ct regions with human MUC4 (2). It is a heterodimeric glycoprotein, composed of an O-glycosylated mucin subunit, ASGP-1, and an N-glycosylated transmembrane subunit, ASGP-2. The ASGP-2 subunit of Muc4 was shown to interact specifically with the receptor tyrosine kinase ErbB2, inducing its phosphorylation (14). ASGP-2 possesses two EGF-like domains, EGF-1 and EGF-2, and it was revealed that the EGF-1 domain of ASGP-2 was important in the receptor-ligand interaction (14). Intriguingly, it was also observed that MUC4 potentiated ErbB2 signaling more effectively when the cells were treated with neuuregulin, a ligand for ErbB3 (14). Moreover, Muc4, alone and in the presence of neuuregulin, produced differential downstream effects. It was reported that Muc4 alone did not lead to the activation of mitogen-activated protein kinase (MAPK) pathway and/or phosphoinositide 3-kinase pathway, but instead up-regulated p27\(^{kip}\) protein (a cyclin-dependent kinase inhibitor). On the other hand, in the presence of neuuregulin, Muc4 augmented the neuuregulin-elicited repression of p27\(^{kip}\) and the activation of MAPK and phosphoinositide 3-kinase pathways, identifying the context-dependent functions of Muc4 in cell signaling (14). Recently, in an effort to expose the mechanism fundamental to the ability of Muc4 to boost the ligand-induced ErbB2-ErbB3 signaling, Funes et al. (15) observed that Muc4 elicited the localization of ErbB2 and ErbB3 to the cell membrane and suppressed the ligand-induced receptor internalization.

In realization of the functional association between Muc4 and ErbB2, we also attempted to observe if human MUC4 could influence the status of ErbB2/HER2. Our study, using a pancreatic cancer cell line (CD18/HPAF), showed that the silencing of MUC4 led to the reduced expression of HER2 (11). This important finding indicated another novel mechanism by which MUC4 can modulate HER2 signaling; however, the molecular basis of this finding is yet to be exposed. Nonetheless, in appreciation of the role of MUC4 in defining HER2 status, either at the expression and/or functional level, there is an increased interest in exploring the yet unknown functions of MUC4 in cancer cell signaling.

**Role of MUC4 in Tumor Growth and Metastasis**

Aberrant expression of MUC4 in various carcinomas has prompted many researchers to elucidate its regulatory mechanisms and to look for its pathologic role (11, 16, 17) so that a MUC4-based intervention therapy can be devised. A tumor originates as a consequence of deregulated cell proliferation and/or disruption of apoptotic machinery, and, to become malignant and succeed in the metastatic process, a tumor cell has to acquire certain additional functional properties (as reviewed in ref. 18). The complicated process of metastasis progresses in several consecutive events, including the detachment of tumor cells from the primary site, intravasation into the blood stream, evasion of immune surveillance, adherence to vascular endothelial cells of distant organs, and finally extravasation into such tissues. In a recent study, we observed that silencing of MUC4 expression resulted in the suppression of pancreatic tumor cell growth and metastasis (11). Importantly, the studies on SMC/Muc4 have also reported that it may influence tumor growth via the suppression of apoptosis and potentiate metastasis via multiple mechanisms (17). During the initial process of tumor cell metastasis, the weakening of cell-matrix and cell-cell interactions is imperative for the detachment of tumor cells from primary sites. Our study revealed that the presence of MUC4 served as the determinant of the adhesive properties of the cell, although no observable changes in the expression of known adhesion molecules were reported (11). The overexpression of the cell-surface Muc4/SMC has also been reported to disrupt integrin-mediated cell adhesions as well as the homotypic cell-cell interactions, causing the dissociation of tumor cells in culture (19). MUC4 mucin is larger in size (ranges between 1.1 and 2.1 \(\mu\)m, depending on the length of the central tandem repeat domain) than its rat counterpart (≈0.5 \(\mu\)m). Hence, MUC4 can more effectively block the interaction of the surface adhesion molecules with their ligands in the extracellular matrix or on the neighboring cell by steric hindrance. In fact, our unpublished observations do indicate that MUC4 modulates the cancer cell binding to the extracellular matrix proteins. Moreover, biochemically modified (O-glycosylated) MUC4 carries an overall anionic charge and, therefore, may disrupt cell-cell interaction due to charge repulsion, in addition to steric hindrance.

Further adding to the metastatic capability of MUC4 was the finding that cell motility correlated with MUC4 expression (11). Cell motility has a central role during the dynamic process of tumor invasion and metastasis. This important phenotypic change may directly result from the MUC4-elicited changes in the actin organization via a yet unknown mechanism and/or indirectly via HER2-mediated pathway(s) as MUC4 seemed to regulate the expression of HER2 (11). Another possibility that we speculated for the MUC4-associated metastatic potency was the interaction of sialyl-oligosaccharides present on the MUC4 with the selectin molecules. Selectin is considered to facilitate the tumor cell binding to the endothelial cells. Additional efforts, however, are needed to confirm and determine the contribution of MUC4-selectin interaction in tumor cell metastasis.

**MUC4 in Therapy Planning**

Cancer is a dreadful disease with limited therapeutic options, especially when it has already metastasized. The inability of cancer drugs to kill tumor cells and resistance to immune therapy are the leading reasons why most therapeutic approaches fail. For this reason, one of the major interests of scientists has been to understand the nature and mechanism(s) of such resistance, so that the underlying obstacle(s) can be overcome and/or an alternate therapy can be planned. In an effort to understand the mechanism of Herceptin resistance in breast and other cancers, it was discovered that MUC4 masked the antibody-binding
Figure 1. Multifaceted roles of MUC4 in cancer development. Cancer results from the interlinked and continued alterations at the molecular and cytoarchitectural levels. An aberrant expression of MUC4 resulting from the genetic/epigenetic changes, alternative splicing, and biochemical modifications may have multiple implications in malignant transformation. Under normal conditions, MUC4 is localized at the apical surface of the epithelial cells. During the course of cancer progression, tumor cells lose polarity, allowing MUC4 to find novel interacting partner(s) such as HER2 and thus participate in cancer cell signaling. In addition, MUC4 may facilitate tumor cell invasion by disrupting the interaction between adhesion molecules and increasing cell motility. Furthermore, MUC4 may protect disseminated tumor cell from anoikis via HER2-mediated mechanisms, prevent immune killing by masking the surface antigens, and assist in its adhesion on endothelial cells, thus aiding the distant metastasis.
epitope of ErbB2, leading to diminished Herceptin binding (20). This finding revealed another interesting attribute of MUC4 that may prove useful in therapeutic planning.

Perspective

All the aforementioned findings add to the growing promise that MUC4 carries as a future diagnostic and therapeutic target (see Fig. 1 for the graphical summary of the data). Knowing the incidence and pathologic association of a molecular alteration may allow us to ensure appropriate treatment and patient monitoring. Past research has shown an aberrant expression of MUC4 in many carcinomas with its pathobiological implications in the disease process, thus supporting its standing as a potential target for cancer diagnosis, prognosis, and therapy. With added knowledge on the MUC4 expression, its pathologic significance in cancer and by delineating the mechanism of MUC4 action, newer approaches for cancer management can be devised. It might be possible to use MUC4 for a refined classification of disease status and prognosis, especially in patients whose outcome can be only insufficiently predicted by the currently established parameters. Moreover, novel MUC4-based cancer therapy can also be developed.

Although recent research has yielded important information on MUC4 and its role in cancer, a lot still remains to be learned. In fact, a more direct role of MUC4 in cancer cell signaling and pathogenesis has only begun to be unraveled and there are many areas that need attention for future investigations. For instance, MUC4 shows extensive alternative splicing that may lead to the generation of three distinct types of MUC4 protein: a family of secreted forms, membrane-associated variant forms, and membrane-bound forms lacking the tandem repeat domain. Actual assessment of their relative expression and pathobiological significance is yet to be established. Moreover, MUC4 exhibits a high degree of variable number of tandem repeat (VNTR) polymorphism. The tandem repeat domain is the largest domain of MUC4, which is hypothesized to contribute significantly to the multifaceted function of MUC4 in normal and cancer cell biology. Thus, it will be interesting to determine the correlation, if any, of VNTR polymorphism in MUC4 with its tumorigenic and metastatic potential. Due to the large size, the cloning of the full-length MUC4 cDNA has not been successful. Moreover, the functional analysis of different domains of MUC4 is also pending.

Recently, novel domains in MUC4, such as NIDO (Nidogen-like) and AMOP (Adhesion-associated domain in MUC4 and Other Proteins), have also been defined. The functional attributes to these domains will bring new impetus to define the multiple implications of MUC4 in cancer and to study its mode of action that can ultimately be translated into wide clinical applications.

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