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Chapter

METCAM/MUC18: A Novel Tumor Suppressor for Some Cancers

Guang-Jer Wu

Abstract

METCAM/MUC18, a component of cellular membrane, is a cell adhesion molecule (CAM) in the Ig-like gene super-family. It is capable of carrying out general functions of CAMs, such as performing intercellular interactions and interaction of cell with extracellular matrix in tumor microenvironment, interacting with various signaling pathways, and regulating social behaviors of cells. METCAM/MUC18 plays the tumor suppressor function in some cancers, such as colorectal cancer, nasopharyngeal carcinoma type I, one mouse melanoma subline K1735-9, ovarian cancer, pancreatic cancer, prostate cancer PC-3 cell line, and perhaps hemangioma. Possible mechanism in the METCAM/MUC18-mediated tumor suppression is proposed. By taking advantage of the tumor suppressor function of METCAM/MUC18, recombinant METCAM/MUC18 proteins and other derived products may be used as therapeutic agents to treat these cancers.

Keywords: METCAM/MUC18, Ig-like CAM, *in vivo* tumor suppression, colorectal cancer, nasopharyngeal carcinoma, mouse melanoma, ovarian cancer, pancreatic cancer, prostate cancer, mouse models

1. Introduction: tumor initiation and malignant progression is mainly controlled by two sets of genes as well as CAMs

Tumor/cancer is a genetic disease due to accumulated mutations or epigenetic alterations in our genetic material, DNA [1]. 80–90% of cancer risk comes from environmental factors and the remaining 10–20% risk from hereditary factors [2]. Environment in a broad sense includes both the physical containment and the social and cultural environment and its associated effects on our lifestyle choices. The environmental factors in the physical containment include chemicals (from polluted drinking water, air and soil, and diet), physical agents (UV and environmental radiation and medical radiation), biological agents (tumor viruses, bacteria, and parasites), and the lifestyle. These agents aim to attack our DNA in the somatic cells and resulting in accumulation of mutations and epigenetic alterations in our genes throughout our life time. Hereditary factors (lineage specific cues) include both the inherited genetic mutations and epigenetic imprinting in the germ cells that pass on from generation to generation. Tumor initiation and malignant progression are mainly caused by two sets of genes, such as the tumor-promoting genes (oncogenes) and the tumor suppressor genes, thus, mutations and epigenetic alterations in these two sets of genes are doom to be responsible for the tumorigenic process [2–4].

In addition to exogenous chemical agents, physical agents, and biological agents in the environment that cause mutations in the genes, endogenous metabolic processes

and chronic inflammation from our lifestyle choices produce free radicals that directly attack our DNA also resulting in mutations [5]. The major sources of free radicals are reactive oxygen species (ROS), which is a collective term for the unstable, reactive, partially reduced oxygen derivatives that are the normal by-products of our metabolic processes. They include hydrogen peroxide (H_2O_2), superoxide anion (O_2^-), hypochlorous acid (HOCl), singlet oxygen ($^1O_2$), and hydroxyl radical ($^{\bullet}HO$). ROS are also produced by the inflammatory macrophages and neutrophils and are spilled out to attack the DNA of bystander cells. ROS acts as the secondary messengers in cell signaling and essential for various biological processes in both normal and cancer cells and as both tumor-promoting and tumor suppressing agents. To keep the system in check, ROS is balanced by intracellular anti-oxidant enzymes, that produce a number of anti-oxidants, such as glutathione (GSH) and thioredoxin (Txn), which are also present in our foodstuffs, to remove ROS. ROS production is a mechanism shared by most chemotherapeutics to trigger cell-death in cancer cells and unfortunately also to some extent in normal cells. Thus, ROS has conflicting roles as a secondary messenger in cancer cells as well as cancer-killers during cancer chemotherapy.

Most of the mutations in the oncogenes are dominant and thus manifest obvious phenotypes of increased proliferation and survival of tumor cells (gain-of-functions). In contrast, most of mutations in the tumor suppressor genes are recessive and thus do not manifest any phenotype until both copies of the gene are mutated or altered epigenetically (loss-of-functions). Some tumor suppressor genes are gate-keepers that directly affect proliferation and death, thus directly open to tumor formation. But some tumor suppressor genes are care-takers that affect DNA repair functions and genomic stability, thus increase mutation rate of all genes and indirectly affect proliferation [2, 6].

Epigenetic alterations may change the extent of methylation (either hypo- or hyper-methylation) in the regulatory regions of both oncogenes and tumor-suppressor genes, thus affect the transcriptionally regulatory region of the genes and directly regulate transcriptional expression of the genes. Epigenetic alterations may also modify histones and non-histone proteins that affect chromosome remodeling, thus indirectly affect the transcription of the genes. Epigenetic alterations may also affect post-transcription processes (namely translational process or stability of mRNA) of the genes via microRNAs [7].

Besides the above traditional two sets of genes, other genes, such as CAMs, also contribute directly to the tumor initiation and progression or orchestrate the tumor microenvironment to affect the tumor progression [8]. CAMs are involved in several biological functions, such as tissue architecture, organ formation, blood vessel generation and angiogenesis, immune and inflammatory reactions, wound healing and social behaviors [8]. An altered expression of CAMs may have implications in tumorigenesis, since CAMs govern cellular social behaviors by directly contributing to cell adhesion and cross-talk with the intracellular signal transduction pathways [8]. As a consequence, an aberrant expression of CAMs is capable of changing mobility and invasiveness, influencing outlasting ability and proliferation of tumor cells, and altering new blood vessel formation [8]. It also affects distant organ-dissemination of carcinoma cells, because CAMs orchestrate complex interactions of tumor cells with various stromal cells in the tumor microenvironment, resulting in augmentation or reduction of the spreading potential of carcinoma cells [8]. Effects of the aberrant expression of the following CAMs on tumorigenesis and malignant progression are better studied, such as cadherin [9], integrins [10], CD44 [11], CEACAM [12], mucins [13], L1CAM [14], EpCAM [15], ALCAM [16] and METCAM/MUC18 [17]. Over the past several years, our team investigated the role of METCAM/MUC18 in several types of tumors, such as melanoma, breast, nasopharyngeal, ovarian and prostate cancers [18–36]. The resulting data showed a dual role of METCAM/MUC18 as a tumor promotor or suppressor in these cancers [17, 37].

2. METCAM/MUC18: an immunoglobulin-like (Ig-like) CAM

Originally, METCAM/MUC18 was first demonstrated to be abundantly expressed on the cellular membrane of most malignant human melanomas, hence named as MUC18. It has been implicated to play a pivotal role in the malignant progression of human melanoma, hence was named as MCAM and Mel-CAM) [38]. However, METCAM/MUC18 was found in subsequent studies not to be exclusively expressed in melanoma, and it did not initiate the transformation of normal cutaneous melanocytes to melanoma either [39]. Instead, METCAM/MUC18 was also expressed in other epithelial tumors and it could initiate or promote the transformation of other epithelial cells into carcinomas [40]. Thus, METCAM/MUC18 also bears other names, such as S-endo1, CD146, A32, or METCAM [40, 41]. Later METCAM/MUC18 was found to be able to suppress tumorigenesis in some cancer cell lines [17, 37, 40].

The human METCAM/MUC18 is a *c*ell *a*dhesion *m*olecule (CAM) belonging to the Ig-like gene superfamily. The naked human METCAM/MUC18 is a single chain transmembrane protein of 65–72 kDa consisting in 646 amino acids with an extracellular N-terminal domain of 558 amino acids, a 24 amino acids-transmembrane domain and a cytoplasmic domain of 64 residues (**Figure 1**) [38, 42].

Figure 1 shows that the N-terminal extra-cellular domain of the protein is composed of a signal peptide sequence (SP) and five immunoglobulin-like domains and one X domain [37, 42]. The intracellular cytoplasmic domain has one, three, and one protein kinase consent sequences that are potentially to be phosphorylated by PKA, PKC, and CK2, respectively [37, 38, 42]. In addition, the METCAM/MUC18 usually has an apparent molecular weight of 110–150,000 because it is heavily glycosylated in all cell types. The amino acid sequence of huMETCAM/MUC18 reveals nine possible N-glycosylation sites, of which six are conserved between human and mouse proteins, in the extracellular domain. METCAM/MUC18 is conserved in mouse, in which the amino acid sequences of mouse METCAM/MUC18 (moMETCAM/MUC18) are 72.6% identical to the huMETCAM/MUC18 [43]. Therefore, both human and mouse METCAM/MUC18's are capable of performing similar general functions of CAMs, such as controlling cellular social behaviors by impacting the adhesion status of cells and modulating signaling. Furthermore, over-expression of both human and mouse METCAM/MUC18's similarly affected tumor cells in *in vitro* motility and invasiveness, *in vitro* and *in vivo* tumorigenesis, and *in vivo* metastasis [42, 43].

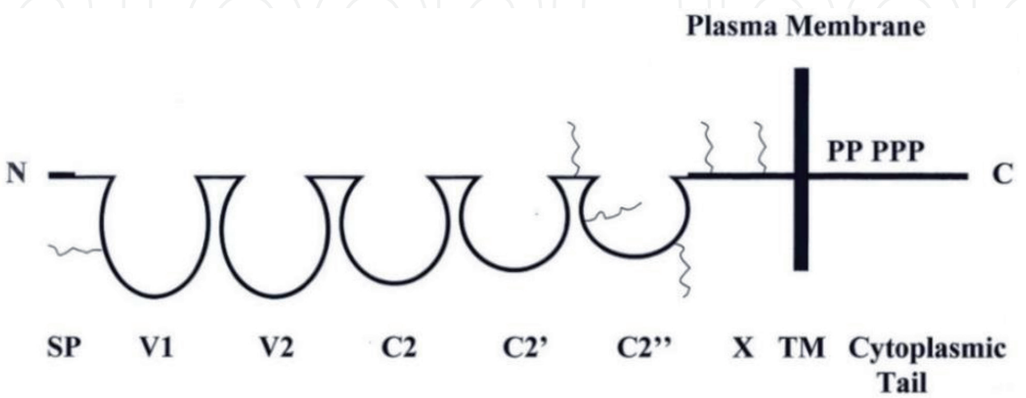


Figure 1.
The human METCAM/MUC18 (huMETCAM/MUC18). The figure represents the protein structure of huMETCAM/MUC18 with its 3 domains: (1) a large extracellular domain showing a signal peptide (SP), the five Ig-like variables (V1 and V2) and conserved (C1, C2, C2' and C2") domains, each of which held together by a disulfide bond, and one X domain; six conserved N-glycosylation sites indicated as wavy lines in V1, the interdomain C2'/C2", C2" and X domains; (2) a short transmembrane domain (TM); and (3) a cytoplasmic domain containing five potential phosphorylation sites (P).

The huMETCAM/MUC18 is expressed in at least 10 normal tissues: hair follicular cells, smooth muscle cells, endothelial cells, cerebellum, basal cells of the lung, activated T cells, intermediate trophoblasts [44], breast epithelium [18, 19], nasopharyngeal epithelium [23], and ovarian epithelium [27]. The protein is also expressed in several carcinomas, such as breast carcinoma, intermediate trophoblast tumors, melanoma, prostate adenocarcinoma, osteosarcoma, and others [17, 44]. Our studies also indicate that over-expression of METCAM/MUC18 augments tumorigenesis of breast carcinoma [18–20], nasopharyngeal carcinoma type III [24, 26], and prostate adenocarcinoma [34], but it does not have an obvious effect on tumorigenesis of most melanoma cell lines [21]. METCAM/MUC18 over-expression also initiates the distant organ-dissemination of prostate cancer [32, 33] and augments the distant organ-dissemination of melanoma [21] and breast carcinoma [45].

In contrast, over-expression of METCAM/MUC18 represses tumorigenesis of a mouse melanoma cell line, K1735-9 [22], nasopharyngeal carcinoma type I [24, 25] and perhaps hemangiomas [46]. METCAM/MUC18 over-expression also represses the distant organ-dissemination of the mouse melanoma cell line, K1735-9 [22].

3. METCAM/MUC18: a tumor suppressor in several types of cancer

3.1 Mouse melanoma

Over-expression of moMETCAM/MUC18 in one mouse melanoma cell line K1735 clone 10 (or K1735-10 subline) has no effect and that in another cell line K1735 clone 3 a slight suppression effect on subcutaneous tumorigenesis [21], but in K1735 clone 9 (or K1735-9 subline) it completely suppresses the subcutaneous tumorigenesis [22]. Thus, METCAM/MUC18 definitely acts as a tumor suppressor for the K1735-9 subline, but may have a less obvious effect on two other K1735 sublines, K1735-3 and K1735-10. In addition to its effect on tumorigenesis, over-expression of moMETCAM/MUC18 in K1735-9 also completely suppressed lung nodule formation in immunocompetent syngeneic C3H brown mouse model. In contrast, over-expression of moMETCAM/MUC18 in K1735-3 and K1735-10 subline has an opposite effect (namely promotion) on lung nodule formation. In conclusion, moMETCAM/MUC18 acts as a tumor suppressor with a different severity on different cell lines in a syngenetic mouse model [21, 22].

3.2 Nasopharyngeal carcinoma

Nasopharyngeal carcinoma (NPC) occurs in the non-lymphomatous, squamous epithelial lining of the posterior nasopharynx [24]. Histologically, three subtypes of NPC are defined according to World Health Organization (WHO) classification: WHO type I (keratinizing squamous cell carcinomas), WHO type II (non-keratinizing squamous cell carcinomas), and WHO type III (undifferentiated carcinomas) [24]. Three major risk factors suggested by epidemiological studies, such as genetic predisposition, dietary and environmental factors, and the Epstein Barr virus (EBV) infection, may cause the unusual occurrence of NPC in endemic areas [24–26]. However, the biological mechanisms of their involvement in cancer initiation, development or malignant progression are not well understood. Nevertheless, it could be hypothesized that altered cell adhesion molecules (CAMs) in NPC lead to tumorigenesis and malignant progression, since aberrant expression of CAMs, such as CD44, connexin 43, E-cadherin, and ICAM, has been associated with the progression of NPC [23]. In order to test this hypothesis, we previously studied the possible role of altered METCAM/MUC18 expression in nasopharyngeal carcinoma [23, 24].

Therefore, we used immunohistochemistry method to determine gene expression at the protein level in seven tissue specimens of normal nasopharynx, 97 specimens of three different types of NPC and also used immunoblot method to determine that in several cell lines established from type I and type III NPC [23]. The results showed a weak expression of the protein METCAM/MUC18 in 27% of the NPC tissues in contrast to all the normal nasopharynx tissues which exhibited high expression of the protein. According to these results, we suggested that METCAM/MUC18 may play a tumor suppressor function in the development of NPC during the progression of the disease [23]. We then tested the hypothesis by transfecting the cDNA into two NPC cell lines which weakly expressed the protein and isolated the high-expressing clones for examining the effect of METCAM/MUC18 over-expression on *in vitro* cellular behavior and *in vivo* tumorigenesis of the two NPC cell lines in athymic nude mice. Consistent with the hypothesis, we indeed observed that METCAM/MUC18 over-expression suppressed the tumor growth of NPC-TW01 cells, which were established from type I NPC [47], as previously shown [24, 25]. We thus conclude that METCAM/MUC18 plays a tumor suppressor role in the development of the type I NPC [24, 25].

Surprisingly, when a different cell line, NPC-TW04, was used for the similar set of the experiments we observed a completely opposite effect of METCAM/MUC18. We observed that over-expression of METCAM/MUC18 promoted *in vitro* and *in vivo* tumor growth of NPC-TW04 cells, which were established from type III NPC [47], as previously reported [24, 26]. We thus conclude that METCAM/MUC18 plays a tumor promoter role in the development of the type III NPC [24, 26].

Taken together we hypothesized that METCAM/MC18 plays a dual suppressor and promotor role in the different types of NPC.

3.3 Ovarian carcinoma

Two independent groups showed that METCAM/MUC18 expression is correlated with the progression of ovarian cancer [27, 48], and it affects the *in vitro* behaviors of ovarian carcinoma cells [49]. However, the role of METCAM/MUC18 in the progression of epithelial ovarian cancer has not been directly tested in animal models. To investigate this, we initiated the studies by testing the effect of over-expression of METCAM/MUC18 on the *in vitro* cellular behaviors and *in vivo* tumorigenesis and malignant progression of human ovarian cancer cell lines in nude mice. First, we used a human ovarian cell line, SK-OV-3, for testing the effects of METCAM/MUC18 expression on their *in vitro* motility and invasiveness, and *in vivo* tumor formation after subcutaneous (SC) injection and also *in vivo* progression after intraperitoneal (IP) injection in athymic nude mice. We observed that over-expression of METCAM/MUC18 reduced *in vitro* motility and invasiveness [28] and suppressed *in vivo* tumorigenesis and malignant progression of the human ovarian cancer cell line SK-OV-3 [28]. Then, we used the other human ovarian cancer cell line, BG-1, for similar tests and also observed similar phenomenon [50].

In summary, we supplied *in vitro* and *in vivo* evidence to definitely support the conclusion that METCAM/MUC18 plays a suppressor role in the tumorigenesis and malignant progression of two human ovarian cancer cell lines [28, 50]. Our results suggest that METCAM/MUC18 is a strong candidate as a new tumor and metastasis suppressor in human ovarian cancer cells.

3.4 Prostate cancer

For the previous two decades, we have firmly established the notion that over-expression of METCAM/MUC18 promotes the tumorigenesis and metastasis

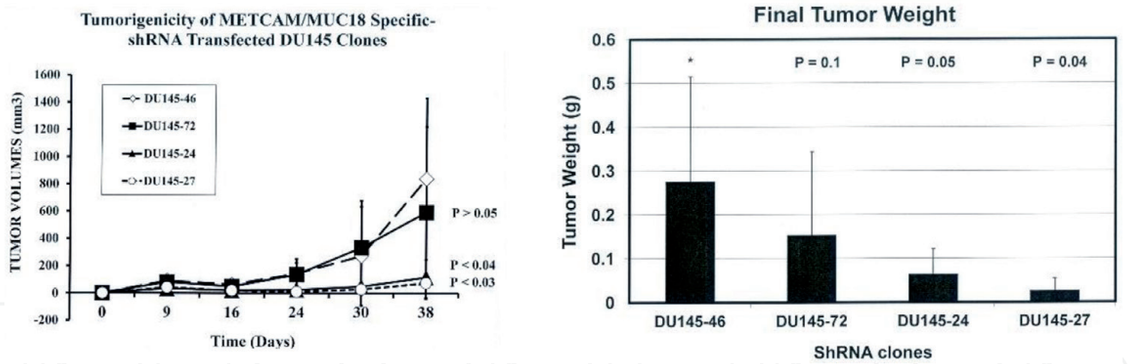


Figure 2.
Tumorigenicity of four shRNA-knockdown clones of DU145. Effect of METCAM/MUC18 expression on in vivo tumorigenicity (Left) and final tumor weight (Right). (Left) Average tumor volumes from five mice S.C. injected with each of the 46 (control), 72, 24, and 27 clones/cells, which were transfected with four corresponding shRNAs in pGIPZ vector, were plotted against time. (Right) Average final tumor weights from five mice S.C. injected with the same clones/cells and standard deviations were plotted at the end point of experiment. P values are shown in the figure by comparing the data to the control clone [51].

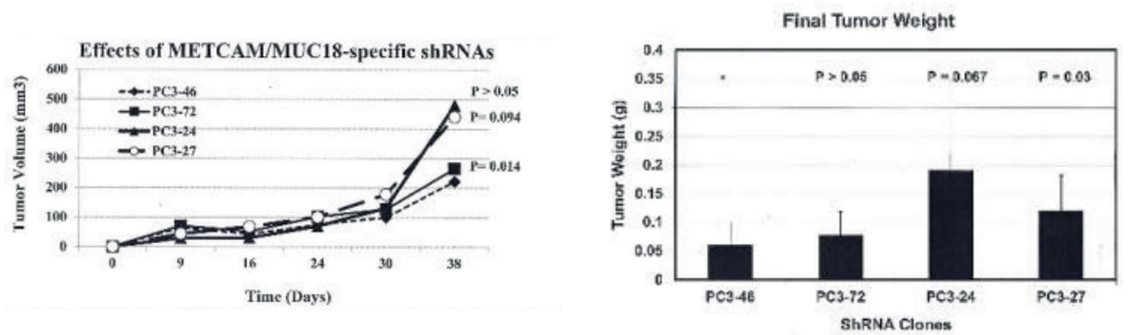


Figure 3.
Tumorigenicity of four shRNA-knockdown clones of PC-3. Effect of METCAM/MUC18 expression on in vivo tumorigenicity (Left) and final tumor weight (Right). (Left) Average tumor volumes from five mice S.C. injected with each of the 46 (control), 72, 24, and 27 clones/cells, which were transfected with the four corresponding shRNAs in pGIPZ vector, were plotted against time. (Right) Average final tumor weights from five mice S.C. injected with the same clones/cells and standard deviations were plotted at the end point of experiment. P values are shown in the figure by comparing the data to the control clone [52].

of human prostate cancer cell line LNCaP, which was established from lymphatic lesions [31–36]. To check if the conclusion is also extended to another human prostate cancer cell line DU145, we recently tested the effect of knocking down the endogenously expressed METCAM/MUC18 on tumorigenesis in a nude mouse system, since DU145 endogenously expressed a high level of METCAM/MUC18 [51]. We found that knocking down of the endogenously expressed METCAM/MUC18 with three shRNAs decreased the subcutaneous tumorigenesis in male nude mice in comparison to a control shRNA, as shown in **Figure 2**. We thus concluded that METCAM/MUC18 expression in DU145 cell line, which was established from brain lesions, plays a positive role in tumorigenesis (and perhaps metastasis) similar to in LNCaP cells.

In contrast, we recently used the similar knocking down strategy to test the effect of decreased the endogenous METCAM/MUC18 expression on *in vivo* tumorigenesis of another human prostate cancer cell line, PC-3, which was established from bone lesions, surprisingly we found that knocking down the endogenously expressed METCAM/MUC18 increased the tumor proliferation of PC-3 cells (which was opposite to that of DU145, as shown above in **Figure 2**), suggesting that expression of METCAM/MUC18 suppressed the tumorigenesis of the human prostate cancer cell line PC-3 [52], as shown in **Figure 3**.

Tumor/cancer cell lines	Tumorigenesis	Metastasis	References
Colorectal cancer human cell lines HT-29, SW480, SW948, SW620, colo205, Lovo320, P6C	Suppression	Not determined	[54]
Hemangioma human cell lines HemEC, HDMEC	Possible suppression	Not determined	[46]
Mouse melanoma cell line K1735-9	Suppression	Suppression	[22]
Mouse melanoma cell lines K1735-3, K1735-10	No effect or slight suppression	Increasing and affecting the late stage	[21]
Nasopharyngeal carcinoma type I cell line NPC-TW01	Suppression	Not determined	[24, 25]
Ovarian cancer cell lines SK-OV3, BG-1	Suppression	Suppression	[28, 50]
Pancreatic cancer human cell lines, UACC-1273, PANC1, C81-61, KP-2, SUIT-2, MIAPaca-2, HS766T and primary CAFs	Suppression	Suppression	[55, 56]
Prostate cancer human cell line PC-3	Suppression	Not determined	[52]

Table 1.
The negative role of METCAM/MUC18 in tumor formation and/or cancer metastasis of seven tumor/cancer cell lines.

We thus conclude that METCAM/MUC18 serves as a tumor suppressor in the PC-3 cell line, different from its role in two other prostate cancer cell lines (LNCaP and DU145), suggesting that prostate cancer cell lines established from different organs may have different intrinsic factors that modulate the function of METCAM/MUC18.

3.5 Colorectal cancer, hemangioma and pancreatic cancer

The protein METCAM/MUC18 is also expressed others cancers, such as angiosarcoma, gestational trophoblastic tumors, Kaposi's sarcoma, leiomyosarcoma, some lung squamous and small cell carcinomas, and some neuroblastoma [44]. However, its role in the development of most of these cancers is not well known. Recent meta-analysis suggests that high METCAM/MUC18 expression in many solid tumors appears to be associated with poor prognosis and patient survival [53]. However, in contrast to the conclusion, reduced expression of METCAM/MUC18 associates with the malignant progression of hemangioma [46]. Likewise, recent results of the effects of METCAM/MUC18 expression on tumorigenesis of colorectal cancer and pancreatic cancer also appear to support the similar conclusion, as described next. Reduced expression of METCAM/MUC18 promotes tumorigenesis and stemness of colorectal cancer [54]. Targeting soluble METCAM/MUC18 with a neutralizing antibody inhibits vascularization, growth and survival of METCAM/MUC18-positive pancreatic tumors [55]. Furthermore, attenuation of METCAM/MUC18 promotes pancreatic cancer progression [56]. Thus, the possible tumor and metastasis suppressor role of METCAM/MUC18 in solid tumors appear to extend from mouse melanoma K1735–9 subline, ovarian cancer, and NPC type I, to colorectal cancer [54] and pancreatic cancer [55, 56], and perhaps hemangioma [46]. **Table 1** summarizes the negative role of METCAM/MUC18 in the tumor formation and/or cancer metastasis of seven tumors/cancers.

4. METCAM/MUC18: a tumor promoter in most solid tumors

In contrast to the above functions of METCAM/MUC18, recent work done on other solid tumors appears to be consistent with the meta-analysis results of solid tumors [53],

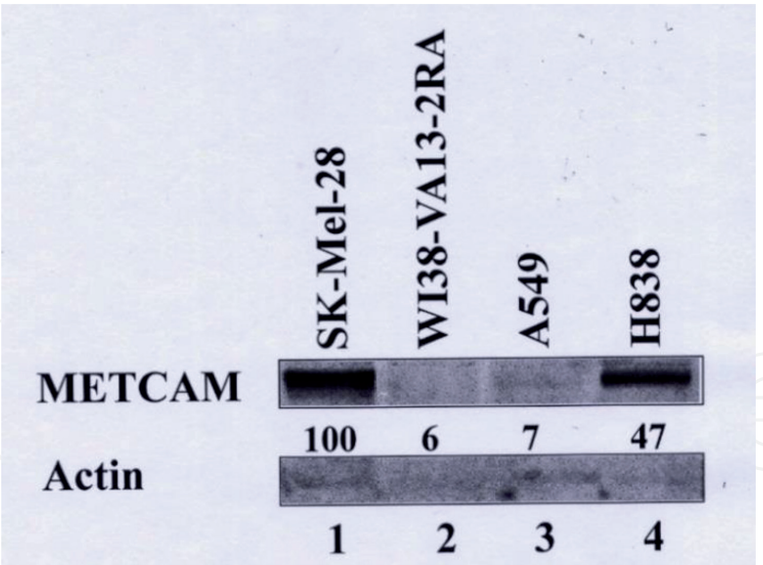


Figure 4.
Expression of METCAM/MUC18 in normal lung tissue (SV40-immortalized normal lung cells (WI38, lane 2) and lung type II alveolar epithelial cell carcinoma cell (A549, lane 3) and lung primary adenocarcinoma (H838, lane 4) (from Guang-Jer Wu, unpublished data).

as described next. For example, METCAM/MUC18 expression correlates with the epithelial-mesenchymal transition (EMT) markers and a poor prognosis in gastric cancer [57]. Tumor up-take of glioma in an orthotopic xenograft mouse model correlates with the expression level of METCAM/MUC18 [58]. METCAM/MUC18 promotes metastasis and predicts poor prognosis of hepatocellular carcinoma [59]. Increased expression of METCAM/MUC18 has been found in hepatocellular carcinoma (HCC) tumor tissues as compared with the matched adjacent normal liver tissues and the METCAM/MUC18^{+} cells purified from HCC tumors and cells have significantly increased colony-forming capacity consistent with the cancer stem cells or the tumor-initiating cells [60]. METCAM/MUC18 expression has been shown to express in 51% of non-small cell lung carcinoma (NSCLC) and positive expression of METCAM/MUC18 has been associated with a shorter survival of patients with adenocarcinomas and used to predict the poor overall survival in patients with lung adenocarcinomas [61–63]. METCAM/MUC18 expression mediates acquisition of cancer stemness and enhances tumor invasion and metastasis in a mouse model [64]. High expression of METCAM/MUC18 correlates with intrapulmonary metastasis of NSCLC cells in a mouse model [65]. Consistent with the results, we showed in **Figure 4** (Guang-Jer Wu, unpublished data) that METCAM/MUC18 is expressed in a lung type II alveolar epithelial cell carcinoma cell, A549, and highly expressed in an adenocarcinoma cell line, H838, in comparison with its no expression in an immortalized normal embryonic WI38 cell line.

Furthermore, METCAM/MUC18 mediates chemoresistance of small cell lung carcinoma (SCLC) [66]. METCAM/MUC18 is expressed in osteosarcoma cell lines, but not in normal osteoblast cells [67]. Osteosarcoma is effectively treated with METCAM/MUC18 monoclonal antibodies [68, 69]. Transcription factor MEIS1 activates METCAM/MUC18 expression to promote migration of mouse pancreatic tumor cell lines [70]. METCAM/MUC18 very likely promotes the formation of angiosarcoma, as supported by our preliminary results as described next. Mouse METCAM/MUC18 was expressed in one angiosarcoma clone, SVR, which was transfected with H-Ras, at a higher level than in the control cell line, an immortalized normal endothelial cell line, MS-1 [71]. Furthermore, the tumorigenicity of the SVR cell line was higher than the control cell line, thus in direct association with the higher expression level of moMETCAM/MUC18 [40, 71]. This suggests that METCAM/MUC18 very likely promotes the formation of angiosarcoma [40, 71].

Tumor/cancer tissues or cell lines	Tumorigenesis	Metastasis	References
Angiosarcoma human cell lines MS1, SVR	Increasing	Not determined	[40, 71]
Human breast cancer cell line MCF-7	Promotion	Not determined	[18]
Human breast cancer cell line SK-BR-3	Promotion	Not determined	[19, 20]
Human breast cancer cell lines MDA-MB-231 and MDA-MB-468	Promotion	Promotion	[19, 45]
Gastric cancer human tissues	Promotion	Not determined	[57]
Glioma cell lines U87MG, U251	Promotion	Not Determined	[58]
Hepatocellular carcinoma human cell lines PLC/PRF/5, Huh7, MHCC97H& 97 L HepG2, SMMC-7721, focus, YY-8103, LM3, HLF and primary HCC cell lines; normal liver cell line LO2	Promotion	Not determined	[59, 60]
Non-small cell lung cancer human cell lines A549, H23, H358, H460, H522, H838, HCC4006, H1650/ER, PC-9, PC9GR, and adenocarcinoma tissues	Promotion	Promotion	[61–65], our unpublished results
Small cell lung cancer human cell lines H69, H69AR, H82, H196, H209, DMS79	Promotion	Not determined	[66]
Clinical melanoma tissues and human melanoma cell lines SB-2, SK, XP-44	No effect	Increasing and affecting the late stage	[38, 72, 73]
Mouse melanoma cell lines K1735-3, K1735-10	No effect or slight suppression	Increasing and affecting the late stage	[21]
Nasopharyngeal carcinoma type III human cell line NPC-TW04	Promotion	Not determined	[24, 26]
Osteosarcoma human cell lines CR9, MNNG-HOS, OHS, KPDX, KRIB, MG-63, shYY1, SaOS, SaOS-2, TE85, U20S	Promotion	Augmentation	[67–69]
Pancreatic cancer mouse cell lines ptf1a, LSL-Kras, LSL-Trp53, Pdx1,	Promotion	Possible augmentation	[70]
Clinical prostate cancer human tissues	Increasing	Increasing and affecting initiation in the early stage (PIN)	[31]
Human prostate cancer cell line LNCaP	Increasing	Increasing and affecting initiation in the early stage	[32, 34–36]
Human prostate cancer cell line DU145	Increasing	Not determined	[51]
Prostate adenocarcinoma in TRAMP mice	Increasing	Increasing and affecting initiation in the early stage	[33]

Table 2.
The positive role of METCAM/MUC18 in tumor formation and/or cancer metastasis of various tumors/cancers.

Hence, the positive role played by the METCAM/MUC18 in the progression of solid tumors have been extended from breast cancer, human and mouse melanoma, prostate cancer to angiosarcoma [40, 71], gastric cancer [57], glioma [58], hepatocellular carcinoma [59, 60], non-small cell lung adenocarcinoma [61–65], small cell

lung cancer [66], osteosarcoma [67–69], and mouse pancreatic cancer [70]. Taken together, METCAM/MCU18 appears to be more prevalently in playing a positive role than a negative role in the tumorigenesis of solid tumors. **Table 2** summarizes the positive role of METCAM/MUC18 in the tumor formation and/or cancer metastasis of various tumors/cancers.

In conclusion, METCAM/MUC18 appears to play a dual role in the tumorigenesis and perhaps also in metastasis of solid tumors. At this point, it is not clear why METCAM/MUC18 plays a dual role in this aspect. Since METCAM/MUC18 only plays a dual role in different cell lines from the same type of cancer or in different type of cancers, but never in the same cancer cell line. It is logical to suggest a possible explanation that the intrinsic properties of each cancer cell line may provide specific co-factors or heterophilic ligands that may positively or negatively modulate the METCAM/MUC18-mediated tumorigenesis and metastasis. This can be readily scrutinized by identifying these specific intrinsic co-factors or heterophilic ligands by using immunological co-precipitation method in the future studies. This approach is feasible as described in one of the following sections, Section 5.1.

5. Putative mechanisms

Since the huMETCAM/MUC18 was first discovered in the 1980s, three groups have worked on the role of huMETCAM/MUC18 in melanoma metastasis [38, 39, 72, 73], another group on the role of huMETCAM/MUC18 in the biology of endothelial cells [41], and our group joined in the effort to study the role of huMETCAM/MUC18 in the progression of mouse melanoma [43] and prostate cancer [31–36, 51, 52], and later breast cancer [18–20], ovarian cancer [27–30], and NPC [23–26], as described above. Recently, more groups have participated in further exploring the possible role of METCAM/MUC18 in other solid tumors in different organs, such as colorectum [54], gastro-organ [57], glioma [58], liver [59, 60], lung [61–66], pancreas [55, 56, 70], and bone [67–69]. Preliminary work in leiomyosarcoma, esophagus squamous cell carcinoma, clear cell renal sarcoma, and gallbladder adenocarcinoma are also beginning to emerge [53].

After many decades of group effort, we are beginning to understand the biology of METCAM/MUC18-mediated tumor progression. However, the biological mechanisms describing the role of METCAM/MUC18 in tumorigenesis and malignant progression are still not well clarified such as: the protein's domain involved in cell adhesion, the domain which mediates the interactions of tumor cells with the tumor microenvironment leading to tumor progression and in the METCAM/MUC18-mediated tumorigenesis and malignant progression, and the effects of N-glycosylation on the functions of METCAM/MUC18 in tumorigenesis. Though the huMETCAM/MUC18-mediated outside-in and inside-out signaling in endothelial cells are understood to some extent, and the METCAM/MUC18-mediated signaling, which is leading to the progression of various cancer cells, are not much known. How METCAM/MUC18 is positively or negatively regulated at the level of transcription in different cancer cells remains minimally known. As such, the following five important aspects are much needed for immediate future studies, such as different kinds or quantities of co-factors or heterophilic ligand(s) in different cancer cell lines, contributions of different domains of the protein, different signaling pathways involved, differential regulation at the transcription level in tumors of different organs, and possible different extent of N-glycosylation in different cancer cell lines, which may critically modulate the function of METCAM/MUC18 in tumor progression.

5.1 The heterophilic ligands of METCAM/MUC18

The heterophilic ligands of METCAM/MUC18 may play an important role in the cell-cell and cell-extra-cellular matrix interactions and cancer metastasis. Our preliminary results suggest that the 72 kDa protein identified by immunoprecipitation method may be one of the heterophilic ligands for METCAM/MUC18, as shown in **Figure 5** [40].

As shown in **Figure 5**, the putative heterophilic ligand 72 kDa is highly expressed in the PC-3 cell line, but much less in the DU145 cell line. This may reveal a possible explanation for the different role of huMETCAM/MUC18 in the tumorigenicity of the two prostate cancer cell lines [40].

5.2 The domains of huMETCAM/MUC18 required for tumorigenesis and metastasis

The relation of the protein structure of huMETCAM/MUC18 to its functions in tumorigenesis and metastasis have not been systematically defined. To begin addressing this question, we have generated mutants deleted different domains of huMETCAM/MUC18 by using a special PCR method [74] and used them to determine their contribution to tumorigenesis. Surprisingly, our results showed that the ecto-domain of huMETCAM/MUC18 induced tumorigenesis in LNCaP cells in nude mice, as well as the whole wild type of cDNA. These preliminary results suggested

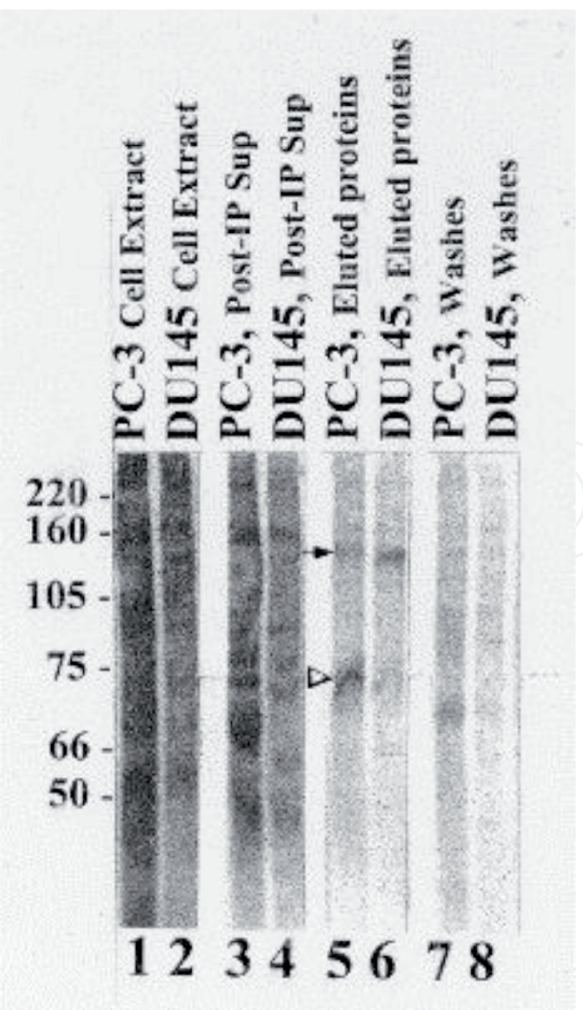


Figure 5.
Putative heterophilic ligand of METCAM/MUC18 in PC-3 and DU145 cell lines.

the key role of the ecto-domain in tumorigenesis induction in prostate cancer cells *in vivo*. This may implicate a puzzling notion that the cytoplasmic domain was not essential for this process (Guang-Jer Wu, data not shown). However, the critical direct test of using only the cytoplasmic domain for inducing tumor has not been performed for LNCaP cells. From the above puzzling observation, it is very clear that a systematic study has also to be performed in other cancer cell lines before a definitive conclusion can be drawn.

5.3 Signaling pathways in the METCAM/MUC18-mediated tumorigenesis and cancer metastasis

The huMETCAM/MUC18 contains three sites which are potentially phosphorylated by PKC, PKA and CK2 in the cytoplasmic tail [38, 42]. However, these putative phosphorylation sites have not been biochemically proven. Thus, the immediate question to be answered is that how many sites in the cytoplasmic tail of the native METCAM/MUC18 protein, which are to be isolated from different cancer cell lines, are actually phosphorylated? Which protein kinase is responsible for the phosphorylation? After this is answered, then we can further study how METCAM/MUC18 mediates crosstalk and networking with different signal pathways and to see if it is similar to or different from the cytoplasmic tails of other CAMs [41, 75–77]. Knowledge learned from other CAMs seem point to one aspect that METCAM/MUC18, as an integral membrane protein and a cell adhesion molecule, should mediate inside-in, inside-out, and outside-in signals to participate in intercellular communication and interaction of cell with extra-cellular matrix, which results in impacting cell motility and invasiveness [78, 79]. Furthermore, its interaction with co-factors or cognate heterophilic ligand(s) may alter these signals, which in turn should affect intrinsic tumor proliferation or impact tumor angiogenesis and/or mediate targeting to specific organs and promoting metastasis. Moreover, METCAM/MUC18 may interact with various hormonal receptors, growth or anti-growth factors/receptors, various chemokines/receptors, and the Ca^{2+}-mediated signaling members, which in turn affect the process of tumor progression. **Figure 6** summarizes the possible preliminary crosstalk of huMETCAM/MUC18 with many members of signal transduction pathways that may affect its function during tumor initiation and development and malignant progression.

5.4 Regulation of the huMETCAM/MUC18 gene transcription

The mechanism of transcriptional control of METCAM/MUC18 gene is minimally studied [17]. Up to now, only the 900 bp sequences in the core promoter region of the huMETCAM/MUC18 gene are well-characterized [80]. This core promoter is rich in GC sequences but does not contain a TATA box. It includes many consensus sequences presumably as putative binding sites for various transcription regulatory factors, such as SP-1, CREB [81], AP-2 [82, 83], c-Myb [84], N-Oct2 (Brn2) [85], Ets [86], CArG [87], and Egr-1 [88]. In addition, it also contains three insulin responsive elements (one Ets and two E-box motifs) [89], suggesting that huMETCAM/MUC18 gene expression may respond to the cue of various growth signals [37, 40], as shown in **Figure 7**.

In addition, some sequences upstream of the minimal core promoter sequences should also be expected for conferring the tissue-specific expression of the huMETCAM/MUC18 gene [90]. Recently this notion has been definitely supported by a finding that Ets sequence in the 10 kilo-bp up-stream region is involved in the regulation of the expression of huMETCAM/MUC18 gene [91]. We have also engaged in this task by searching the sequence of the up-stream region of the huMETCAM/MUC18 promoter in the Celera or other web sites. By taking advantage of the

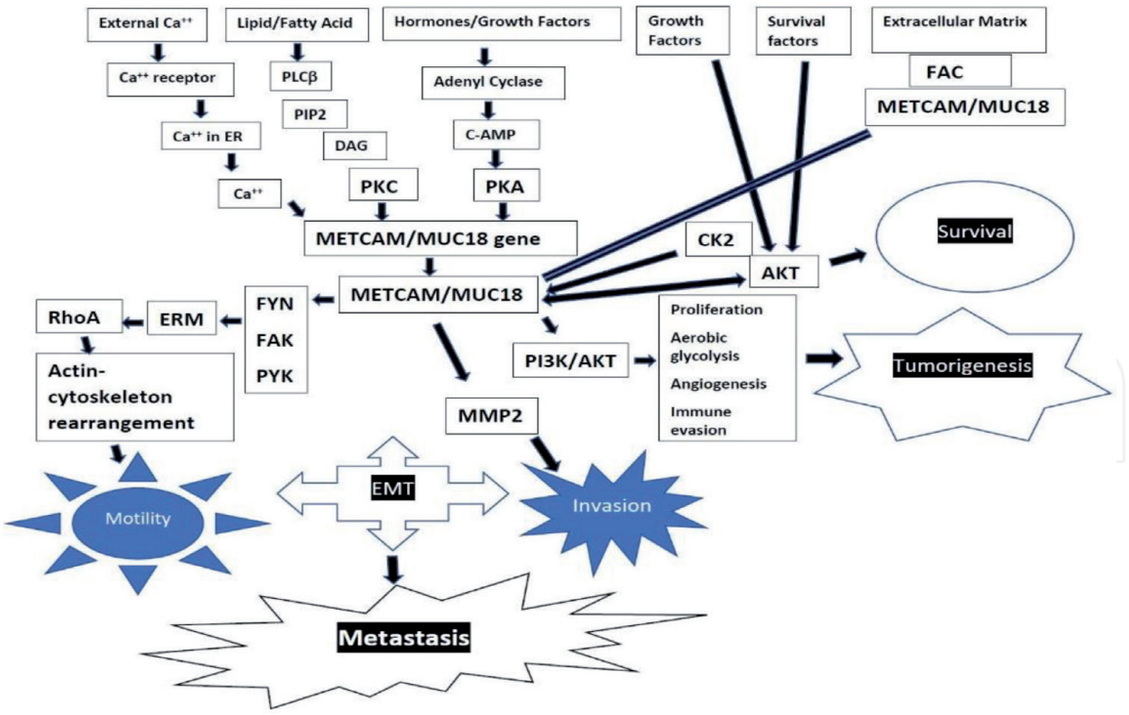


Figure 6.
METCAM/MUC18-mediated signal transduction in tumorigenesis and malignant progression.

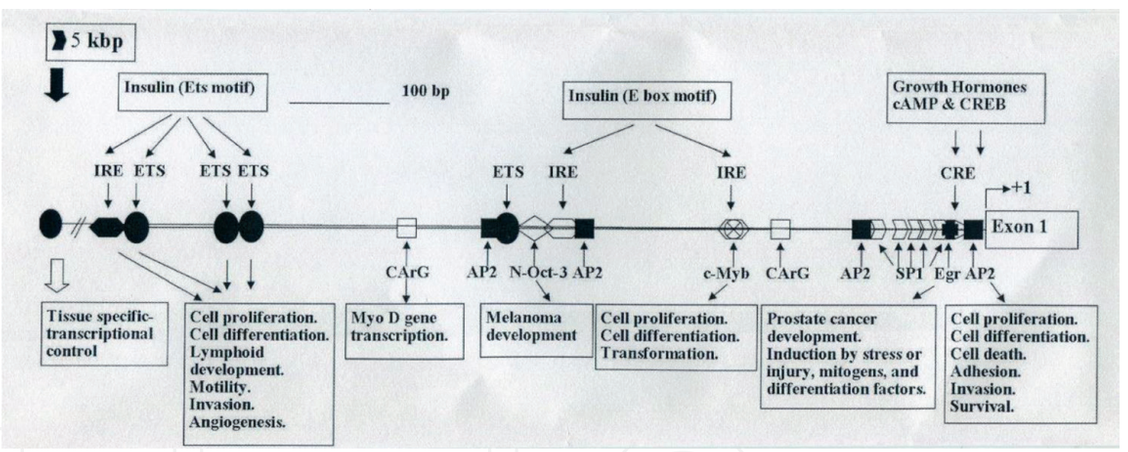


Figure 7.
Putative transcription factor-recognized motifs in the 900 bp core promoter and 5–10 kilo bp up-stream region of the huMETCAM/MUC18 gene.

known sequence searched, we designed many pairs of primers to screening a genomic library and obtained several phage clones which contain at least 4 kilo-bp of the gene for future studies (Guang-Jer Wu, unpublished data).

The epigenetic control of the expression of huMETCAM/MUC18 gene has not been extensively studied in NPC, though it has been implicated [92]. This is because huMETCAM/MUC18 gene is located at the locus of human chromosome 11q23.3 that has been shown to be hypermethylated in NPC, suggesting that the expression of this gene may be regulated by epigenetic controls [93]. To support this notion, our preliminary results of treating NPC cell lines with 5-Aza-2′-deoxycytidine (Aza-C) showed that after the treatment with Aza-C, METCAM/MUC18 expression was somewhat elevated in the NPC-TW01 cell line, but not in the NPC-TW04 cell line (Guang-Jer Wu, unpublished data). METCAM/MUC18 has also been shown to be methylated in most of the early stage of prostate cancer [94]. Further systematic studies in this aspect should be very interesting and rewarding in the future.

5.5 The possible roles of glycosylation on the protein of METCAM/MUC18 in tumorigenesis and tumor progression

Glycosylation of a protein may affect the proper folding, stability, and/or activity of a protein [95], however, the possible roles of glycosylation in the function of MCETCAM/MUC18 protein have not been explored. The glycosylation of METCAM/MUC18 may also affect its ability in inducing/promoting or suppressing the metastasis of cancer cells [95–99]. Both huMETCAM/MUC18 and moMETCAM/MUC18 may very likely to be heavily glycosylated, sialylated, and post-translationally modified, because both have an apparent molecular weight of about 110–150 kDa, which is much more than the naked protein with a molecular weight of about 65–70 kDa [100]. To initiate the study, we subjected the huMETCAM/MUC18, which was expressed in a human cancer cell line, to the digestion with N-glycosidase F, neuraminidase (sialidase), O-glycosidase, or endoglycosidase H, and we observed that the apparent molecular weight of the protein was decreased after digestion with N-glycosidase F and neuraminidase (sialidase), but not with O-glycosidase or endoglycosidase H [37, 40]. From this, we suggested that both sialic acid and N-glycans are probably the major carbohydrate side chains of huMETCAM/MUC18. It is also possible that glycosylation may differ depending on the type of cancers. Thus, we suggested that different N-glycans at the N-glycosylation sites of huMETCAM/MUC18 may differ in different cancer cell lines, which may have significant positive or negative impacts on their EMT abilities as well as tumorigenesis and metastasis. According to our hypothesis, a recent study described GCNT3 as an up-stream regulator of METCAM/MUC18. Moreover, GCNT3 glycosylates METCAM/MUC18 and extends its half-life which results in further elevation of S100A8/A9-mediated cellular motility in melanoma cells [101].

By searching in the primary sequence of the human huMETCAM/MUC18 protein, nine potential N-glycosylation sites (Asn-X-Ser/Thr or N-X-S/T sites) have been revealed [37, 38, 40, 42], whereas only seven sites found in the mouse METCAM/MUC18 [43]. Six N-glycosylation sites are conserved between the two proteins: 56/58 NL/FS, 418/420NRT, 449/451NLS, 467NGT/469NGS, 507NTS/509NTT, and 544/546NST [37, 38, 40, 42]. We suggest that only these six conserved N-glycosylation sites are actually glycosylated, because the apparent molecular weights of human METCAM/MUC18 and mouse METCAM/MUC18 are similar in the SDS gel. All the N-glycosylation sites are located in the external region of the protein, such as the domains of V1, C′, C″ and X. First, all these six sites should be biochemically identified before further molecular genetic task. Then, we will use genetic tools to alter the N-glycosylation sites. The mutants will be transfected back into cancer cell lines without the endogenous expression of the protein. The clones, which only express these mutated METCAM/MUC18, will be used for various *in vitro* and *in vivo* experiments to test the effect of N-glycosylation on the function huMETCAM/MUC18. They also will be used for testing effects on *in vitro* cell–cell aggregation and cell-extracellular matrix adhesion and on *in vivo* tumorigenesis and metastasis of human cancer cells. We anticipate that systematic studies on this aspect should be very informative to reveal the essential role of N-glycosylation played in the METCAM/MUC18-mediated tumor progression.

6. Conclusions

METCAM/MUC18 plays a key role in suppressing the progression of colorectal cancer, one mouse melanoma cell line, NPC type I, ovarian cancer, pancreatic cancer, prostate cancer PC-3 cell line, and perhaps hemangioma and possibly in other cancers.

On the other hand, METCAM/MUC18 also play a key positive function in the progression of breast cancer, gastric cancer, hepatocellular carcinoma, lung cancer, melanoma, NPC type III, pancreatic cancer, and prostate cancer. To further understand its role in these processes, it is essential to further identify its co-factor regulators and cognate heterophilic ligands, define its functional domains, and study its crosstalk with members of various signal transduction pathways, the regulation of its expression at the level of transcription, and effects of N-glycosylation on the functions of the protein.

7. Research perspectives and clinical applications

7.1 Research perspectives

The current studies have laid an important biological basis for inspiring future intense investigation to further understand the detailed knowledge of METCAM/MUC18-mediated suppression of tumorigenesis and metastasis of various cancer cell lines. For this purpose besides those have been described above, other future endeavors may include: (a) understanding three major mechanisms involved in METCAM/MUC18-induced tumor and metastasis dormancy, such as key players participated in inhibition of intrinsic growth capability, key chemokines and cytokines participated in suppression of immunological responses, and key pro-angiogenic and anti-angiogenic factors participated in the reduction of angiogenesis [102], (b) identification of possible miRNAs and non-coding RNAs participated in the process upstream and downstream of METCAM/MUC18 [103], and (c) possible clinical applications should be explored. Precaution should be taken that a complete picture may only be possibly constructed after all the above studies are successfully executed.

7.2 Clinical applications

The majority of the cancer-associated mortality is due to dissemination of primary tumor to distant organs (metastasis). If we are able to decrease or stop the metastatic propensity of cancer cells and keep them stayed only at the primary site, it should be a major success in cancer therapy. Alternatively, it is also a major success if we are able to control cancer cells at the state of dormancy or remaining them at the stage of micro-metastatic lesions [104]. Thus, similar to other tumor and metastasis suppressors, such as KISS1, KAI1, nm23, MAP2K4, and some micro-RNAs, METCAM/MUC18 may be used as a new therapeutic target for some clinical cancer treatments [105]. Strategically four major approaches may be taken for this purpose: (a) use gene therapeutic method to restore the functional copy of the suppressor genes or use epigenetic method to re-activate the genes. For gene therapy, the METCAM/MUC18 cDNA gene may be transported by an adenovirus-associated virus vector or a replication-defective adenovirus [106]. The human METCAM/MUC18 gene, located on 11q23-3 chromosome may be targeted with clinical reagents to reverse epigenetic repression, like Aza-C [107], or to change histone modifications to induce remodeling of the chromosome [108], (b) dispense recombinant proteins directly to the patients. For this approach, a complete copy or a partial portion of the METCAM/MUC18 recombinant protein, oligopeptides, or small molecule mimetics of METCAM/MUC18 may be directly dispensed to cancer patients, (c) target at downstream key members in the signaling pathways which are activated by the loss of the suppressor function, and (d) the co-factors or the cognate heterophilic ligand(s) of METCAM/MUC18 may be targeted. The above strategies may be used in single, or better in combination for treating the patients for the purpose of holding tumor cells at the primary sites, stopping them

in a dormant state, or keeping the disseminating cancer cells at the state of micro-metastases. However, the dual role of METCAM/MUC18 in cancer progression may limit the above clinical applications to only cancers exhibiting an anti-tumor activity mediated by METCAM/MUC18.

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Conflict of interests

The author has no conflict of interests.

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