Chapter

METCAM/MUC18 Promotes Tumor Progression and Metastasis in Most Human Cancers

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Abstract

In addition to oncogenes and tumor suppressor genes, cell adhesion molecules (CAMs) also significantly contribute to tumor progression and metastasis. For the past two decades, we have demonstrated that METCAM/MUC18, a cell adhesion molecule in the immunoglobulin-like gene superfamily, orchestrates complex interactions of tumor cells with various stromal cells in the tumor microenvironment, resulting in augmentation or reduction of the metastatic potential of carcinoma cells. Here we show that METCAM/MUC18 plays a positive role in the tumor progression and metastasis in most human cancers, such as breast cancer, human melanoma and most mouse melanoma, nasopharyngeal carcinoma type III, prostate cancer LNCaP and DU145 cell lines, and perhaps angiosarcoma, gastric cancer, glioma, hepatocellular carcinoma, non-small cell lung adenocarcinoma, small cell lung cancer (SCLC), osteosarcoma, and human and mouse pancreatic cancer. Possible mechanisms in the METCAM/MUC18-mediated tumor progression and metastasis are proposed. Anti-METCAM/MUC18 antibodies and siRNAs may be used as therapeutic agents to treat these cancers.

Keywords: METCAM/MUC18, Ig-like CAM, tumor promotion, metastasis, breast cancer, melanoma, nasopharyngeal carcinoma, prostate cancer, many solid tumors, mouse models

1. Introduction: cancer and CAM-mediated tumor progression and metastasis

Tumor/cancer is a chronic disease resulting from gradually accumulation of mutations or epigenetic alterations in our genetic material, DNA [1]. Ten to twenty percent cancer risk comes from hereditary factors and 80–90% of cancer risk from environmental factors [2]. The environmental factors in the physical containment include (a) chemically polluted drinking water, air and soil, and diet; (b) irradiation from solar UV, artificial sources, and environmental radioactive elements; (c) pathological agents (tumor viruses, bacteria, and parasites); and (d) the lifestyle (stress, chronic inflammation from obesity, and free radicals from metabolism) [3–5]. These agents aim to attack our DNA in the somatic cells resulting in slow accumulation of mutations and epigenetic alterations in our genes throughout the life span [6]. The question of "Is cancer a metabolic disease or a genetic disease?" cannot be easily answered. Prior to 1970, most cancer researchers thought cancer is a metabolic disease because of the Warburg effect. After 1970 when Warburg died and after 1971 when oncogenes

were discovered, most researchers shifted their thinking to view cancer as a genetic disease. After 2010–2015 when cancer was rediscovered as a metabolic disorder, the view was shifted back to "cancer is a metabolic disease." While cancer as a genetic disease looks to be impossibly complex, tumor cells are a genetic "train wreck" with an infinite number of mutations and epigenetic alterations in ~250 oncogenes and ~700 tumor suppressor genes. In contrast, cancer as a metabolic disease with only seven different "metabotypes" appears to be remarkably simple to deal with, since all the above mutations mainly affect three major metabolic pathways: aerobic glycolysis, glutaminolysis, and one-carbon metabolism [7].

Besides the traditional oncogenes and tumor suppressor genes [6], cell adhesion molecules (CAMs) also contribute directly to the tumor initiation and metastasis or orchestrate the tumor microenvironment to affect the tumor progression [8]. CAMs are involved in several biological functions, such as cellular social behaviors, tissue architecture, organ formation, blood vessel generation and angiogenesis, immune and inflammatory reactions, and wound healing [8]. An altered expression of CAMs has implications in tumor progression and metastasis, since most CAMs govern cellular social behaviors by directly contributing to cell adhesion, epithelial-to-mesenchymal transition (EMT), and cross talk with the intracellular signal transduction pathways affecting other tumor progression-related processes [8]. As a consequence, the 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 25 years, our team investigated the role of METCAM/MUC18 in several types of tumors, such as melanoma and breast, nasopharyngeal, ovarian, and prostate cancers [17–37].

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

METCAM/MUC18 was first demonstrated to be abundantly expressed on the cellular membrane of most malignant human melanomas and hence was named as MUC18 [38] and MCAM [37]. It has been implicated to play a pivotal role in the malignant progression of human melanoma and hence was named as Mel-CAM [39]. However, subsequent studies showed that METCAM/MUC18 was not found to be exclusively expressed in melanoma, and furthermore, it did not initiate the transformation of normal cutaneous melanocytes to melanoma [39]. Instead METCAM/MUC18 was also expressed in endothelial cells and 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 also found to act as a suppressor in tumorigenesis and metastasis in some cancer cell lines [17, 37, 40].

The human METCAM/MUC18 (huMETCAM/MUC18) is a cell adhesion molecule (CAM) belonging to the Ig-like gene superfamily. The METCAM/MUC18 usually has an apparent molecular weight of 110–150,000 due to its high glycosylation in all cell types. The naked huMETCAM/MUC18 is a single-chain transmembrane protein of 65–72 kDa consisting of 646 amino acids with an extracellular

N-terminal domain of 558 amino acids, a transmembrane domain with 24 amino acids, and a cytoplasmic domain of 64 residues (**Figure 1**) [38, 42].

Figure 1 shows that the N-terminal extracellular domain of the protein is composed of a signal peptide sequence (SP) and five immunoglobulin-like domains and one X domain [37, 38, 40, 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, 40, 42]. The amino acid sequence of huMETCAM/MUC18 reveals nine potential 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 huMETCAM/MUC18 and moMETCAM/MUC18 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, overexpression of both human and mouse METCAM/MUC18s similarly affected tumor cells in in vitro motility and invasiveness, in vitro and in vivo tumorigenesis, and in vivo metastasis [42, 43].

The huMETCAM/MUC18 is expressed in at least ten 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 overexpression 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 overexpression 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, overexpression 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 overexpression also represses the distant organ dissemination of the mouse melanoma cell line, K1735-9 [22]. Thus, METCAM/MUC18 plays a dual role in some of these cancers [17, 37].

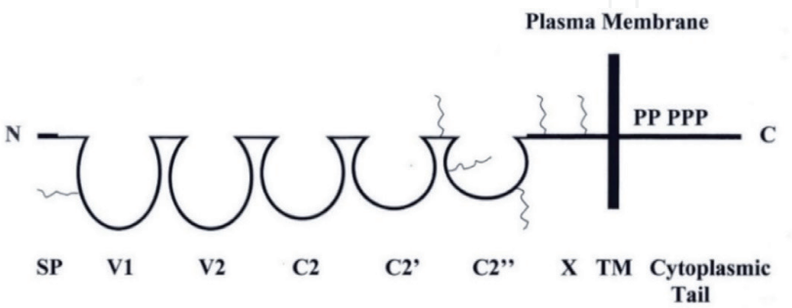

Figure 1.
The human METCAM/MUC18 (huMETCAM/MUC18). The figure represents the protein structure of huMETCAM/MUC18 with its three domains: (1) A large extracellular domain showing a signal peptide (SP), the five Ig-like variables (V1 and V2) and conserved (C1, C2, C2', and C2") domains, each of which held 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). (3) A cytoplasmic domain containing five potential phosphorylation sites (P).

3. METCAM/MUC18: a promoter in tumor progression and metastasis of human cancers

The protein METCAM/MUC18 is expressed in breast cancer, melanoma, nasopharyngeal carcinoma, and prostate cancer and also expressed in others cancers, such as angiosarcoma, gestational trophoblastic tumors, Kaposi's sarcoma, leiomyosarcoma, some lung adenocarcinoma and squamous and small cell carcinomas, and some neuroblastomas [44]. However, its role in the progression 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 [47]. In addition, METCAM/MUC18 expression and its possible role in other solid tumors began to emerge, such as angiosarcoma, gastric cancer, hepatocellular carcinoma, glioma, non-small cell lung cancer (NSCLC), small cell lung cancer (SCLC), osteosarcoma, and pancreatic cancer, as described in the following.

3.1 Breast cancer

Breast carcinomas were heterogenous with three histological subtypes (ER+, PR+, and ERBB2 receptor (HER)) ([48] for a review), with at least five distinct molecular subtypes (luminal A (ER+ and PR+), luminal B (ER+ and PR±), basal-like (ER-, PR-, AR-), HER2-enriched (HER2+), and normal-like) [49], or with ten combined genomic/transcriptomic subtypes [50]. HuMETCAM/MUC18 was found to be expressed in breast cancer cell lines and tissues of basal-like and mesenchymal subtypes at much higher levels than in luminal subtypes, which poorly or very weakly expressed the protein [18, 51]. METCAM/MUC18 was suggested by two groups to play a tumor suppressor role [52, 53] but by two other groups as a tumor promoter in the progression of human breast cancer [51, 54]. To resolve this controversy, we started separate studies to explore the real role of METCAM/MUC18 in the tumor progression of human breast cancer. We demonstrated that ectopic expression of METCAM/MUC18 in two breast cancer cell lines (MCF-7 and SK-BR-3) augmented their ability in epithelial-to-mesenchymal transition (EMT) and formation of colony in vitro and increased tumor-take and tumorigenesis (in vivo tumorigenesis) in athymic nude mice [18–20].

Treatment with an anti-METCAM/MUC18 antibody decreased the motility and invasiveness of the two basal-like cell lines, MDA-MB-231 and MDA-MB-468, which endogenously express the protein [19]. Overexpression of huMETCAM/MUC18 could also induce metastasis of the MCF7 cells in SCID/beige mice with the supplement of estrogen [45]. Furthermore, enforced expression of METCAM/MUC18 increases the metastasis of both basal-like cell lines in athymic nude mice [45]. The tumor suppression role of huMETCAM/MUC18 in tumorigenesis of human breast cancer cells previously observed by one group [52] has not been supported by evidence published later [18, 45]. The most likely reason may be due to the artifact of including fetal bovine serum in their injection mixtures, as extensively discussed in our published paper [18]. The other discrepancy may be because only in vitro experiments were done, but no in vivo animal test [51, 53, 54]. Taken together, METCAM/MUC18 plays a positive role in the tumor progression of four human breast cancer cell lines. From the results of further preliminary mechanical study, we suggest that METCAM/MUC18 promotes the progression of human breast cancer cells by increasing proliferation, angiogenesis, epithelial-to-mesenchymal transition (EMT), and switching to aerobic glycolysis [18–20]. METCAM/MUC18's downstream signaling molecules may also be used as therapeutic targets for the treatment of breast cancer.

3.2 Melanoma

Most malignant human melanomas overly expressed huMETCAM/MUC18 on the cellular surface, suggesting that it may promote the malignant progression of human melanoma [38]. This notion is supported by the evidence that enforced expression of the huMETCAM/MUC18 increases the metastatic ability of three nonmetastatic human melanoma cell lines in the immune-incomplete mouse models [55, 56]. It is further corroborated by our results that enforced expression of moMETCAM/MUC18 also augments the lung nodule formation ability of two low-metastatic mouse melanoma cell lines, K1735-3 and K1735-10, in a syngeneic mouse model with the complete immunity [21]. However, overexpression of moMETCAM/MUC18 in K1735-3 and K1735-10 subline has a minimal effect on tumor formation.

METCAM/MUC18 enables melanoma cells to establish pulmonary metastasis only when the METCAM/MUC18-expressing melanoma cells are injected into the tail vein (experimental metastasis) [18–20, 55, 56], but not when the cells were injected subcutaneously (spontaneous metastasis) either in immune-deficient mouse models [55, 56] or in immune-competent syngeneic mouse models [21]. Thus, it bypassed the initial stages of metastasis, suggesting that METCAM/MUC18 may promote melanoma metastasis only in the later stage of metastasis. This result is consistent with a later observation in that huMETCAM/MUC18 does not confer melanocytes the ability to initiate the tumor progression into melanoma [39]. Surprisingly when another mouse melanoma cell line, K1735-9, was used for the similar test in the syngeneic brown mouse model, a totally opposite result was obtained [22], to be described in Section 4. The exact reason for the dual role of METCAM/MUC18 in the tumor progression and metastasis is not clear, but one possibility is suggested in Section 5.

Taken together, our syngeneic mouse system should be more useful than the immune-incomplete mouse system to comprehend the complex mechanisms played by METCAM/MUC18 in the malignant progression of melanoma cells. Furthermore, the knowledge learned from our syngeneic mouse systems should also be useful for testing the real efficacy of various therapeutic strategies before the treatment of clinical melanoma, because they should more closely mimic the clinical melanoma cases than the xenograft models.

3.3 Nasopharyngeal carcinoma

Most (90%) nasopharyngeal carcinoma (NPC) occurs in the non-lymphomatous, squamous epithelial lining of the posterior nasopharynx [23, 24]. Three histological subtypes of NPC are defined according to World Health Organization (WHO) classification: WHO type I (keratinizing squamous cell carcinomas), WHO type II (nonkeratinizing squamous cell carcinomas), and WHO type III (undifferentiated carcinomas) [23, 24]. Epidemiological studies suggested three major risk factors, such as genetic predisposition, dietary and environmental factors, and the Epstein-Barr virus (EBV) infection, that may induce the unusual incidence of NPC in endemic areas [23–26]. However, the biological mechanisms of their contribution to tumor initiation, development, and malignant progression remain elusive. Since aberrant expression of CAMs, such as CD44, connexin 43, E-cadherin, and ICAM, has been associated with the progression of NPC ([23] for a review), it is highly probable that these risk factors may alter cell adhesion molecule (CAM) expression and lead to tumorigenesis and malignant progression of NPC. In order to test this hypothesis, we initiated the studies on the possible role of altered METCAM/MUC18 expression in the malignant progression of nasopharyngeal carcinoma. First, we investigated if an aberrant expression of METCAM/MUC18 was associated with NPC

[23] and then the effect of METCAM/MUC18 overexpression on the tumorigenesis of two NPC cell lines in an athymic nude mouse model [24–26], as described next.

We used immunohistochemistry (IHC) method to determine the expression level of huMETCAM/MUC18 in 7 tissue specimens of normal nasopharynx and 97 specimens of three different types of NPC and also used immunoblot method to determine several cell lines established from type I to type III NPC [23]. The results showed a weak expression of the METCAM/MUC18 protein in only 27% of the NPC tissues (no expression in 73% of the NPC tissues), in contrast to all the normal nasopharynx tissues which exhibited a high expression of the protein, suggesting that METCAM/MUC18 may play a tumor suppressor role in the development of NPC during the progression of cancer [23]. Then, we further tested the hypothesis by examining the effect of ectopic METCAM/MUC18 expression on in vitro cellular behavior and in vivo tumorigenesis of the two NPC cell lines in athymic nude mice. Indeed, the predicted hypothesis was supported by the results when NPC-TW01 cells were used for the tests [24–26], as described in Section 4. Surprisingly, contrary to the hypothesis, when NPC-TW04 cell line was used for similar in vitro and in vivo tests, we observed that overexpression of METCAM/MUC18 actually promoted in vitro and in vivo tumor growth of NPC-TW04 cells [24, 26], which were established from type III NPC [57]. We thus conclude that METCAM/MUC18 plays a positive role in the tumor progression of the type III NPC [24, 26]. Overall, METCAM/MUC18 plays a dual role in the tumor progression of NPC.

3.4 Prostate cancer

For the past two decades, we have first demonstrated that METCAM/MUC18 expression in human tissues was associated with the progression of human prostate cancer [31] and also with that of mouse adenocarcinoma in a transgenic model, TRAMP [33]. We further showed that overexpression of METCAM/MUC18 promotes the progression of a human prostate cancer cell line, LNCaP, which was established from lymph node lesions [32, 34], as described next.

First, by using IHC and immunoblot assays to determine the expression of huMETCAM/MUC18 in the tissues of human normal prostate gland, patients with BPH, and patients with prostate cancer and metastatic lesions, we found that METCAM/MUC18 was highly expressed in all of the high-grade PINs and most of prostate carcinoma at advanced pathological stages and metastatic lesions, but it was not expressed in most normal prostate glands and in all BPH lesions. Thus, huMETCAM/MUC18 expression is associated with the progression of human prostate cancer [31].

Second, by using similar immunological methods to determine the expression of moMETCAM/MUC18 in the prostatic tissues of a transgenic mouse model, TRAMP, at different times of life span, we found that moMETCAM/MUC18 expression was increased with the progression of the mouse adenocarcinoma in this transgenic mouse model. Thus, moMETCAM/MUC18 overexpression is associated with the progression of mouse prostate adenocarcinoma in a transgenic mouse model, TRAMP [33].

Third, we tested the effect of overexpression of huMETCAM/MUC18 in a human prostate cancer cell line LNCaP on its tumorigenesis when the cells were injected at the non-orthotopic *SC* sites in nude mice. We observed that huMETCAM/MUC18 overexpression promoted the tumorigenesis of the cell line at the non-orthotopic sites [34]. Then, we tested the effect of overexpression of huMETCAM/MUC18 in the LNCaP cell line on its tumorigenesis and establishing metastatic lesions when the cells were injected at the orthotopic site (in the dorsal and lateral lobes of mouse prostate gland) in a male nude mouse model [32]. We found that

huMETCAM/MUC18 overexpression promoted the tumorigenesis at the orthotopic prostate gland and also initiates metastatic lesions at periaortic lymph nodes and multiple distant sites (such as seminal vesicles, ureters, and the kidney). From the results, we conclude that ectopic overexpression of huMETCAM/MUC18 promotes in vivo tumorigenesis of the cells at either at non-orthotopic *SC* sites or at orthotopic prostate gland and also that it also initiates metastasis of the cells to multiple distant sites when cells were injected at the orthotopic mouse prostate gland. Taken together, we concluded that huMETCAM/MUC18 expression promotes the tumor progression of LNCaP cells in an athymic nude mouse model [31–36].

Fourth, to check if the above 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 expresses a high level of METCAM/MUC18 [58]. 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 summary, we conclude that METCAM/MUC18 plays a positive role in the tumor progression and metastasis of two human prostate cancer cell lines, LNCaP and DU145. However, we recently observed an opposite result when the third human prostate cancer cell line PC-3 was used for the similar test, as described in Section 4, suggesting that METCAM/MUC18 also plays a dual role in the tumor progression of human prostate cancer.

3.5 Other solid tumors

3.5.1 Angiosarcoma

METCAM/MUC18 very likely promotes the formation of angiosarcoma, as supported by our preliminary results as described next. MoMETCAM/MUC18 was expressed at a higher level in one angiosarcoma clone, SVR, which was transfected with H-Ras, than in the control cell line, MS-1, an immortalized normal endothelial cell line [59]. 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

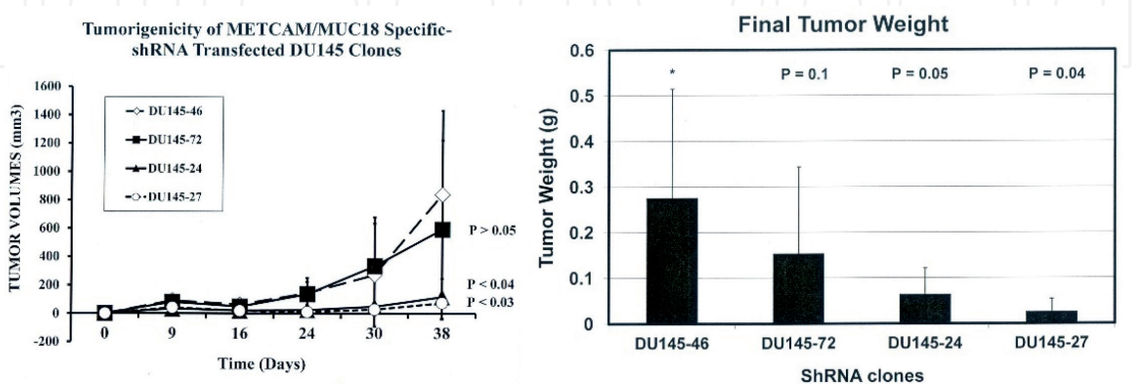

Figure 2.
Tumorigenicity of four shRNA knockdown clones of DU145. Effect of METCAM/MUC18 expression on in vivo tumorigenicity (left) and final tumor weight (right). (Left) Average tumor volumes from 5 mice S.C. injected with each of the 46 (control), 72, 24, and 27 clones/cells, which were transfected with 4 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 [58].

moMETCAM/MUC18 [40, 59]. This suggests that METCAM/MUC18 very likely promotes the tumor progression of angiosarcoma [40, 59].

3.5.2 Gastric cancer

The expression of huMETCAM/MUC18 in gastric cancer was investigated to evaluate its clinical-pathological and prognostic significance [60]. The expression of huMETCAM/MUC18 and three EMT-related proteins (E-cadherin, β-catenin, and vimentin) was examined by IHC method in 144 gastric cancers. Forty-one percent of the gastric cancer specimens were positive for the huMETCAM/MUC18 expression. HuMETCAM/MUC18 was also correlated positively with lymph node involvement and a poor prognosis. Furthermore, the huMETCAM/MUC18 expression was directly correlated with the lost expression of the epithelial marker, E-cadherin, and the gained expression of the mesenchymal markers, nuclear β-catenin and vimentin, suggesting that huMETCAM/MUC18 promotes EMT and also tumor progression in gastric cancer. It is possibly used as an independent index for a poor prognosis in gastric cancer and as a potential therapeutic target for patients with gastric cancers [60].

3.5.3 Glioblastoma

Glioblastoma multiforme (GBM) is the most common brain malignancy, accounting for more than 45% of all primary malignant brain tumors. YY146, an anti-METCAM/MUC18 monoclonal antibody, was created and radiolabeled for the noninvasive positron-emission tomography (PET) imaging of orthotopic GBM models. ^{64}Cu-labeled YY146 was demonstrated to be preferentially accumulated in the U87MG xenografted tumors, which permitted the obtaining of high-contrast PET images of small tumor nodules (~2 mm). Furthermore, tumor-take of glioblastoma in an orthotopic xenograft mouse model correlates with the expression level of METCAM/MUC18 in a highly specific manner. Furthermore, YY146 can mitigate the EMT of these U87MG cells. Moreover, using YY146 as the primary antibody for histological studies of the World Health Organization, grades I through IV primary gliomas showed that there was a positive correlation between METCAM/MUC18-positive staining and high tumor grade, which concurred with the GBM data available in The Cancer Genome Atlas (TCGA). Taken together, METCAM/MUC18 appears to promote the aggressive phenotypes and hence the tumor progression of glioblastoma U87MG cells [61].

3.5.4 Hepatocellular carcinoma

Hepatocellular carcinoma (HCC) remains the fifth most common malignant cancer and as the third leading cause of cancer-related mortality [62]. High-throughput flow cytometry (HT-FC) profiling was used to characterize the expression of METCAM/MUC18 in the tumor cells from 30 human HCC samples. Increased expression of METCAM/MUC18 expression was significantly increased in hepatocellular carcinoma (HCC) tumor tissues as compared with the matched adjacent normal liver tissues. The METCAM/MUC18+ cells purified from HCC tumors have significantly increased colony-forming capacity, consistent with the characteristics of the cancer stem cells or the tumor-initiating cells, which are considered to contribute to the pathogenesis of HCC [63]. The high expression of METCAM/MUC18 in HCC samples and in HCC cell lines isolated from HCC samples was also confirmed by RT-PCR and Western blot analyses [64]. The HCC cell lines, which stably expressed METCAM/MUC18, had been shown to promote EMT, IL8 upregulation, and STAT1 downregulation, suggesting that METCAM/

MUC18 promotes tumor progression and metastasis and predicts poor prognosis of hepatocellular carcinoma [64].

3.5.5 Non-small cell lung cancer

Lung cancer is the cancer with the highest mortality rate in the world [62], and non-small cell lung cancer (NSCLC) is the cause for about 80% of all lung cancer. The frequency of occurrence of adenocarcinoma, which is one of the major histological subtypes of NSCLC, has recently increased [65]. Eighty-five specimens of NSCLC were immunohistochemically analyzed by using an anti-METCAM/MUC18 monoclonal antibody (clone N1238) on an NSCLC tissue microarray, and the staining was semiquantitatively scored. METCAM/MUC18 has been shown to express in 51% of NSCLC, preferentially squamous cell carcinomas. Positive expression of METCAM/MUC18 has also been associated with a shorter survival of patients with adenocarcinomas and used to predict the poor overall survival in patients with lung adenocarcinomas [65, 66]. Another group also used IHC to show that METCAM/MUC18 expression was more frequently detected in males than in females. The positive expression of METCAM/MUC18 was associated with a poorer 5-year overall survival rate according to the survival analysis, suggesting that METCAM/MUC18 may be a useful marker for predicting poor prognosis in patients with NSCLC following complete resection [65]. The third group reported that METCAM/MUC18 protein expression was found in 46.61% of squamous cell carcinomas and 37.47% of adenocarcinomas. METCAM/MUC18 expression positively correlated with vimentin but inversely with E-cadherin, indicating a positive correlation with EMT. METCAM/MUC18 expression in surgically treated primary tumor NSCLC is clearly associated with lymph node metastasis and is a statistically significant prognostic factor [67]. Consistent with the results and also supporting the conclusion described above, we also showed 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 [68], as shown in **Figure 3**.

Furthermore, the fourth group observed that METCAM/MUC18 expression mediates acquisition of cancer stemness and enhances tumor invasion and metastasis in a mouse model [69–70]. High expression of METCAM/MUC18 correlates with intrapulmonary metastasis of NSCLC cells in a mouse model [69–70]. Taken

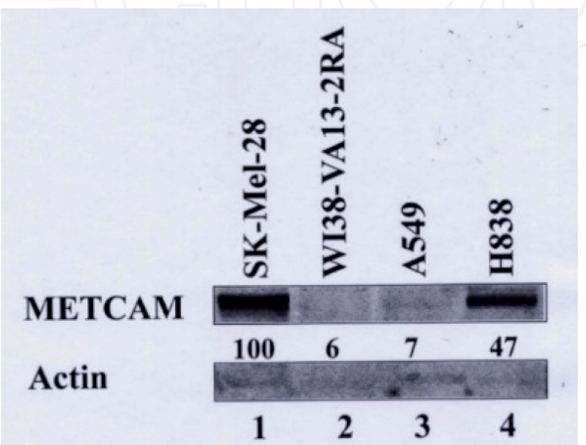

Figure 3.
Expression of METCAM/MUC18 in normal lung tissue (SV40-immortalized normal lung cells) (WI38, lane 2) and lung type II alveolar epithelial cell carcinoma cell (A549, lane 3) and lung primary adenocarcinoma (H838, lane 4) [From [68]].

together, METCAM/MUC18 plays a positive role in the tumor progression and metastasis of NSCLC.

3.5.6 Small cell lung cancer

Small cell lung cancer (SCLC), a lung cancer subtype with an aggressive and highly metastatic nature, is the cause for about 10–20% of lung cancer incidence. The 5-year survival rate is at 7%, still very gloomy because choices of systemic treatment for SCLC patients have not been much increased. SCLC is highly responsive to chemotherapy at the start of treatment. Despite favorable responses to initial therapy, SCLC relapse occurs within a year exhibiting a multidrug-resistant phenotype, which eventually contributes strongly to poor prognosis. Through in-depth proteomic profiling, METCAM/MUC18 was identified as a markedly upregulated surface receptor in chemoresistant SCLC cell lines that exhibited a mesenchymal phenotype as well as in chemoresistant patient-derived xenografts compared to matched treatment-naïve tumors. METCAM/MUC18 knockdown in chemoresistant cells reduced cell proliferation and decreased the IC50 inhibitory concentration of chemotherapeutic drugs. METCAM/MUC18 was found to modulate sensitivity of SCLC cells to chemotherapeutic drugs through upregulation of MRP1/ABCC1 expression and of the PI3/AKT pathway in a SOX2-dependent manner. Metabolomic profiling revealed that METCAM/MUC18 modulates lactate production in chemoresistant cells that exhibit a distinct metabolic phenotype characterized by low oxidative phosphorylation. METCAM/MUC18 may serve as a novel therapeutic target to overcome chemoresistance in SCLC [71]. In summary, the above results point to the positive role played by METCAM/MUC18 in the tumor progression and metastasis of SCLC.

3.5.7 Osteosarcoma

Osteosarcoma is the most common primary malignant bone tumor in children. Clinically evident metastatic disease is present in 10–20% of patients at diagnosis. Despite advancements in multimodality treatment, 5-year survival rates are ~40–50% [72]. METCAM/MUC18 was widely expressed on both osteosarcoma and Ewing's sarcoma cells. METCAM/MUC18 protein and RNA are highly expressed in osteosarcoma cell lines (SaOS, MG-63, U-2OS), but not in normal osteoblast cells [72–73]. ABX-MA1, an anti-METCAM/MUC18 antibody, did not appear to inhibit the in vitro proliferation of osteosarcoma cells, and neither did it significantly inhibit the in vivo growth of KRIB human osteosarcoma cells in the tibias of nude mice. Nevertheless, after 1½ months, a noticeably fewer number of ABX-MA1-treated mice spontaneously developed pulmonary metastatic lesions than the control antibody-treated mice. Furthermore, ABX-MA1 reduced the in vitro invasiveness of osteosarcoma cells in the Matrigel-coated trans-well assay and disturbed the homotypic adhesion among osteosarcoma cells and the heterotypic interaction of them with vascular endothelial cells. Osteosarcoma is effectively treated with anti-METCAM/MUC18 monoclonal antibodies [73–74]. Taken together, METCAM/MUC18 plays a positive role in the metastasis of osteosarcoma [72–74].

3.5.8 Human and mouse pancreatic cancer

Pancreatic ductal adenocarcinoma (PDAC) is the cancer that has the fourth highest mortality rate in the western countries [62]. The 5-year survival rate of all PDAC patients is 6%. Since this tumor is highly aggressive, cancer incidence is almost equivalent to its mortality rate [62]. Most pancreatic cancer deaths are due to metastasis. Especially, the events of tumor spreading are heterogeneous,

Tumor/cancer tissues or cell lines	Tumorigenesis	Metastasis	References
Angiosarcoma human cell lines MS1, SVR	Increasing	Not determined	[40, 59]
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	[60]
Glioma cell lines U87MG, U251	Promotion	Not Determined	[61]
Hepatocellular carcinoma human cell lines PLC/PRF/5, Huh7, MHCC97H and 97L HepG2, SMMC-7721, FOCUS, YY-8103, LM3, HLF, and primary HCC cell lines; normal liver cell line LO2	Promotion	Not determined	[63, 64]
Non-small cell lung cancer human cell lines A549, H23, H358, H460, H522, H838, HCC4006, H1650/ER, PC-9, and PC9GR and adenocarcinoma tissues	Promotion	Promotion	[65–70]
Small cell lung cancer human cell lines H69, H69AR, H82, H196, H209, DMS79	Promotion	Not determined	[71]
Clinical melanoma tissues and human melanoma cell lines SB-2, SK, XP-44	No effect	Increasing and affecting the late stage	[38, 55, 56]
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	[72–74]
Pancreatic cancer human cell lines and mouse cell lines ptf1a, LSL-Kras, LSL-Trp53, Pdx1	Promotion	Possible augmentation	[75, 76]
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	[58]
Prostate adenocarcinoma in TRAMP mice	Increasing	Increasing and affecting initiation in the early stage	[33]

Table 1.
The positive role of METCAM/MUC18 in the tumor progression of various solid tumors/cancers.

thus limiting the therapeutic choices for patients at late stages. METCAM/MUC18 expression in cancer cells is associated with a secretion of soluble METCAM/MUC18 (sMETCAM/MUC18) that plays an active role in tumor development. For example, sMETCAM/MUC18 causes the overproduction of its binding partner, angiomotin, in cancer cells and endothelial cells in the tumor micro-*milieu*,

which augments angiogenesis and proliferation and survival of cancer cells. These are mediated in part by the promotion and activation of c-myc in cancer cells. Dispensation of a new specific monoclonal antibody pinpointing on sMETCAM/MUC18 represses tumor angiogenesis and growth of huMETCAM/MUC18+ pancreatic cancer cell xenografts in mice models. Taken together, sMETCAM/MUC18 secreted by METCAM/MUC18+ tumors exhibit promoting effects on tumor angiogenesis and growth. Thus, an antibody pinpointing on sMETCAM/MUC18 successfully represses vascularization, growth, and survival of METCAM/MUC18-positive pancreatic tumors [75].

The expression of a homeodomain transcription factor MEIS1 (myeloid ecotropic viral integration site) has been associated with a ductal phenotype in pancreatic tissue architecture. To investigate a possible role of MEIS1 in the malignant progression of PDAC, pancreatic cancer cell clones/lines, which overexpress MEIS1, were generated and tested for in vitro proliferation rate and motility. Overexpression of MEIS1 had no effect on in vitro proliferation rate but augmented motility. Furthermore, an upregulation of the MTCAM/MUC18 gene in the migrating cells has been found in the subsequent expression analysis. The interaction of MEIS1 with the enhancer DNA of METCAM/MUC18 is revealed by employing DNA pulldown and chromatin immunoprecipitation (ChIP) assay. Furthermore, the transcriptional activation of METCAM/MUC18 also facilitates migration of pancreatic cancer cells in vitro. Activation of METCAM/MUC18 through MEIS1 occurs in a cell type-dependent fashion, reflecting the different routes that lead to metastasis in vivo. Thus, the transcription factor MEIS1 activates METCAM/MUC18 expression to promote migration of mouse pancreatic tumor cell lines [76].

In summary, the positive role played by the METCAM/MUC18 in the progression of solid tumors has been extended from breast cancer, human and mouse melanoma, and prostate cancer to angiosarcoma [40, 59], gastric cancer [60], glioblastoma [61], hepatocellular carcinoma [63, 64], non-small cell lung adenocarcinoma [65–70], small cell lung cancer [71], osteosarcoma [72–74], human and mouse pancreatic cancer [75, 76], and prostate cancer [32–36, 58]. Taken together, METCAM/MCU18 appears to be more prevalently playing a positive role than a negative role in the tumor formation and/or cancer metastasis of various tumors/cancers. **Table 1** summarizes the positive role of METCAM/MUC18 in the tumor progression of many solid tumors/cancers.

4. METCAM/MUC18: a tumor suppressor and metastasis suppressor in some cancers

In contrast to the positive role played by METCAM/MUC18 in the above cancers, recent results of testing the effects of METCAM/MUC18 expression on tumorigenesis of other cancer types revealed that it also plays a negative role in the tumor progression and metastasis in some cancers, such as colorectal cancer, hemangioma, one mouse melanoma cell line K1735-9, NPC type I, ovarian cancer, human pancreatic cancer, and one human prostate cancer cell line PC-3, as described next.

4.1 Colorectal cancer

Colorectal cancer (CRC) is the third leading cause of cancer deaths in recent years [62]. Cancer stemness contributes to carcinogenesis, tumor relapse, and chemoresistance in traditional cancer therapeutics ([77] for a review). Stemness, which is the cell state with the properties of self-renewal, differentiation, and tumor-initiating potential, might be characterized by a set of more dynamic features

influenced by the nature of the microenvironment. Various extrinsic cues and intrinsic signaling pathways, such as Wnt, Notch, and Hedgehog signals, are involved in the maintenance of stemness. Since METCAM/MUC18 has also been identified as a pluripotent marker for mesenchymal stem cells (MSCs), it was also hypothesized to exert potential effects on cancer cell stemness. One group of investigators has provided evidence to demonstrate that reduced expression of METCAM/MUC18 actually functions as a positive regulator of stem cell properties in colorectal cancer through augmenting the Wnt/β-catenin signaling pathway. METCAM/MUC18 may actually manifest multifaceted effects on tumor progression in a context-dependent manner. The above evidence suggests that METCAM/MUC18 expression suppresses the tumor progression and metastasis of colorectal cancer [77].

4.2 Hemangioma

The expression of METCAM/M/MUC18 in hemangioma is inversely proportional to the progression of hemangioma, suggesting that METCAM/MUC18 plays a negative role in the progression of hemangioma [46].

4.3 Mouse melanoma

As shown in the above section, moMETCAM/MUC18 does not have an obvious effect on the tumorigenesis of the two K1735 cell lines, such as K1735-3 and K1735-10 [21]. In contrast, moMETCAM/MUC18 definitely acts as a tumor suppressor for the K1735-9 cell line. Overexpression of moMETCAM/MUC18 in K1735-9 also completely suppressed lung nodule formation in immunocompetent syngeneic C3H brown mouse model [22]. Thus, METCAM/MUC18 expression suppresses the tumor progression and metastasis of the mouse melanoma K1735-9 cell line [22].

4.4 Nasopharyngeal carcinoma (NPC)

According to the IHC results as shown in the above section, we suggested a hypothesis that METCAM/MUC18 may play a tumor suppressor function in the development of NPC during the progression of the cancer [23]. Then, we further tested the hypothesis by examining the effect of METCAM/MUC18 overexpression on in vitro cellular behavior and in vivo tumorigenesis of the two NPC cell lines in athymic nude mice. When the METCAM/MUC18-overexpressing NPC-TW01 clones/cells, which were established from NPC type I, were used for the animal test, indeed tumor suppression was observed [24, 25]. However, the opposite results were obtained when the METCAM/MUC18-overexpressing NPC-TW04 clones/cells, which were established from NPC type III, were used for the similar tests. Thus, METCAM/MUC18 plays a dual role in the tumor progression of NPC [23, 24–26].

4.5 Ovarian cancer

Two independent groups showed that METCAM/MUC18 expression is correlated with the progression of ovarian cancer [27, 78] and it affects in vitro behaviors of ovarian carcinoma cells [79]; however, the role of METCAM/MUC18 in the progression of epithelial ovarian cancer has not been directly tested in animal models. For this purpose, we initiated testing the effect of METCAM/MUC18 overexpression 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 the in vitro and in vivo tests. We observed that overexpression of METCAM/MUC18 reduced in vitro motility and

invasiveness [28] and suppressed in vivo tumorigenesis on subcutaneous (SC) sites and in intraperitoneal cavity as well as in vivo malignant progression of the human ovarian cancer cell line SK-OV-3 in intraperitoneal (IP) cavity in female athymic nude mice [28]. When the other human ovarian cancer cell line, BG-1, was similarly tested, similar results were also observed [80]. 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 [28–30, 80], suggesting that METCAM/MUC18 is a strong candidate as a new tumor and metastasis suppressor in human ovarian cancer cells.

4.6 Human pancreatic cancer

In contrast to the above results of human and mouse pancreatic cancer that METCAM/MUC18 expression plays a positive role in the malignant progression of PDAC, a group has demonstrated that METCAM/MUC18 expression in cancer-associated fibroblasts (CAFs) has been correlated with the pre-pancreatic intraepithelial neoplasia (PIN) and the invasive ductal pancreatic cancer with a low histological grade. Furthermore, the prognosis for the patients with a low METCAM/MUC18 expression is poorer than those with a high METCAM/MUC18 expression. Suppressing METCAM/MUC18 expression in CAFs augmented tumor cell in vitro motility and invasiveness in a co-culture system that includes both tumor cells and CAFs. Knockdown of METCAM/MUC18 also augmented CAF activation, possibly via regulation of NF-kB activity, which in turn induces the yield of factors for tumorigenesis. In line with this notion, METCAM/MUC18 overexpression in CAFs decreased in vitro motility and invasiveness of the cancer cells co-cultured with CAFs. Moreover, METCAM/MUC18 expression in CAFs was decreased by interaction with cancer cells. Taken together, reduced METCAM/MUC18 expression in CAFs and reduction of METCAM/MUC18 augment tumor progression of pancreatic cancer [81]. Therefore, METCAM/MUC18 expression suppresses the tumor progression and metastasis of pancreatic cancer. In comparison with the results from Section 3.5.8, METCAM/MUC18 expression also plays a dual role in pancreatic cancer.

4.7 Prostate cancer

We recently used the knocking down strategy similar to that of DU145 cell line to test the effect of decreased 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, suggesting that expression of METCAM/MUC18 suppressed the tumorigenesis of the human prostate cancer cell line PC-3 [82]. We thus conclude that METCAM/MUC18 serves as a tumor suppressor in the PC-3 cell line. Thus, similar to mouse melanoma, NPC, and pancreatic cancer, METCAM/MUC18 expression also plays a dual role in tumor progression and metastasis in human prostate cancer.

In summary, METCAM/MUC18 may also suppress tumor progression and metastasis of the following solid tumors, such as colorectal cancer [77], mouse melanoma K1735-9 subline [22], NPC type I [24, 25], ovarian cancer [28–30], human pancreatic cancer [81], one prostate cancer cell line PC-3 [82], and perhaps hemangioma [46]. Thus, METCAM/MUC18 appears to play a negative role in tumor progression and metastasis of some solid tumors but a dual role in some other solid tumors. It is not clear why METCAM/MUC18 plays a dual role. 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 cofactors or heterophilic ligands by using immunological coprecipitation method in the future studies, which is feasible as described in Section 5.5.

5. Preliminary and possible 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, 55, 56], another group on the role of huMETCAM/MUC18 in the biology of endothelial cells [41, 83], and one group on breast cancer [45], 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] and later breast cancer [18–20], ovarian cancer [27–30], and NPC [23–26], as described above. Recently, more research groups have participated in further exploring the possible role of METCAM/MUC18 in other solid tumors in different organs, such as the colorectum [77], gastro-organ [60], glial cells [61], liver [63, 64], lung [65–71], bone [72–74], and pancreas [75, 76, 81]. Furthermore, preliminary work in leiomyosarcoma, esophagus squamous cell carcinoma, clear cell renal sarcoma, and gallbladder adenocarcinoma are also beginning to emerge [47]. Thus, after decades of group effort, we are beginning to understand the biology of METCAM/MUC18-mediated tumor progression.

However, we still know very little how METCAM/MUC18 mediates or regulates tumor progression and metastasis of cancer cells. Thus, the biological mechanisms describing the role of METCAM/MUC18 in tumorigenesis and malignant progression are still not well clarified. By deducing knowledge learned from the tumorigenesis of other tumors [6, 17, 37, 40] and angiogenesis [41, 83], we may be able to find some common clues to begin understanding its mechanisms. As such, the following five important aspects are much needed for immediate future studies, such as differential regulation at the transcription level in tumors of different organs; different signaling pathways involved; contributions of different domains of the protein; possible different extent of N-glycosylation in different cancer cell lines, which may critically modulate the function of METCAM/MUC18 in tumor progression; and different kinds or quantities of cofactors or heterophilic ligand(s) in different cancer cell lines.

5.1 Transcriptional regulation

The mechanism of transcriptional control of METCAM/MUC18 gene is minimally studied [17]. So far, only 900 bp of the core promoter region of the huMETCAM/MUC18 gene are sequenced [84]. The core promoter reflects a typical housekeeping gene, which is rich in GC sequences but does not contain a TATA box. Nevertheless, it includes many consensus sequences presumably as putative binding sites for various transcription regulatory factors, such as SP-1, CREB [85], AP-2 [86–87], c-Myb [88], N-Oct2 (Brn2) [89], Ets [90], CArG [91], and Egr-1 [92], and three insulin-responsive elements (one Ets and two E-box motifs) [93], suggesting that transcriptional control of the huMETCAM/MUC18 gene is regulated by various growth signals [37, 40]. For example, the huMETCAM/MUC18 gene is positively regulated by PKA/CREB (cAMP-responsive element binding protein) and negatively regulated by AP-2α [94]. Having a longer DNA containing sequences for tissue-specific expression of the gene is essential for further understanding the roles

of other regulators [17]. In line with this hypothesis, recently, the Ets sequence in the 10 kilo-bp upstream region has been shown to regulate the expression of huMETCAM/MUC18 gene [95]. We have also engaged in this task by screening in a phage library containing the human genomic sequences and obtained several phage clones which contain at least 4 kilo-bp of in the upstream region of the promoter region of the gene for future studies [96]. Thus, the regulatory mechanism of tissue-specific expression of the METCAMMUC18 gene may be forthcoming.

The epigenetic control of the expression of huMETCAM/MUC18 gene has been implicated in human cancers, because huMETCAM/MUC18 gene is located at the locus of human chromosome 11q23.3 [97] that has been shown to be hypermethylated in NPC [98], suggesting that the expression of this gene may be regulated by epigenetic controls. 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 [99]. METCAM/MUC18 has also been shown to be methylated in the early stage of most prostate cancer [100]. Thus, it is highly possible that the gene is epigenetically controlled in other cancers.

5.2 Signaling pathways

The cytoplasmic tail of huMETCAM/MUC18 contains consensus sequences potentially to be phosphorylated by PKA, PKC, and CK2, suggesting that its functions may be mediated by these protein kinases and regulated by cross talk with various signaling pathways [17, 37, 38, 40, 42]. First, it is necessary to biochemically prove how many sites are actually phosphorylated in the cytoplasmic tail of the METCAM/MUC18 protein purified from different cancer cell lines and which protein kinase is responsible for the phosphorylation. After this is answered, then we can further study how METCAM/MUC18 mediates cross talk and networking with different signal pathways and is to be compared with the cytoplasmic tails of other CAMs [6, 8, 41]. Knowledge learned from the impact of other CAMs on tumor progression suggests that METCAM/MUC18, as an integral membrane Ig-like CAM, should mediate inside-in, inside-out, and outside-in signals to participate in intercellular communication and interaction of cell with the extracellular matrix, which results in impacting EMT [6, 8, 41, 101]. Furthermore, huMETCAM/MUC18 has been shown to express in normal mesenchymal cells (smooth muscle, endothelium, and Schwann cells) in the tissue stroma and be a marker for the mesenchymal stem cells [102]; thus, expression of METCAM/MUC18 may augment the EMT of cancer cells and hence the progression of many cancers. Moreover, METCAM/MUC18 may affect cancer cell progression by cross talk with signaling pathways that affect apoptosis, survival and proliferation, angiogenesis, and energy metabolism of tumor cells [6, 8, 101]. This is indeed found in our preliminary mechanical studies in breast cancer [19, 20], melanoma [21, 22], NPC [24–26], ovarian cancer [28–30], and prostate cancer [34–37]. Further systematic studies by using specific RNAi's to knockdown the downstream effectors one by one in the METCAM/MUC18-expressing clones may be necessary to further understand this aspect of mechanism. Moreover, its interaction with cofactors 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. Finally, METCAM/MUC18 may interact with various hormonal receptors, growth or anti-growth factors/receptors, various chemokines/receptors, and the Ca++-mediated signaling members and affect tumor progression [17].

HuMETCAM/MUC18 expression in melanoma cells is reciprocally regulated by AKT, in which AKT upregulates the level huMETCAM/MUC18 and overexpression of huMETCAM/MUC18 activates endogenous AKT, which in turn inhibits apoptosis and increases survival ability [103]. A similar mechanism is also likely to be used in other cancers; however, the detailed mechanism of how AKT upregulates the expression of METCAM/MUC18 in most cancers has not been reported. After the cytoplasmic tail is phosphorylated, then it may facilitate its interaction with FAK, thus promoting cytoskeleton remodeling, which in turn augments tumor cell motility and invasiveness [83]. Alternatively, after phosphorylation, huMETCAM/MUC18 may interact with the downstream effectors of Ras, activating ERK and JNK, which in turn may transcriptionally activate the expression of AKT or other genes that promote the proliferation and angiogenesis of tumor cells. Moreover, by predicting from the relatively less selectivity of CK2 for its substrate and many CAMs which are phosphorylated by CK2, such as CD44, E-cadherin, and L1-CAM, and one of the integrin receptors in the extracellular matrix protein, vitronectin [104], huMETCAM/MUC18 is very likely to be phosphorylated by CK2 and linked to AKT to affect the proliferation, survival, and other tumorigenesis-related functions [105]. Recent findings appear to support this mechanism in that METCAM/MUC18 may promote EMT of breast cancer cells via activation of RhoA and upregulation of slug [45]. HuMETCAM/MUC18 may play an important role in regulating tumor dormancy or awakening, driving or preventing cancer cells to pre-metastatic niche, and formatting a microenvironment for favorable or unfavorable tumor growth in secondary sites [17, 37].

HuMETCAM/MUC18 may mediate hematogenous spreading of melanoma cells, as implicated by its expression in endothelial cells and malignant melanoma cells [106] and presence in junctions of endothelial cells [107, 108], essential for tumor angiogenesis in three tumor cell lines [109] and human prostate cancer LNCaP cells [110], and it is highly likely for that in other cancers [111, 112]. HuMETCAM/MUC18 may also be implicated in promoting lymphatic metastasis of cancer cells, since it is one of the lymphatic metastasis-associated genes, which are upregulated in malignant mouse hepatocellular carcinoma [113]. However, the detailed mechanisms of huMETCAM/MUC18-mediated hematogenous and lymphatic spreading of cancer cells remain to be investigated. For this purpose, labeling cells with viable dyes and employing a newly developed non-intruding, but highly photo-penetrating imaging photoacoustic tomography (PAT) to monitor each step of the process in real time in hairless nude mice may be helpful to provide some answers [114].

HuMETCAM/MUC18 may interact with the host immune system and affect tumor progression, though the immune system may have a contradictory role in the process [115]. This notion is positively supported by a recent finding that a subset of host B lymphocytes may be implicated in regulating melanoma malignant progression via interaction with huMETCAM/MUC18 [116]. Our syngeneic mouse system for mouse melanoma should be useful for exploring the role of immune T and B cells in the progression of METCAM/MUC18-expressing melanoma cells. However, the role of B and T cells in the progression of most human cancer cells may not be explored in the athymic nude mouse models since most human cancer cells can only grow as xenografts in these immunodeficient mouse models. Nevertheless, to investigate the effect of huMETCAM/MUC18 expression on mediating NK cells in metastasis may be possible in these nude mouse models, which can be tested by pretreatment of nude mice with anti-NK surface marker antibodies to deplete the NK cells prior to injection of the huMETCAM/MUC18-expressing cancer cells. This possibility is supported by the finding that the surface huMETCAM/MUC18 expressed in cancer cells may have a homophilic interaction with the NK cells, which also express huMETCAM/MUC18 and enhance cytotoxic functions of NK cells [117].

5.3 Functional domain

To begin addressing the relation of the protein structure of huMETCAM/MUC18 to its functions in tumorigenesis and metastasis, we have generated mutant-deleted different domains of huMETCAM/MUC18 by using a special PCR method [118] and used them to determine their contribution to tumorigenesis. Surprisingly, our preliminary results showed that the ecto-domain and the intact copy of huMETCAM/MUC18 cDNA equally efficiently induced tumorigenesis in LNCaP cells in nude mice, suggesting the key role of the ecto-domain in inducing tumorigenesis of prostate cancer cells in vivo. However, this stirs up a puzzling question that the cytoplasmic domain was not essential for this process [119]. Nevertheless, critical direct test of using only the cytoplasmic domain for inducing tumor should be performed. It is essential that a systematic study has also to be performed in other cancer cell lines before a definitive conclusion can be drawn.

5.4 Glycosylation

Malignant progression of cancer cells has been shown to associate with an abnormal glycosylation, resulting in expression of altered carbohydrate determinants [120]. Thus, the glycosylated status of huMETCAM/MUC18 in different cancer types may be different from normal cells and may manifest either a positive or negative effect on the progression of different cancer types, which should be very intriguing since huMETCAM/MUC18 possesses six conserved N-glycosylation sites in the extracellular domain [17, 37, 40].

Glycosylation of a protein may affect the proper folding, stability, and/or activity of a protein [121]. Both huMETCAM/MUC18 and moMETCAM/MUC18 are very likely heavily glycosylated, sialylated, and/or posttranslationally modified, because both have an apparent molecular weight of about 110–150 kDa, in comparison with the naked protein with a molecular weight of about 65–70 kDa [122]. The possible roles of METCAM/MUC18 glycosylation in inducing/promoting or suppressing the metastasis of cancer cells should be explored [123]. To initiate the study, we subjected the huMETCAM/MUC18, which was isolated from one human cancer cell line, to the digestion with N-glycosidase F, neuraminidase (sialidase), O-glycosidase, or endoglycosidase H. 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], suggesting 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. Our hypothesis is supported by a recent report that described GCNT3 as an upstream regulator of METCAM/MUC18 in that it glycosylates METCAM/MUC18 and extends its half-life which results in further elevation of S100A8/A9-mediated cellular motility in melanoma cells [124]. The role of glycosylation in the six N-glycosylation sites should be genetically altered to explore their effects on the functions of METCAM/MUC18 in tumor progression and metastasis.

5.5 Heterophilic ligands and cofactors that modulate the function of METCAM/MUC18

Since METCAM/MUC18 only plays a dual role in different cell lines from the same type of cancer or in different types 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 cofactors or heterophilic ligands that may positively or negatively modulate the METCAM/MUC18-mediated tumor progression and metastasis. This can be readily scrutinized by identifying these specific intrinsic cofactors or heterophilic ligands in different cancer cell lines by using immunological coprecipitation method. This approach appears to be feasible as shown in our preliminary result in that a putative heterophilic ligand, 72 kDa protein, is identified [17, 37, 40]. This protein is present at a higher concentration in PC-3 cells than in DU145 that may be responsible for an opposite role of METCAM/MUC18 in tumor progression of these two cell lines. Thus, it is possible that mechanisms of huMETCAM/MUC18-mediated cancer progression may be different in different cancer cell lines due to their different intrinsic properties, which possess different concentration or completely different heterophilic different ligands and/or cofactors. The heterophilic ligands and/or cofactors of METCAM/MUC18 may contribute to the cellular intrinsic properties, such as adhesion-associated signaling cascades and cytoskeleton rearrangement, leading to different EMT of these cells and modulating the huMETCAM/MUC18-mediated tumor progression and metastasis. Different intrinsic cofactors in different cancer cell lines may modulate METCAM/MUC18 and alter cell-to-cell and cell-extracellular matrix interactions in the tumor microenvironment, resulting in affecting tumor progression and metastasis in vivo. Finally, these cofactors/ligands may interact differently with METCAM/MUC18 in different cell lines and affect other host physiological factors, which may augment or suppress in vivo tumor progression and metastasis by affecting metabolic switch, pro-apoptosis/anti-apoptosis, tumor angiogenesis, and host immune system in the tumor micro-*milieu* and in various metastatic sites [17, 37, 40, 111, 112]. Thus, the identification of the cofactors and the huMETCAM/MUC18-cognate heterophilic ligand(s) is critical for understanding the mechanism.

6. Conclusions

METCAM/MUC18 also plays a key positive function in the progression of angiosarcoma, breast cancer, gastric cancer, glioblastoma, hepatocellular carcinoma, lung cancer, melanoma, NPC type III, osteosarcoma, pancreatic cancer, prostate cancer, and possibly other cancers. On the other hand, 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. To further understand its role in these processes, it is essential to further identify its cofactor regulators and cognate heterophilic ligands, define its functional domains, and study its cross talk 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 foundation for future intriguing investigation to further understand the detailed mechanism of METCAM/MUC18-mediated tumor progression and metastasis of various cancer cell lines. For this purpose besides those have been described above, other future endeavors may include (a) understanding the mechanisms in the METCAM/MUC18-mediated tumor progression and metastasis, such as intrinsic growth capability, key chemokines and cytokines

participating in the evasion of immunological responses, and key pro-angiogenic and anti-angiogenic factors participating in the augmentation of angiogenesis, (b) identification of possible miRNAs and noncoding RNAs participating in the process upstream and downstream of METCAM/MUC18 [126], and (c) possible clinical applications that should be explored. Precaution should be taken that a thorough picture may be possibly revealed only after the comprehensive studies are successfully executed.

7.2 Clinical applications

Four major approaches may be taken to decrease or stop the progression and metastatic propensity of cancer cells and keep them staying at the primary site, stopping them in a dormant state or keeping the disseminating cancer cells at the state of micrometastases: (a) Dispense humanized anti-METCAM/MUC18 antibodies to the cancer patients [125]. (b) Knocking down the METCAM/MUC18 expression by siRNAs to silence the genes [35, 36]. For knocking down therapy, the METCAM/MUC18 gene-specific siRNAs may be delivered by liposomes or other delivery methods [126]. (c) Target at downstream key members in the signaling pathways which are activated by the promotion. (d) Target at the cofactors or the cognate heterophilic ligand(s) of METCAM/MUC18. The above strategies may be used in single or better in combination for treating the patients. However, the dual role of METCAM/MUC18 in cancer progression may limit the above clinical applications to only cancers exhibiting a positive METCAM/MUC18-mediated tumor progression and metastasis.

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Conflict of interest

The author has no conflict of interests.

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