Objectives: Mucoepidermoid carcinoma (MEC) accounts for 35% of all malignant salivary gland tumors. Previous investigations have shown that vasculogenic mimicry (VM) exists in many cancers which can be used as a prognostic factor of poor prognosis. Elevated expression level of vascular endothelial (VE)-cadherin has been implicated in cancer neovascularization, growth, and progression. The current study aimed to study the presence of VE-cadherin in VM channels and tumor cells in different grades of MEC.

Materials and Methods: A total of 63 MEC samples (21 samples in each grade) were collected from the archive of pathology department of Besat Educational Hospital, Hamadan, Iran, from 2002 to 2016. Hematoxylin and eosin staining was performed to confirm the previous diagnosis. The specimens were then processed for immunohistochemistry analysis. Then, periodic acid–Schiff staining was performed. Analyses were conducted through SPSS software version 22.0 (SPSS, Inc., Chicago, IL, USA). Chi-square test was used to examine the differences between categorical variables. Significance level was set at 0.05. Pearson’s correlation was used to assess the co-localization of the marker.

Results: A total of 63 samples (35 men; 55.6%, and 28 women; 44.4%) were used for immunohistochemical study. There were statistically significant differences between tumor grade and the expression levels of VE-cadherin ($P = 0.000$), between tumor grade and VM formation ($P = 0.000$), and also between tumor grade and microvessel density (MVD) ($P = 0.000$). Additionally, there was a strong positive correlation between tumor grade and VE-cadherin expression level (Pearson’s $r = 0.875$, $P < 0.000$).

Conclusions: Our results may disclose a definite relationship between VE-cadherin expression level, VM, epithelial–mesenchymal transition, cancer stem cells, and MVD in MEC samples. Thus, it is reasonable to suggest that VE-cadherin is related to angiogenesis and VM formation in MECs.

Keywords: Mucoepidermoid carcinoma, salivary gland tumors, vasculogenic mimicry, vascular endothelial-cadherin

Introduction

Malignant salivary gland tumors make up <5% of all head and neck malignancies. Mucoepidermoid carcinoma (MEC) accounts for 35% of all malignant salivary gland tumors.[1,2] The origin of MEC is not clear. Some investigators suggested the myoepithelial cells, excretory or the intercalated duct cells, or intermediate cells as the origin of MEC.[3] Regarding the histopathology, MECs are classified as low, intermediate, and high grade according to the relative number of epidermoid, mucous, and intermediate cell types, growth pattern, type of invasion, and cytological atypia.[2,4-6]

In 1971, Folkman first proposed a theory regarding tumor angiogenesis. According to his hypothesis, a
tumor produces its own new vasculature from the existing blood vessels. Although several studies have indicated that tumor blood vessels develop from endothelial cells, a growing body of evidence has proved that some cancer blood vessels are not lined by endothelial cells.[7] Maniotis et al. found that the aggressive melanoma cells form vascular-like channels which function as tumor blood vessels to supply nutrition. This phenomenon was called “vasculogenic mimicry” (VM).[8] VM formation enhances tumor growth and cancer metastasis.[7] Previous investigations have shown that VM exists in many cancers such as oral squamous cell carcinoma (OSCC) which can be used as a prognostic factor of poor prognosis.[9] VM is positive for the periodic acid–Schiff (PAS) reaction.[7] Vascular endothelial-cadherin (VE-cadherin) is an adhesive protein which belongs to the cadherin family of transmembrane proteins and promotes cell-to-cell interaction. Recently, VE-cadherin has been shown to be expressed by both endothelial cells and highly aggressive melanoma cells.[10] VE-cadherin is an important gene for both VM and endothelial-lined vessels. Elevated expression level of VE-cadherin has been implicated in the cancer neovascularization, growth, and progression.[11] Indeed, the cancer cells lining the VM vessels secrete matrix metalloproteinases and express VE-cadherin and laminin to promote the formation of VM.[12] The ability of cancer cells to find alternative growth signaling pathways also needs to be considered. Therefore, the inhibition of angiogenesis has become a new strategy for anticancer therapy. Recently, anti-tumor angiogenic therapies have been challenged. Thus, new drugs are needed. A previous study on esophageal cancer found that VM formation can be inhibited by targeting VE-cadherin.[13]

The role of VE-cadherin is not clear in VM formation and cancer development in MEC. The current study aimed to study the presence of VE-cadherin in VM channels and tumor cells in different grades of MEC.

**Materials and Methods**

PASS software (Power Analysis and Sample Size) software (version 11.0.7; PASS, NCSS, LLC) was used to calculate the sample size using the following information:

DF = 4, effect size = 0.5, power (1–β) = 0.9, and alpha (significance level) = 0.05.

A total of 63 MEC samples (21 samples in each grade) were collected from the archive of Pathology Department of Besat Educational Hospital, Hamadan, Iran, from 2002 to 2016.

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There were 30 cases from the parotid gland, 20 cases from submandibular gland, and 13 cases from minor salivary glands. Adjacent normal salivary gland tissue (from parotid, submandibular, and minor salivary glands) served as the control group. Hematoxylin and eosin staining was performed to confirm the previous diagnosis. MECs were classified as low, intermediate, and high grade on the basis of presence of cystic spaces, proportion of mucous cells, growth pattern, type of invasion, and cytological atypia.[4]

**Detection and Scoring**

VE-cadherin expression was detected in the membrane of the tumor cells. The abundance of positive cells was graded as follows: 1 (weak) for <20% positive cells, 2 (moderate) for 20%–50% positive cells, and 3 (strong) for >50% positive cells.[14] VM was identified by the detection of PAS-positive loops surrounded by tumor cells (not endothelial cells), with or without red blood cells in it. Microvessel density (MVD) was determined by the light microscopy examination of stained sections at the “hot spot.” Fields of the greatest neovascularization were identified by the light microscope at low power (×100). The average vessel count of the five fields (×400) was regarded as the MVD. MVD was classified as either high (≥15.0) or low (<15.0); 15.0 was considered as the median value.

**Statistical Analysis**

Analyses were conducted through SPSS software version 22.0 (SPSS, Inc., Chicago, IL, USA). Chi-square test was used to examine the differences between categorical variables. The significance level was set
at 0.05. Pearson’s correlation was used to assess the co-localization of the marker.

RESULTS

A total of 63 samples (35 men; 55.6%, and 28 women; 44.4%) were used for immunohistochemical study. Age ranged from 20 to 70 years, with a mean age of 50.3 years. There were statistically significant differences between tumor grade and the expression levels of VE-cadherin ($P = 0.000$), between tumor grade and VM formation ($P = 0.000$), and also between tumor grade and MVD count ($P = 0.000$). Additionally, there was a strong positive correlation between the tumor grade and VE-cadherin expression level (Pearson’s $r = 0.875$, $P < 0.000$).

The details are summarized in Table 1.

DISCUSSION

In this study, the expression level of VE-cadherin was examined in the normal salivary gland tissue and in different histological grades of MEC. The present study demonstrated that the high-grade tumors [Figure 1] showed higher VE-cadherin expression level compared to low-grade [Figure 2] and intermediate-grade samples [Figure 3]. This result is consistent with that of a previous report. Our study showed the strong VE-cadherin positivity in 31 cases (41.9%); 10 cases of intermediate-grade tumors and all cases of high-grade tumors. A previous study on aggressive melanoma showed the high expression of VE-cadherin by tumor cells. The authors proposed that VE-cadherin expression by tumor cells results in their abilities to mimic endothelial cells and form vasculogenic-like networks. Thus, it can be proposed that VE-cadherin expression is correlated to the formation of VM channels in highly aggressive tumors and tumor plasticity allows VM to occur. It has been suggested that cancer cells migrate through the endothelium. Four methods have been proposed for migration. First, cancer cells migrate via the endothelial cell body. Second, cancer cells induce endothelial cell apoptosis. Third, cancer cells migrate through endothelial cell membrane staining of tumor cells around the cystic space (×400).
cell–cell junction without permanently destroying endothelial cell layer. Finally, the very recent hypothesis, which suggests that cancer cells push the endothelial cells to deeper extracellular matrix to migrate. Apparently, cancer cell migration is a complex process which requires more studies.[16] VE-cadherin, expressed by endothelial cells at cell–cell junction, is one of the key adhesion molecules and the integrity of the endothelium is dependent on them. These findings indicate that cancer cells affect VE-cadherin at the early stage of transmigration, but they may provide an easier route for migration. For instance, a previous study on breast cancer demonstrated that cancer cells become a part of endothelium. This phenomenon is called “incorporation.” It starts by creating a small hole in the VE-cadherin molecule between endothelial cells, becoming much larger by the time. Then, cancer cells are exposed to the fibronectin-coated matrix. During incorporation, VE-cadherin is not expressed by endothelial cells along borders with cancer cells, but the neighboring endothelial cells continue to express VE-cadherin. It means that the endothelium may detach and/or displace by cancer cells during incorporation.[16] Elevated expression of VE-cadherin in cancers such as melanoma and breast cancer is associated with a poor prognosis.[17] In the present study, strong expression level of VE-cadherin was also indicated in high- and intermediate-grade samples. In a published work, Fry et al. found an increased expression level of VE-cadherin in serum samples of breast cancer. The authors suggested VE-cadherin as a metastatic biomarker in breast cancer.[18] The expression of VE-cadherin and Ephrin type-A receptor 2 by aggressive melanoma cells may serve as a vasculogenic switch.[19] In triple-negative breast cancer, CD133+ cancer stem cells (CSCs) expressed a higher level of VE-cadherin compared with CD133- cells.[20] It has been suggested that CD133+ CSCs might gain endothelial cell phenotype and express VE-cadherin to promote VM formation.[21] Besides, another publication has shown an elevated expression of VE-cadherin in lung cancer cells related to increased angiogenesis and metastasis.[22] We also found that the cancer cells at the invasive front were positive for VE-cadherin [Figure 4]. In addition, VE-cadherin positivity was found in the detached cells, especially around the vessels [Figure 5]. Accumulated evidence has indicated that the epithelial–mesenchymal transition (EMT) is closely correlated with CSCs, and tumor cells which have the ability of undergoing EMT also have the characteristics of CSCs. A growing body of evidence found the presence of CSCs predominantly at the tumor–host interface which had acquired EMT phenotype as well as stemness. Furthermore, other reports have demonstrated that EMT is sufficient to

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**Figure 3:** Paraffin section of intermediate-grade tumor. The high-power magnification view shows strong cell membrane staining of tumor cells.

**Figure 4:** Immunohistochemical analysis indicating significantly increased expression level of vascular endothelial-cadherin at invasive front. Black arrow indicates the tumor–stromal interface (×400)

**Figure 5:** High-power photomicrograph from high-grade mucoepidermoid carcinoma. Note the cell membrane positivity in detached tumor cells, especially around the vessels.
provoke a cell population with stem cell characteristics.[23] CSCs can be found in specialized areas known as the “niche.” In some tissues such as brain, CSCs aggregate in the perivascular areas which are called “perivascular niche.”[24] The present study may indicate cancer cells which gained the characteristics of EMT and CSCs can express VE-cadherin. Besides, our study may confirm the invasive front and perivascular areas as the “niche” in MECs.

Angiogenesis is a hallmark of cancer, and VM is another way to supply oxygen and nutrition to the cancer cells. VM vessels are lined by tumor cells and do not require endothelial cells.[25] Previous studies have shown that VM has a crucial role in the tumor progression and metastasis. For instance, aggressive melanoma contains a lot of tumor cell-lined vasculatures.[26] In our series, VM was present in 9/21 (42.9%) of intermediate-grade samples and 14/21 (66.7%) of high-grade samples (altogether 23/63; 36.5%), which were significantly associated with tumor grade [Figures 6 and 7]. Additionally, all cancer cells lined by VM channels expressed VE-cadherin [Figures 8 and 9]. VM has been shown to present in 21/84 (25%) of gastrointestinal stromal tumors, which were significantly associated with tumor grade and liver metastasis.[27] In another study on OSCC, tumor cell-lined vessels were found in 18/33 (54.5%) of cases.[9] In addition, VM formation was found in 40% of adenoid cystic carcinoma (ACC) samples, mainly in solid pattern.[21] More VM formation in the solid type may express the worst prognosis of the solid subtype of ACC. In a study on 99 cases of hepatocellular carcinoma, VM formation was observed in 12 cases (12%). In this study, a positive correlation was indicated between VM rate and histopathology grade.[28] Hypoxia has an important role in VM. Cancer cells that form VM channels can express VE-cadherin which is an important

Figure 6: Close section of intermediate-grade tumor showing vascular channel lined by tumor cells (yellow arrow) (H and E)

Figure 7: High-power section of high-grade tumor demonstrating vascular channels. Yellow arrows indicate tumor cells (H and E)

Figure 8: High-power photomicrograph of intermediate-grade sample demonstrating vascular channels lined by vascular endothelial-cadherin-positive tumor cells (yellow arrows)

Figure 9: High-power histopathologic presentation of high-grade tumor showing multiple vascular channels lined (yellow arrow) by vascular endothelial-cadherin-positive tumor cells
VE-cadherin expression is regulated by hypoxia-inducible factors under hypoxic conditions. Bel-2 also plays a critical role in angiogenesis in hypoxic conditions. VE-cadherin expression is regulated by Bel-2. In cancers such as melanoma, the overexpression of Bel-2 results in elevated VE-cadherin expression and VM formation. Importantly, a published work found that CD133+ phenotype was positively associated with VM in ACC specimens. These data indicated that CD133+ CSCs might promote migration and invasion via VM process. In triple-negative breast cancer, the expression of VE-cadherin in CD133+ CSCs was significantly higher than that in CD133- cells. The authors suggested that CD133+ CSCs might have the capacity of acquisition of endothelial cell phenotype and VE-cadherin expression to promote VM formation.

MVD may give useful information about tumor behavior. In the present study, MVD count was higher in intermediate- and high-grade samples compared to that of low-grade cases. Similar to our study, previous studies on prostate cancer found a significant association between microvessel count and tumor grade. MVD was correlated significantly with the clinical stage, vascular invasion, and metastasis in patients with ACC. Previous reports found intense angiogenesis at the periphery of malignant salivary gland tumors. Besides, a moderate vascular endothelial growth factor (VEGF)-positive staining was found in low-grade MECS while intensity was increased in high-grade MECS. In this study, the VEGF expression was mainly observed in epidermoid and intermediate cells and was mild/absent in mucous cells. This study also found a positive association between histologic grade of MEC tumor samples and the expression level of caveolin-1. In our study, the high counts of MVD were determined in intermediate- and high-grade tumors. In addition, intratumoral MVD was high in all tumor grades. These findings explain the role of angiogenesis in the tumor progression, invasion, and metastasis.

Conclusions

Our results may disclose a definite relationship between VE-cadherin expression level, VM, EMT, CSCs, and MVC in MEC samples. Thus, it is reasonable to suggest that VE-cadherin is related to angiogenesis and VM formation in MECS. Taken together, it could be demonstrated that MECs contain the CSCs since they share the same markers in the tumor cells, in the stroma, and at the invasive front of the tumor. Detection of VE-cadherin, to some extent, has a significantly increased value for determining MEC prognosis. While VE-cadherin plays an important role in the control of angiogenesis, it is not the only gene that is involved in the control of angiogenesis. Although we have identified a link between VE-cadherin expression and MEC aggression, more research is needed to determine whether the expression level of VE-cadherin in MEC could serve as prognostic markers or an indicator of response to therapy. Besides, our results profoundly indicated that a targeting strategy against VM is most urgently needed, and VE-cadherin would be an ideal target for therapy, as it would target multiple aspects of tumor biology.

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Conflicts of Interest

There are no conflicts of interest.

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