Abstract. Background/Aim: To study the expression of the pro-angiogenic factor carcinoembryonic antigen-related cell adhesion molecule-1 (CEACAM1) in epididymal adenomatoid tumor tissue, a very rare benign neoplasia, in relation to its vascularization. Materials and Methods: Immunohistochemistry for CEACAM1 and for both endothelial markers CD31 and CD34 was performed in normal human epididymal and epididymal adenomatoid tumor tissue. The vessel density was calculated in four tumor regions with different degrees of vascularization in comparison to the vascularization of the normal epididymal tissue. Results: CEACAM1 was found in normal epididymal epithelium, while the epithelium of tumor glands was mostly negative. Only few blood vessels and lymphatics in adenomatoid tumor tissue expressed CEACAM1. The assessment of vascularization revealed either equal or a significantly lower vessel density in some adenomatoid tumor regions in comparison to normal epididymal tissue. Discussion: CEACAM1 was found in normal epididymal epithelium, while the epithelium of tumor glands was mostly negative. Only few blood vessels and lymphatics in adenomatoid tumor tissue expressed CEACAM1. The assessment of vascularization revealed either equal or a significantly lower vessel density in some adenomatoid tumor regions in comparison to normal epididymal tissue. Discussion: These data demonstrate that despite its epithelial down-regulation, CEACAM1 is not present in the majority of adenomatoid tumor blood vessels, which might be related to the lower angiogenic activity and benign behaviour of this tumor. Tumor growth and metastasis depend on neovascularization provided by angiogenesis and postnatal vasculogenesis (1, 2). The processes of new vessel formation are regulated by the balance between angiogenic activators and inhibitors (3, 4). In addition, cell–cell adhesion molecules are involved in the signaling and morphogenetic events of new vessel formation (5, 6). Tumors of the male reproductive organs, such as seminoma, teratocarcinoma and Leydig cell tumors, are almost exclusively limited to the testis. Despite their predominantly benign behavior, Leydig cell tumors of the testis are highly vascularized (7, 8). Similarly, the primary tumors of epididymis are usually benign and so rare that there is only little attention given to these tumors in literature (9, 10). Benign paratesticular tumors are most commonly adenomatoid, while the most common malignant para-testicular tumors are rhabdomyosarcomas (10, 11).

The expression of vascular endothelial growth factor (VEGF) and VEGF receptors, the most potent signaling system initiating and promoting angiogenesis and tumor vascularization, has been demonstrated in normal human testicular, as well as epididymal tissues (12, 13). The expression of collagen 18 and angiogenesis inhibitor endostatin was shown in human normal testicular tissue and testicular cancer (14). In contrast, the vascularization of epididymal tumors has not been studied sufficiently. Recently, we demonstrated the expression of collagen18 and endostatin in the epididymal adenoma and showed that, surprisingly, the tumor cell groups within the adenoma expressed high levels of endostatin, which was also localized in the wall of blood vessels in close vicinity to the tumor tissue (15). The switch of the angiogenic balance towards a predominance of pro-angiogenic activators is crucial for the initiation and maintenance of tumor vascularization (16), which is essential for progression of tumor growth and metastasis (3). The carcinoembryonic antigen-related cell adhesion molecule 1 (CEACAM1) is expressed in endothelial cells of angiogenically activated tumor vessels and acts pro-angiogenically by direct effects on endothelial migration and tube formation as well as by supporting VEGF-A-induced angiogenesis (14). Furthermore, it has recently been shown that CEACAM1 also activates lymphangiogenesis by re-programming vascular endothelial cells to a lymphatic phenotype (17).

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Given the fact that human epididymis is very rarely the primary localization of tumor development (18) or the target organ of metastasis (19) and contains high levels of the angiogenesis inhibitor endostatin, we sought to study the CEACAM1 expression pattern in epithelium versus tumor endothelium of epididymal adenomatoid tumors, as well as the relation between endothelial CEACAM1 expression and vessel density in epididymal adenomatoid tumor tissue.

Materials and Methods

Tissue samples. Normal epididymal tissue (n=10) and epididymal tumor tissue (n=4) were obtained from patients who underwent operations at the University Hospital of Hamburg, associated hospitals and the University Hospital of Munich.

Antibodies. CEACAM1 antibody 4D1/C2 was provided by Professor Dr. Wagener C (Department of Clinical Chemistry, University Hospital Hamburg Eppendorf, Hamburg, Germany). The monoclonal antibodies against CD34 and CD31 were purchased from Dako (Glostrup, Denmark).

Immunohistochemistry. From Bouin-fixed and paraffin-embedded normal epididymal tissue (n=10) and tissue of epididymal adenomatoid tumor (n=4), sections of 7 μm thickness were obtained and prepared for immunohistochemistry for human CEACAM1 using the species-specific monoclonal antibody 4D1/C2 (2.5 μg/ml), and for CD34 and CD31 (dilutions 1:100) using the monoclonal antibodies purchased from Dako.

After deparaffinization and rehydration, the sections were incubated with the CEACAM1 antibody 4D1/C2 and the antibodies against CD34 and CD31 (final dilution for both 1:100) overnight. The sections were washed in phosphate-buffered saline (PBS) and subsequently incubated with the biotinylated secondary antibody against mouse IgG recognizing 4D1/C2, CD34 and CD31 antibodies. After further steps of washing and incubation with peroxidase-
antiperoxidase and with ABC kit (Dako), the development of the specific peroxidase reaction was achieved by means of the modified nickel-glucose oxidase technique as described previously (23, 24). To visualize the tissue structure for a better localization of the specific reaction, the sections were then counterstained with Calcium Red.

Histological and statistical evaluation. The assessment of vessel density of normal epididymal tissue and epididymal adenomatoid tumor tissue was performed on tissue sections which were immunohistochemically stained for both endothelial markers CD34 and CD31. Since CD34 staining was also found in some stromal cells of the normal epididymal tissue, the counting of blood vessels was carried out on sections stained only for CD31. The number of blood vessels was counted in three different tissue areas of each section marked by a microscopic grid using the microscopic magnification of ×200. The number of blood vessels in normal epididymal tissue was compared to that in epididymal tumor tissue using the Wilcoxon rank sum test. A \( p \)-value of less than 0.05 was considered as significant.

Results

In normal human epididymal tissue, CEACAM1 was found to be localized in the epithelium of the efferent ducts and the epididymal duct (Figure 1A) as previously shown (25), while no staining was observed in the interstitial tissue including blood vessels and lymphatics. Analyses at higher magnification revealed that CEACAM1 staining was limited...
to the luminal surface of the epithelium (not shown) and no specific staining was seen at the basolateral side of the epithelium. To visualize the blood vessels in the normal human epididymis, immunohistochemistry for both CD34 (Figure 1B) and CD31 (Figure 1C) was performed on sections obtained from the same tissue blocks as used for CEACAM1 staining. These analyses revealed an intense vascularization, particularly in close vicinity to the lamina propria of the efferent ducts and epididymal duct (Figure 1B-C), whereas neither CD31 nor CD34 immunostaining were seen within the epithelium. Interestingly, numerous interstitial stromal cells in addition to blood vessels were also positive for CD34 (Figure 1C). In control sections exposed to the secondary antibody only, no specific staining was observed (Figure 1D).

In contrast to normal epididymal tissue, major parts of the epididymal adenomatoid tumor tissue were negative for CEACAM1 (Figure 2A). The adenomatoid tumor tissue was composed of a solid cell cluster or of glandular tubules. In a
few small areas (approximately 3-5% of the whole adenomatoid tumor area) of the adenomatoid tissue, CEACAM1 was present in the epithelium of some glands (Figure 2B). Detailed studies of such areas revealed that in contrast to the normal epididymal epithelium, CEACAM1 staining was not prominent at the luminal surface but at the basolateral side of the adenomatoid tumor epithelium (Figure 2B). In major parts of the adenomatoid tissue, most of the blood vessels were negative for CEACAM1. Neither the great majority of blood vessels within the adenomatoid tumor, nor within the tissue surrounding it exhibited CEACAM1 staining, except for a few small blood vessels within the adenomatoid tumor tissue (Figure 2C). Furthermore, a few lymphatics within tissue areas around the adenomatoid tissue and in close vicinity to large blood vessels were positive for CEACAM1 (Figure 2D). In control sections which were only treated with the secondary antibody, as shown in Figure 1D, no specific staining was found.

Next we wanted to determine the vascular density of adenomatoid tumor tissue in comparison to that of normal epididymal tissue based on the immunostaining for both CD34 and CD31. In addition to blood vessels, CD34-positive stromal cells were present within the glands of epididymal adenomatoid tumor (Figure 3A), but their number seemed to be reduced in comparison to that of normal epididymal tissue as shown in Figure 1B. Interestingly, a heterogeneous pattern of tissue vascularization with a different degree of vascular density was found within the adenomatoid tumor tissue where areas with relatively high vessel density (Figure 3A) could be distinguished from those with low blood vessel density (Figure 3B). The same pattern was confirmed by staining for CD31 (Figure 3C-D). The counting of blood vessels was performed on sections of normal epididymal and epididymal adenomatoid tissue stained for CD31. Considering the differences in the vascularization of different adenomatoid tissue areas, vessel density of four tumor regions was compared to that of normal epididymal tissue. This evaluation surprisingly revealed that the vascular density in the strongest vascularized adenomatoid tumor tissue area was only as high as in normal epididymal tissue (Figure 4). Moreover, in some adenomatoid tumor areas, the vessel density was lower than that of normal epididymal tissue (Figure 4). However, no correlation was found between CEACAM1 presence in a few blood vessels and vascular density of the adenomatoid tissue.

Discussion

Here, we demonstrate that the cell adhesion molecule CEACAM1, which is present in normal epithelium of efferent ducts and epididymal duct, is absent from the epithelium of epididymal adenomatoid tumor, a benign and very rare neoplasia of the epididymis. These data show for the first time that epithelial CEACAM1 is not only down-regulated in several malignancies, as has been previously shown (20, 22, 26), but also in benign neoplastic tumors as shown here for epididymal adenomatoid tumor. Interestingly, this epithelial down-regulation or loss of CEACAM1 is not accompanied by its up-regulation in vascular endothelial cells as has been previously shown for several malignant tumor types (22, 26). Briefly, our present data show that i) CEACAM1 is localized at the luminal surface of the efferent ducts and the epididymal duct of normal human epididymis but is absent from their associated blood vessels; ii) in contrast, in epididymal adenomatoid tumor tissue, CEACAM1 is not detectable in solid tumor cell clusters or in glands formed by tumor cells, with the exception of a few ducts within the adenomatoid tumor tissue; iii) the vascular density in the adenomatoid tumor tissue is not significantly changed in comparison to normal epididymal tissue, and finally iv) only a few intra-tumoral blood vessels and a few lymphatics around the adenomatoid tumor tissue are positive for CEACAM1.

CEACAM1 is normally present in epithelium of several organs including colon, prostate, breast and urinary bladder tissue (22, 26-28). In these organs CEACAM1 is predominantly localized at the luminal epithelial surface. However, this epithelial expression of CEACAM1 disappears at an early stage of tumor development as has been reported for several organs (22, 26-28). Thus, CEACAM1 has been considered as a tumor suppressor. However, in a few malignancies, such as adenocarcinoma of the lung and malignant melanoma, the expression of CEACAM1 in tumor tissue was associated with a poor prognosis (29, 30). On the other hand, it was shown that CEACAM1 is up-regulated in endothelial cells of tumor-associated blood vessels concurrently with its epithelial down-regulation (22, 26). Once expressed in the endothelial cells, CEACAM1 acts pro-angiogenically and supports VEGF-induced angiogenesis (14, 22, 26).

However, in the epididymal adenomatoid tumor the epithelial down-regulation or loss of CEACAM1 is apparently not accompanied by its concurrent endothelial up-regulation. Our present data show that blood vessels within the adenomatoid tissue are practically negative for CEACAM1. In comparison to previously published results showing a high number of CEACAM1-positive blood vessels in several malignant tumor types (22, 26), only few sporadically distributed small vessels within the adenomatoid tumor tissue exhibited CEACAM1 staining. Since the counting of the blood vessels revealed an either equal or regionally significantly lower vessel density in epididymal adenomatoid tissue in comparison to normal epididymal tissue, one can postulate that CEACAM1 negativity might be associated with reduced angiogenic activity. However, we were unable to find any
correlation between the sporadically distributed CEACAM1-positive blood vessels and the degree of vascularization within the adenomatoid tumor. Due to the rarity of this tumor entity, our analyses could only be performed on restricted tissue material and therefore the explanatory power of this statement is limited. Another explanation for less vascularization of the epididymal adenomatoid tumor tissue might be the presence of the endogenous angiogenesis inhibitor endostatin in spherical bodies within epididymal adenomatoid tumor tissue as we have recently shown (15). Endostatin and/or other endogenous inhibitors which are produced or processed by the adenomatoid cells themselves may suppress angiogenic activity leading to less vascularization. This might also be responsible for the lower proportion of CEACAM1-positive blood vessels within the adenomatoid tumor. Further supporting evidence for this explanation is the less chaotic organization of blood vessels observed in the adenomatoid tumor tissue as compared to that of malignant tumors.

Another indicator for moderate angiogenic activity is the fact that we did not find CEACAM1-positive blood vessels in close vicinity to the adenomatoid area. In tumor surrounding tissue the vessel density was not changed when compared to normal tissue or to adenomatoid tissue.

In summary, whatever accounts for reduced angiogenesis and probably also lymphangiogenesis, CEACAM1 positivity versus negativity in vascular and lymphatic endothelial cells seems to be a useful parameter to assess the degree of angiogenesis in tumors. Whether CEACAM1 is mechanistically involved in the lower degree of vascularization in epididymal adenomatoid tumors remains elusive and needs further analyses.

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