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Review

The Epithelial Cell Adhesion Molecule (Ep-CAM) as a Morphoregulatory Molecule Is a Tool in Surgical Pathology

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Cell adhesion receptors (CAMs) are actively involved in regulating various cell processes, including growth, differentiation, and cell death. Therefore, CAMs represent a large group of morphoregulating molecules, mediating cross-talk between cells and of cells with their environment. From this perspective, CAMs do contribute to cells and tissue organization, and in diseased tissue, to the disease development and biological characteristics. Therefore, observed changes in expression patterns of adhesion molecules may contribute to establish a diagnosis. A distinct shift in expression patterns in neoplastic epithelium has been described, for example for cadherins, integrins, and CD44. A relatively novel cell CAM, Ep-CAM, was first reported to be a pan-carcinoma antigen, although it is rather a marker of epithelial lineage. Several antibodies directed to Ep-CAM have been generated, and many epithelial tissues and their neoplastic appendages have been studied. This article outlines the results of these studies. Based on the results of these studies, we conclude that Ep-CAM immunohistochemistry can be a useful tool in the diagnosis of disturbed epithelial tissues. *(Am J Pathol 2003, 163:2139–2148)*

Four major families of cell adhesion molecules (CAMs) are recognized on the basis of their structure: integrins, selectins, CAMs of the immunoglobulin gene (IgG-like) super family, and cadherins. Also other types of molecules with adhesion properties have been reported, for example, syndecans, CD44, and Ep-CAM. Nowadays, CAMs defined as morphoregulatory molecules that affect cellular processes, based on data about inside-out and outside-in signaling and signal transduction pathways.

During embryogenesis, but also in tumor development, the maturation and differentiation of epithelial cells is regulated by signals within the epithelium and between epithelia and other tissues. Every tissue type and state of maturation can be defined by specific expression patterns of adhesion molecules. Changes in expression patterns of one or several adhesion molecules may suggest altered tissue differentiation or maturation. In other words, a disturbed tissue maintenance may be concomitant by ectopic or overexpression of adhesion molecules, and this can be used as a tool in surgical pathology. For example, in epithelial tissues many studies have been conducted to study the morphoregulatory role of E-cadherin.[1] A tumor suppressor function for E-cadherin has been proposed frequently, and has been demonstrated in breast and gastric cancer. Inactivation of E-cadherin is an early and crucial step in the formation of lobular carcinoma *in situ*, as a precursor of invasive lobular breast cancer and hereditary gastric cancer.[2–6] Furthermore, co-expression of E-, N-, and P-cadherin was demonstrated for several breast tumors, but unique expression patterns were distinguishable for each type of tumor.[7]

Here we analyze in detail the biological significance and diagnostic value of the expression changes of a novel adhesion receptor, Ep-CAM.

Ep-CAM

Ep-CAM has first been identified as a tumor-specific antigen on several carcinomas of different origin. Several independent studies generated different antibodies directed against the tumor-specific molecule expressed on carcinomas (Figure 1). In addition, the corresponding cDNA had been independently cloned by a number of groups.[8–11] Therefore, the molecule was first known by many different names, ie, the human pan-antigen epithe-

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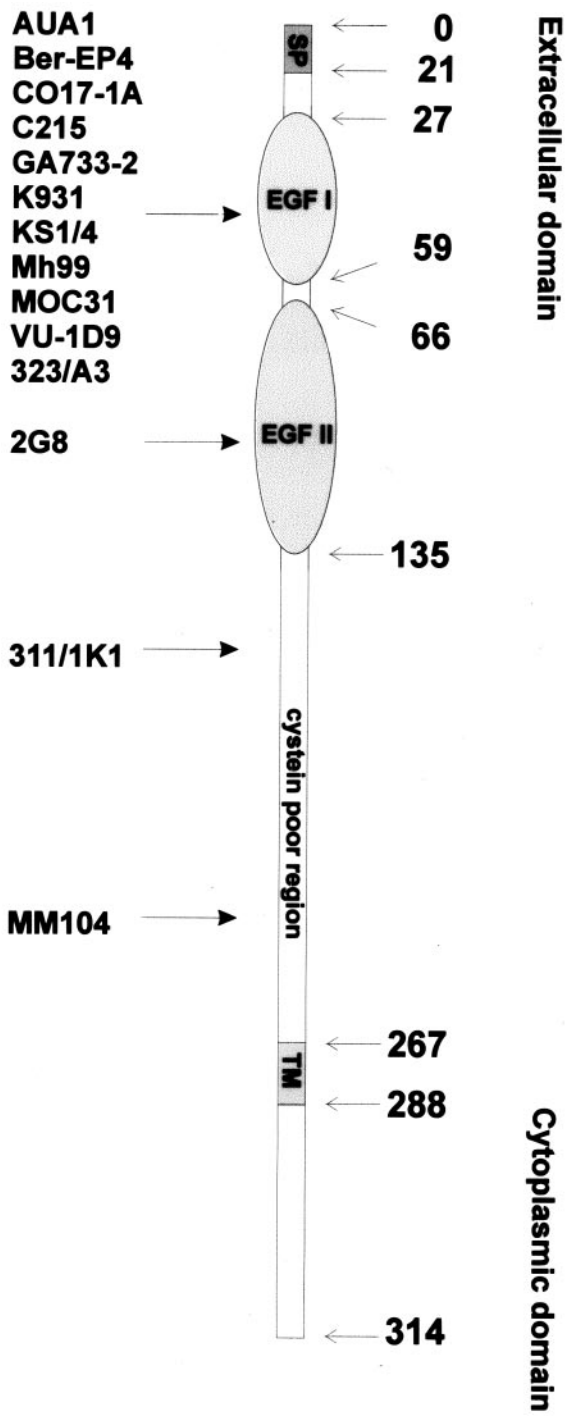

Figure 1. Schematic composition of Ep-CAM. SP, signal peptide; EGF, EGF-like domain; TM, transmembrane. The numbers indicate the amino acid residues that mark the regions in the molecule.

lial glycoprotein EGP40, CO17-1A antigen, KSA1/4, ESA, GA733-2, MOC31, Ber-EP4, and so forth (Table 1). In the early 1990s, the reports on the carcinoma antigens and the cloning of the cDNA were combined and it became clear that the described molecules were virtually identical. Initial studies on the characteristics of the molecule revealed that the molecule is a marker of epithelial lineages.

Table 1. Known Antibodies Directed against the Epithelial Adhesion Molecule Ep-CAM

Antibody	Epitope	Reference
AUA1	EGF-like domain I	Durbin et al[69]
Ber-EP4	EGF-like domain I	Latza et al[70]
CO 17-1A	EGF-like domain I	Herlyn et al[71]
C215	EGF-like domain I	Bjork et al[72]
ESA, EGP-2, EGP40	Not established	Simon et al[10]
FU-MK-1	Not established	Watanabe et al[27]
GA733-2	EGF-like domain I	Szala et al[11]
HEA125	Not established	Momburg et al[25]
K928	Not established	Quak et al[73]
K931	EGF-like domain I	Copper MP[74]
KSA, KS-1, KS1/4	EGF-like domain I	Varki et al[75]
MM104	Cysteine-poor region	Schön et al[76]
MH99	EGF-like domain I	Mattes et al[77]
MOC31	EGF-like domain I	Myklebust et al[78]
MT201	Not established	Naundorf et al[79]
VU-1D9	EGF-like domain I	Tsubura et al[56]
2G8	EGF-like domain II	Unpublished data
311-1K1	Cysteine-poor region	Helfrich et al[80]
323/A3	EGF-like domain I	Edwards et al[81]

It is a type I transmembrane glycoprotein, not structurally related to one of four the major families of the adhesion molecules. The molecule consists of an extracellular domain containing two epidermal growth factor (EGF)-like repeats, and a short intracellular domain of 26 amino acids in which two binding sites for α-actinin are present for linkage to the actin cytoskeleton (Figure 1).[12] It is a relatively small protein that is highly conserved during evolution and mediates calcium-independent homotypic cell-cell adhesions.[13,14] It is normally expressed at the basolateral membrane of cells by the majority of epithelial tissues, except in adult squamous epithelium and some specific epithelium cell types, such as hepatocytes.[15–17] Based on the properties of this molecule, it was renamed epithelial cell adhesion molecule, Ep-CAM.[15,16]

Further studies revealed that in murine fibroblasts transfected with Ep-CAM, the expression of Ep-CAM is associated with proliferation.[15,16] On (over-) expression of Ep-CAM, cadherin adhesions dissociate, which leads to accumulation of detergent soluble E-cadherin/β-catenin complexes, and to a decrease in total cellular α-catenin.[18] This suggests that during cell division, the strong, tight E-cadherin-mediated cellular adhesion is abrogated, while the weaker intercellular adhesion mediated by Ep-CAM still holds the cell in place.[18,19] After the proliferative phase, Ep-CAM expression declines and higher levels of E-cadherin mediate intercellular adhesions and direct cellular differentiation.

Based on a large study on the expression of Ep-CAM, the possibilities to target Ep-CAM for immunotherapy were explored.[20] Patients with Dukes' C colorectal carcinoma who had undergone curative surgery, were treated in a monotherapy in the adjuvant setting with edrecolomab, the murine IgG2a monoclonal antibody that recognizes and binds with low affinity to Ep-CAM. After 7 years of follow-up, the edrecolomab-treated group had a 32% reduction in mortality, and a 23% reduction in recur-

Table 2. Expression Pattern of Ep-CAM in Normal Tissue, Dysplasia, and Carcinoma (ca)

Epithelium (species)	Antibody	Expression pattern Ep-CAM		
		Normal tissue	Premalignant	Carcinoma
Oral mucosa, glottic squamous epithelium	KS1/4 323/A3	Negative Positive	Dysplasia: positive	Positive Positive
Esophagus	KS-1	Negative (columnar cells) Negative (squamous)	Metaplasia: positive (BE)	
Gastric	323/A3	Positive only in crypts Negative (mucosa)		Positive constitutive from crypts till villae
Colon	KS1/4 323/A3	Positive	Adenomas: enhanced	Enhanced (colorectal ca)
Liver	323/A3 17-1A MOC31 Ber-EP4	Negative Positive (regenerating/Proliferating hepatocytes)	Positive	Positive (CHC) Negative (HCC)
Pancreas	KS1/4	Positive		Enhanced
Kidney	Ber-EP4 FU-MK-1	Positive*		Heterogeneous (clear cell ca)
Bladder	AUA1 FU-MK-1	Positive		Enhanced
Testes	HEA125	Positive*		Positive*
Prostate	323/A3 FU-MK-1	Positive		Enhanced
Mammary	2G8 ESA 323/A3 17-1A	Positive		Enhanced
Ovary	AUA-1 Ber-EP4	Low-positive (oocyte)		Enhanced (serous) Enhanced (mucinous)
Uterine cervix	323/A3	Negative	Dysplasia: positive	Positive
Lung	Ber-EP4	Low positive		Positive (SCC) Negative (mesothelioma) Lymph node metastasis from NSCLC: positive
Skin	Ber-EP4 MH99	Negative (squamous) Positive (sweat ducts/ Proliferating squamous cells)		Positive (BCC) Positive (basosquamous) Negative (SCC)

*For details on cell types and tumor types: see text.

rence, as compared to the observation arm.[20] More clinical trials are ongoing.

Ep-CAM expression is believed to be an early marker for (pre-) malignancies.[21] Immunohistological stainings of dysplastic colon cells showed overexpression of Ep-CAM. Not only the basolateral membrane was Ep-CAM-positive, apical positivity was also observed. For mature squamous epithelium, a *de novo* expression has been described in weak, mild, and severe dysplasia.[21] Because it is important to diagnose (pre-) malignancies at early stages, Ep-CAM immunohistochemistry can be of use to diagnose aberrant tissue morphology.

Ep-CAM in Various Malignant Tissues

As mentioned, throughout the last 3 decades many antibodies were raised against a widely detected tumor antigen that later was designated Ep-CAM. Several histological studies of expression patterns of Ep-CAM were conducted on different tissues, but because of the variety of names for antibodies and types of studies a comprehensive overview of the results is lacking. These are listed below and summarized in Table 2. The findings for Ep-CAM expression patterns in adult tissue, premalignancy, and malignancy will be discussed.

Head and Neck Region

In the squamous epithelium of the oral cavity expression of Ep-CAM is a reliable marker for the development of neoplasia. Weak, mild, and severe oral mucosal dysplasias displayed high expression levels of Ep-CAM in dysplastic basal and suprabasal cells, whereas normal epithelial cells are Ep-CAM-negative.[22]

In glottic squamous epithelium, Ep-CAM [using monoclonal antibody (mAb) 323/A3] was expressed in all dysplastic areas with the border of the expression corresponding to the border of the dysplasia. In all dysplasia a full layer expression of Ep-CAM was observed, indicating complete dysplasia of the epithelium (Sjögren EV, unpublished results).

In invasive tumors, a strong heterogeneity in Ep-CAM expression within and between tumors was observed. Besides proliferation, this heterogeneity was found to correspond to keratinization, with keratinizing areas being low or negative in Ep-CAM expression (Sjögren EV, unpublished results).

Nodal metastases and their corresponding primary tumors of head and neck squamous carcinoma were examined for gene expression.[23] The expression of most genes involved in tumorigenesis, for example E-cadherin, was similar in primary tumors and metastases. Surprisingly, Ep-CAM expression was detected less frequently in metastases, compared to the corresponding primary tumor, suggesting involvement in metastasis. To identify high-risk patients having small numbers of disseminated tumor cells in early tumor stages, a reverse transcriptase-polymerase chain reaction assay for Ep-CAM expression that detects a single tumor cell within normal cells was successfully developed.[24]

Esophagus

The squamous epithelium of the esophagus is clearly negative for Ep-CAM, while the columnar epithelium in Barret's esophagus displays a diffuse and low expression pattern for Ep-CAM.[25] In biopsies of Barret's esophagus a heterogeneous pattern of Ep-CAM staining is present. Within several patients, the expression of Ep-CAM (mAb KS-1) differed among various regions of the columnar esophageal epithelium of the intestinal type.[26]

Preliminary data of Kumble and colleagues[26] showed high expression levels of Ep-CAM (mAb KS-1) in four tested adenocarcinomas of the esophagus. The authors hypothesized that Ep-CAM is positively correlated with the progression to adenocarcinoma of the esophagus.

Gastric

In normal gastric epithelium no Ep-CAM expression can be observed, only in the basal layer of crypts. However, with the development of intestinal metaplasia, a strong up-regulation of Ep-CAM expression is observed in all cases studied with 323/A3 (De Boer CJ, unpublished results). Ep-CAM expression appeared throughout the crypts and is constitutive to the foveola. The authors found that expression of Ep-CAM can already be detected on some cells on the border of normal and metaplastic cells that have no metaplastic phenotype yet, and suggested that expression of Ep-CAM may be an early event in the development of gastric metaplasia that corresponded completely with increased proliferation as measured by an increase in Ki-67-positive cells. Using the FU-MK1 antibody, similar results were obtained.[27]

Songun and colleagues[28] studied whether Ep-CAM expression in primary tumor specimens from primary gastric adenocarcinoma was indicative for the presence of lymph node metastases, but it was not. However, loss of Ep-CAM expression is an independent prognostic value for poor survival prognosis. This can be explained by the fact that loss of Ep-CAM expression, as an epithelial adhesion molecule, may reflect a loss of epithelial differentiation. Furthermore, low expression levels of E-cadherin in carcinoma, increases the role for Ep-CAM adhesions in interconnecting cells. The loss of Ep-CAM expression probably results in loss of cell-cell adhesion, which promotes metastasis.[29]

Colon

Ep-CAM is widely expressed in the highly proliferative cells of the intestinal epithelium. Ep-CAM is expressed from cells in the basal cells throughout the crypts at the basolateral membranes, and only the apical membrane facing the lumen is negative.[30] The development of adenomas is accompanied by an increased Ep-CAM expression and Ep-CAM overexpression (mAb GA733) has been frequently demonstrated in colorectal carcinomas.[31,32]

In clinical trials, colorectal cancer has been targeted with the monoclonal antibody CO17-1A and anti-idiotypic antibodies mimicking the CO17-1A or GA733-2 epitope. An improved survival was accompanied by a prolonged systemic immune reaction to the antibody.[33] Presently, its anti-tumor effect is being studied as monotherapy after resection of stage II colon cancer, and in combination with chemotherapy in patients with stage II or III rectal cancer.[34] Patients with resected Dukes' C colorectal cancer were randomly allocated to infusions of CO17-1A antibody.[20] The follow-up study shows that 17-1A antibody administered after surgery prevents the development of distant metastasis in approximately one-third of patients. The therapeutic effect is maintained after 7 years of follow-up.[20] Various mechanisms can be responsible for the clinical observed effects of Ep-CAM immunotherapy. According to Haller,[34] the murine IgG2a mAb against Ep-CAM mediates an antibody-dependent cellular cytotoxicity, complement-mediated cytolysis, and anti-idiotypic network.

Liver

Ep-CAM (mAb 323/A3) is expressed on hepatocytes in embryonic liver and maturing liver cells, but is absent in adult hepatocytes.[35] Ep-CAM does mark a pluripotent stem cell, the progenitor for both bile duct cells and hepatocytes. The *de novo* expression of Ep-CAM in regenerating/proliferating hepatocytes is explained by the fact that these stem cells replace the damaged cells, and decreased intercellular adhesion by E-cadherin is required for proliferation. On maturation of the new cells, ie, on differentiation, Ep-CAM expression is lost again. The down-regulation of Ep-CAM and thereby the signal for proliferation precedes the restoration of cadherin-mediated cellular adhesion.

Diseased liver tissue displayed a strong Ep-CAM expression (mAb 17-1A) in the epithelium of typical and atypical bile ducts.[36] In addition, periportal or periseptal hepatocytes revealed variable staining of Ep-CAM, which is directly related to acute and chronic inflammatory changes. The Ep-CAM expression in hepatocytes was most pronounced in acute and chronic active hepatitis, with Ep-CAM expression levels that are common to bile

ductular cells. This suggests that the hepatocytes in diseased liver represent transformed hepatocytes.

It was demonstrated that all hepatocellular carcinomas (HCCs), including the pseudoalveolar type, were uniformly negative for Ep-CAM.[37,38] In the mixed HCC-cholangiocarcinoma cases, Ep-CAm (mAb MOC31) highlighted the glandular component, but did not stain the HCC portion of the neoplasm.

Furthermore, Ep-CAM differentiated between HCC and metastatic adenocarcinoma from the colon, lung, breast, pancreas, small intestine, kidney, or ovary.[37,38] However, according to Sansonno and Dammacco,[36] neoplastic bile duct epithelium did not react for Ep-CAM (mAb17-1A) in cholangiocarcinoma, whereas neoplastic liver cells acquired cytoplasmic-positive staining in clustered areas in HCC. The intensity of staining and Ep-CAM distribution were inversely related to the grade of tumor differentiation.

Pancreas

In the mature pancreas, the ductal compartment strongly stained for Ep-CAM exhibited the highest proliferation index.[39] The authors established a correlation between frequency of proliferating cells and increased expression of Ep-CAM (mAb KS1/4) in each cell compartment. The highest Ep-CAM expression was recorded at the cell-cell boundaries of intercalar ductal cells, in interlobular ducts, and in main ducts. Islets of Langerhans, identified by the insulin- and glucagon-specific antibodies, exhibit a significantly less intense Ep-CAM expression. The authors suggest that Ep-CAM expression negatively regulates the endocrine differentiation in pancreatic islet cells.

In cell lysates, increased expression levels of Ep-CAM were detected in human islet β-cell tumors (insulinoma).[39] This increase is most likely also detectable with immunohistochemistry on tissue sections, but this has not yet been performed.

Kidney

Few studies have described Ep-CAM in normal and neoplastic kidney. Normal renal tubules are in general strongly positive, while clear cell carcinomas show a more heterogeneous pattern. Five of twelve cases were positive for Ep-CAM (mAb Ber-EP4), whereas only one of five cases of renal carcinoma was weakly positive with the FU-MK1 antibody.[40,41] Concluding from the stained sections presented in the study, the use of the FU-MK1 antibody may not be the best suitable antibody to use for diagnostic purposes.

Urothelium

Transitional epithelium of the bladder is only slightly positive for Ep-CAM (mAb AUA1/FU-MK1). In dysplastic lesions of urothelium and transitional cell carcinoma, enhanced expression of Ep-CAM was observed, although antigenic heterogeneity exists between tumors of the same grade and within the same tumor.[42] Using the FU-MK-1 antibody, only two of five bladder carcinomas were positive.[41]

Testes

In tissues of the male genital tract, some of the cells in testis (spermatogonia, low Ep-CAM expression), epididymis (ciliated, basal and cuboidal cells, intermediate expression), and seminal vesicle (positive expression) reveal Ep-CAM expression when using the HEA125 antibody.[25]

Kommoss and colleagues[43] concluded that among other antibodies, immunohistochemical staining for Ep-CAM in testicular neoplasms are helpful in the differential diagnosis when distinction on morphological grounds is difficult. Using HEA125, he demonstrated Ep-CAM reactivity in cases of seminoma (3 of 12, 25%), embryonal carcinoma (3 of 12, 25%), yolk sac tumor (6 of 8, 75%), teratoma (1 of 2, 50%), whereas juvenile granulosa cell tumor, Sertoli cell tumor, primary and metastatic Leydig cell tumor, choriocarcinoma, and sex cord tumor all were negative.[43]

Prostate

Secretary, basal, and ductal cells of the prostate reveal an intermediate Ep-CAM expression when using the HEA125 antibody.[25]

Positive Ep-CAM staining (mAb FU-MK-1) was detected in normal prostate and in adenocarcinoma, although a small number of cases was studied.[41] However, no clear staining pattern was observed with this antibody.

A low immunoreactivity was found for Ep-CAM (mAb 323/A3) in benign prostatic epithelium, concentrated on the luminal cells.[44] Strong immunopositivity was detected in luminal cells of high-grade prostatic intraepithelial neoplasias, as well as in adenocarcinomas, suggesting that increasing levels of Ep-CAM expression represent early events in the development of prostatic adenocarcinoma. However, Ep-CAM positivity was not correlated to the clinical outcome of patients.

Mammary Gland

The mammary gland epithelium undergoes several stages of development and dedifferentiation. In normal human mammary glands, Ep-CAM is mainly expressed in luminal epithelium.[45] Ep-CAM expression during the developmental phases was extensively studied in C57BL6 mice by Balzar and colleagues.[46] Using the mAb G8.8, it was clearly demonstrated that endogenous Ep-CAM expression very well correlated with the proliferative state of the mammary gland postnatal development, while the E-cadherin expression was unchanged during this period. With the start of milk production, the epithelium is differentiated and Ep-CAM expression decreases. Mice transfected with human Ep-CAM under the control of the MMTV-LTR promotor showed not only an association of Ep-CAM with regulation of mammary gland morphogenesis, but also direct involvement. The virgin mammary glands of transgenic mice displayed increased budding and secondary branching as compared to their nontransgenic littermates.

The staining pattern of mAb 323/A3 in benign breast disease was analyzed by Courtney and colleagues.[47] Patients who have had both a benign biopsy and a later biopsy for breast carcinoma were screened. In apocrine

metaplasia, the cytoplasm of benign tissue did not stain with 323/A3, whereas in the biopsies with associated breast cancer did (five of seven). The authors noted a positive predictive value of 100% for strong cytoplasmic staining to indicate the presence of carcinoma.

An immunohistochemical study on breast cancer biopsies showed that Ep-CAM (mAb 17-1A) was expressed in the majority of the breast carcinomas, especially on paraffin sections.[32] Spizzo and colleagues[48] stated that the overexpression of Ep-CAM, detected with mAb ESA, in 205 cases of localized invasive breast cancer was an independent prognostic marker by multivariate analysis. Ep-CAM overexpression correlated significantly with disease-free and overall survival, independent of tumor size, nodal status, histological grade, and hormone receptor expression. Specific immunotherapy with mAbs against Ep-CAM in minimal residual stages of breast cancer should be considered.[32]

Ovary

In ovaries, the oocytes display a moderate Ep-CAM-positive staining (Ab HEA125), but the follicular epithelial cells are negative. In the oviduct, (non-) ciliated cells show a low reactivity.[25]

Ovarian clear cell carcinomas showed Ep-CAM positivity with both AUA1 and Ber-Ep4.[40,49] Cherchi and coworkers[50] showed 50% and 79% Ep-CAM positivity (mAb Ber-EP4) in ovarian cancer of serous and mucinous type. Furthermore, Ep-CAM positivity (mAb Ber-EP4) was directly proportional to tumor differentiation; 70% of the well-differentiated tumors were Ep-CAM-positive, compared to 37.5% of the poorly differentiated tumors. No positivity was observed for Ep-CAM (mAb Ber-EP4) in either metastatic ovarian tumors or germ cell tumors.

Uterine Cervix

Normal, mature squamous epithelium of the uterine cervix does not express any Ep-CAM (323/A3).[21] Squamous differentiation marker cytokeratin 13-positive staining appears from parabasal cells, and the staining intensity increased toward the lumen in normal squamous epithelium. This is also observed for the squamous terminal differentiation marker involucrin.

Sections of the uterine cervix stained for Ep-CAM and Ki67 have demonstrated that in squamous dysplasia, both low and high grade, Ep-CAM is associated with proliferation.[21] In cervical intraepithelial neoplasia (CIN) grade I, Ep-CAM-positive areas were found in the parabasal layer, where now cytokeratin 13 was absent (Figure 2). In progressing grades of CIN, grade II and III, larger layers of Ep-CAM expression were observed, while cytokeratin 13 almost disappeared. Similar staining patterns were found for the terminal differentiation marker involucrin. In progressing CIN lesions, involucrin staining is lost and Ep-CAM expression expanded. The highly proliferative activity in undifferentiated cells of CIN layers is associated with Ep-CAM expression. The Ep-CAM expression is inversely correlated with E-cadherin participating in cell-cell junctions.[17] Ep-CAM can be used as an early marker for disturbed tissue proliferation and differentiation in cervical premalignant stages. In the majority of both squamous and adenocarcinomas of the cervix a strong expression of Ep-CAM was observed, although some decrease in the expression (both the intensity and the number of positive cells), as compared with CIN III lesions, was observed in the areas of squamous differentiation.[21] Because it is unlikely that E-cadherin-mediated adhesion had returned and the tissue was differentiating, the population of cells that are less positive for Ep-CAM may be submitted to genetic imbalance where Ep-CAM transcription was lost.

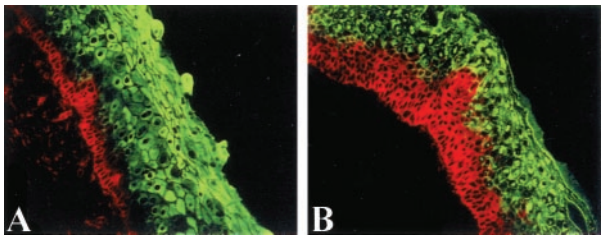

Figure 2. Immunofluorescent double staining for Ep-CAM (red) and squamous differentiation marker cytokeratin 13 (green) in uterine cervix, stage CIN 1 (**A**), and stage 2 (**B**). The area where Ep-CAM is expressed is larger in CIN 2 as compared to CIN 1. For details see text and Litvinov and colleagues.[21]

Lung

In normal lung tissue, the ciliated bronchial epithelium, alveolar duct, and alveolar epithelial cells type I and II all show a low Ep-CAM expression. In pulmonary fibrosis, Ep-CAM could further be detected on the cell surface of epithelial remnants.[25,51]

Two studies by Piyathilake and colleagues,[52,53] reported strong Ep-CAM positivity in 98% of the squamous cell cancers (SCC) and uninvolved bronchial mucosa and in 100% of the hyperplasias and dysplasias. There was increased Ep-CAM expression in luminal cells as compared to basal cells and was more consistent in hyperplasia than in uninvolved mucosa. The authors described a statistically significant stepwise increase in Ep-CAM expression from uninvolved bronchial mucosa to epithelial dysplasia to SCC. A significant association was detected with lower tumor differentiation, advancing nodal status, and advancing clinical stage. Well-differentiated SCCs expressed more Ep-CAM than poorly to moderately differentiated SCCs, and the increase in the Ep-CAM expression tends to correspond with increasing size or local extent of the primary tumor and involvement of regional lymph nodes. In contrast to squamous carcinomas, Ep-CAM is not expressed in mesothelioma. Using MOC31, a distinction between carcinoma and mesothelioma can be made on the basis of Ep-CAM expression.[54]

The Ber-EP4 antibody was used to discover small tumor cell deposits in regional lymph nodes in patients with resected non-small cell lung cancer. In a prospective study of 125 patients, the detection of single Ep-CAM-

positive (mAb Ber-EP4) cells proved to be an independent prognostic factor for the overall survival.[55]

Skin

In the skin, the keratinocytes and melanocytes are Ep-CAM-negative, while the sweat ducts (eccrine and apocrine coils, apocrine ducts) and the proliferative zone of the hair follicle are Ep-CAM-positive.[56,57] However, within the basal layers of the epidermis, some Ep-CAM reactivity can be observed in the reserve cells with mAb MH99.[58]

Cutaneous neoplasms reported to stain for Ep-CAM (mAb Ber-EP4) include basal cell carcinoma (BCC), Merkel cell carcinoma, and mixed tumor of skin (chondroid syringoma). In BCC, Ep-CAM (mAb Ber-EP4) is constantly and diffusely expressed, while SCC, squamous intraepithelial neoplasia, and actinic keratosis are Ep-CAM-negative.[57,59,60] This pattern was observed in nodular, cystic, superficial, and infiltrative BCC, but not in SCC, irrespective of the degree of differentiation. Using Ber-EP4, the identification of basosquamous carcinoma is also possible, because the studied tumors all showed at least some areas of Ep-CAM positivity.[61]

Discussion and Conclusions

This report has presented an overview of the expression patterns for Ep-CAM in several epithelial tissues, and the pathology thereof. Ep-CAM can be detected in all simple, columnar, and pseudostratified epithelia, but is absent in adult squamous epithelium. *In vitro*, a clear association was demonstrated between Ep-CAM and cell proliferation. Overexpression of Ep-CAM, as well as a *de novo* expression was observed in colon carcinoma and in squamous carcinoma of the uterine cervix.[15,16] Simultaneously, Ep-CAM expression abrogates the cadherin-mediated adhesions, which has serious implications for differentiation of epithelial tissues, and may by itself be the reason behind increased cell proliferation. We believe that the enhanced expression and *de novo* expression is an early step in the malignant transformation of epithelium, and can be used as a marker for diagnostic purposes.

The discussed studies here all used antibodies that have their epitope in the EGF-like domain I. Other antibodies are known to have their epitope in the EGF-like domain II or in the cysteine-poor region (Table 1) but were apparently not suited for immunohistochemistry because we retrieved no published reports in which reliable results on histological slides with these antibodies were described. To our knowledge, no antibodies have been developed that have their epitope in the intracellular domain of Ep-CAM.

The most frequently used antibodies are the monoclonal antibodies 323/A3, KS1/4, and Ber-EP4. They are known to have a high affinity and specificity for human Ep-CAM. Observed reported heterogeneity in reactivity of Ep-CAM-specific antibodies with subpopulations of Ep-CAM with cell or tissue suggests that intracellular and cell surface Ep-CAM differ in the conformational state of the protein, and that some epitopes may be masked on the molecules participating in intercellular adhesions.[62] One study described the favor of MOC31 to Ber-EP4: one case of HCC (Ep-CAM-negative) was detected with Ber-EP4, but not with MOC31.[37] This was independently confirmed, and the authors concluded that the MOC31 staining was readily interpretable with rare exceptions.[38]

For our research, the 323/A3 antibody is routinely used on both frozen and paraffin-embedded tissue samples. After standard xylene and graded alcohol series, formalin-fixed and paraffin-embedded tissues are fixed in methanol and incubated in 0.3% H_2O_2 in methanol. For 323/A3 staining, tissues are pretreated with 0.1% trypsin (w/v) in 0.1% $CaCl_2$. Standard two-step biotin/streptavidin labeling is often used for detection. Fresh tissue samples can be stained with standard methods, without any pretreatments (see Table 3). Immunofluorescent labeling for co-localization studies has been reported frequently by Balzar and colleagues,[12,30] Litvinov and colleagues,[18] Cirulli and colleagues,[39] and Winter and colleagues.[9] The staining can be scored easily; for normal squamous tissues, staining is negative, whereas premalignant lesions display positive cell membranes. For other epithelial cell types, aberrant cells show a more intense Ep-CAM positivity than normal tissue at the basolateral membranes. Furthermore, the cytoplasm and apical membranes can be positive as well in case of Ep-CAM overexpression.

To summarize the discussed studies, Ep-CAM expression can be detected at membranes of proliferating (epithelial) cells of colon, pancreas, mammary gland, lung, and regenerating liver, and is absent in normal liver, oral mucosa, gastric mucosa, skin, and uterine cervix. In those tissues with pre-existing Ep-CAM expression, Ep-CAM positivity is enhanced during neoplastic development. In tissues where Ep-CAM is absent in the normal situation, *de novo* expression of Ep-CAM indicates dysplasia or malignancy. The dysplastic squamous epithelia start to express Ep-CAM *de novo* at the basal layer. Tumors of epithelial origin virtually all express Ep-CAM at a high level, often the apical membrane is also Ep-CAM-positive. In some tumors, intracellular Ep-CAM-positive vesicles can be detected. Cervical dysplasia is correlated with Ep-CAM positivity; in low-grade dysplasia Ep-CAM is confined to the basal and parabasal cell layers, whereas in severe dysplasia the luminar cells are positive as well.

The association of Ep-CAM with metastases is less clear. One would expect to find higher Ep-CAM expression in metastasized cells, because these cells are more likely to escape the epithelium than well-differentiated cells anchored by E-cadherin-mediated junctions. Momburg and co-workers[25] demonstrated that micrometastases originating from carcinomas could be detected with for instance the HEA125 antibody. However, in nodal metastasis originating from head and neck squamous carcinomas, Ep-CAM expression was found to be reduced compared to the primary tumor.[23] In contrast, Chaubal and co-workers[24] concluded that Ep-CAM gene expression could be used as a useful tool to identify disseminated tumor cells. In both SCC and non-small cell cancer of the lung, Ep-CAM-positive cells were detected in the regional lymph nodes. In metastases from primary

tumors in the ovary, Ep-CAM expression is decreased. Although loss of Ep-CAM expression is associated with the progression of intestinal metaplasia, it is not an indicative marker for the presence of lymph node metastases in patients with adenocarcinoma of the stomach.

Ep-CAM expression in immunohistological diagnostics may have additional value over the use of Ki-67 in suspected neoplasias. While using Ki-67, proliferative cells can always be detected in the basal layers of squamous tissues, Ep-CAM positivity is only found in aberrant tissue. To discriminate between (hyper-) proliferative squamous tissue and premalignant squamous tissue, Ep-CAM is only expressed in the latter. In simple epithelia, Ep-CAM is always detectable on the basolateral sides of the cell. Premalignancies display overexpression of Ep-CAM and the apical membrane becomes Ep-CAM-positive as well, for instance in colon.

A useful application of Ep-CAM immunohistochemistry is to discriminate tumors of epithelial and nonepithelial origin. In human tissue, Ep-CAM is only expressed in epithelium or neoplasias from epithelial origin. Most squamous carcinomas are positive for Ep-CAM, except for squamous carcinoma of the skin. BCC can therefore be distinguished from SCC of the skin, squamous intraepithelial neoplasia, and actinic keratosis.[57,60] The positive staining pattern in BCC is a useful tool to locate latent BCC in inflammatory Mohs margins.[63] In liver, surprisingly, not all liver neoplasias are positive for Ep-CAM. Almost all analyzed cholangiocellular carcinomas were Ep-CAM-positive, whereas the majority of HCCs were not.[27,35,37,38] One of the two Ep-CAM-positive HCC cases in our own study was diagnosed as fibrolamellar carcinoma, a rare variant of primary liver carcinoma.[35] In combined type tumors consisting of a mixture of HCC and cholangiocellular carcinoma, only the cholangiocellular carcinoma areas react Ep-CAM-positive.[27] Sheibani and co-workers[64] used the Ber-EP4 mAb, which may have great use in the differential diagnosis of mesothelioma *versus* adenocarcinoma, particularly when only formalin-fixed tissue is available.

According to Friedman and co-workers,[65] using the combination of mAbs Ber-EP4, carcinoembryonic antigen, and vimentin are useful immunohistochemical markers in differentiating malignant mesotheliomas from adenocarcinomas, whereas immunohistochemistry does not reliably distinguish malignant from benign hyperplastic mesothelial cells. The addition of DNA ploidy studies is useful for differentiating the latter two groups. Roberts and colleagues[66] postulated that mesotheliomas, adenocarcinomas, and reactive pleura could only be accurately diagnosed with a panel of antibodies, in which the Ber-EP4 is only positive in adenocarcinomas. To distinguish peritoneal mesothelioma in women from serous papillary ovarian and peritoneal carcinoma, the use of Ber-EP4 is discriminative in contrast to other mesothelial markers thrombomodulin, cytokeratin 5/6, and CD44H and carcinoma markers polyclonal and monoclonal CEA, and Leu-M1.[67] Also, the AUA1 antibody was demonstrated to distinguish between carcinoma cells and mesothelial cells in serous effusion.[68]

Taken together the above-described findings, it is clear that expression of (epithelial) adhesion molecules may represent different stages in tissue development. Extending the definition of adhesion molecules to morphoregulating molecules is nowadays accepted. To diagnose disturbed or suspected lesions in epithelium, the expression pattern of epithelial adhesion molecule Ep-CAM can be of help. Normal, Ep-CAM-negative epithelia (squamous tissue) show a *de novo* expression in metaplasia, whereas an enhanced Ep-CAM expression can be found in other preneoplastic epithelia. The advantage of Ep-CAM staining over Ki-67 staining is described above. Also, Ep-CAM can serve in determining the tissue origin of tumors. Therefore, we conclude that Ep-CAM immunohistology proves to be a useful tool in the diagnostics of epithelial lesions.

Table 3: Staining for the Three Most Frequently Used Antibodies Directed against Ep-CAM

Antibody	Staining method	
	Paraffin	Cryostat
323/A3	*Fixation:* methanol (5 minute; Tr) and 0.3% H_2O_2 (20 minute; Tr)	*Fixation:* acetone (10 minute; Tr)
	Pretreatment: 0.1% trypsin (w/v) in 0.1% $CaCl_2$ (w/v) (20 minute; Tr)	*Pretreatment:* -
	Incubation 1st Ab: 1 to 5 μg/ml (overnight; Tr)	*Incubation 1st Ab:* 5 μg/ml in PBS/1.0% BSA (1 hour; Tr)
	Detection: two-step biotin/streptavidin system	*Detection:* two-step biotin/streptavidin system or envision
Ber-EP4	*Fixation:* formalin	*Fixation:* acetone (10 minute; Tr)
	Pretreatment: 0.1% trypsin (w/v) in 0.1% $CaCl_2$ (w/v) (20 minute; Tr)	*Pretreatment:* -
	Incubation 1st Ab: 6 μg/ml (overnight; Tr)	*Incubation 1st Ab:* 5 μg/ml (45 minute; 40 °C)
	Detection: two-step biotin/streptavidin system	*Detection:* Vectorstain Elite kit
KS1/4	*Fixation:* formalin	*Fixation:* 4.0% formaldehyde (20 minute; 4 °C)
	Pretreatment: 0.1% trypsin (w/v) in 0.1% $CaCl_2$ (w/v) (20 minute; Tr)	*Permabilization:* 0.1% saponin (5 minute; Tr), 50 mM glycine in PBS
	Incubation 1st Ab: 5 to 10 μg/ml (overnight; Tr)	*Pretreatment:* -
		Incubation 1st Ab: 5 μg/ml (1 hour; Tr)
	Detection: two-step biotin/streptavidin system	*Detection:* immunofluorescent labeled 2nd Ab

Tr: room temperature.

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