J Mol Med (1999) 77:699–712
Digital Object Identifier (DOI) 10.1007/s001099900038

REVIEW

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The biology of the 17–1A antigen (Ep-CAM)

Received: 7 June 1999 / Accepted: 27 July 1999 / Published online: 13 October 1999

Abstract The glycoprotein recognized by the monoclonal antibody (mAb) 17–1A is present on most carcinomas, which makes it an attractive target for immunotherapy. Indeed, adjuvant treatment with mAb 17–1A did successfully reduce the 5 years mortality among colorectal cancer patients with minimal residual disease. Currently the antibody is approved for clinical use in Germany, and is on its way to approval in a number of other countries. New immunotherapeutic strategies targeting the 17–1A antigen are in development or even in early-phase clinical trials. Therefore, a better understanding of the biology of the 17–1A antigen may result in improved strategies for the treatment and diagnosis of human carcinomas. In this review the properties of the 17–1A antigen are discussed concerning tumor biology and the function of the molecule. This 40-kDa glycoprotein functions as an Epithelial Cell Adhesion Molecule, therefore the name Ep-CAM was suggested. Ep-CAM mediates Ca^{2+}-independent homotypic cell–cell adhesions. Formation of Ep-CAM-mediated adhesions has a negative regulatory effect on adhesions mediated by classic cadherins, which may have strong effects on the differentiation and growth of epithelial cells. Indeed, in vivo expression of Ep-CAM is related to increased epithelial proliferation and negatively correlates with cell differentiation. A regulatory function of Ep-CAM in the morphogenesis of epithelial tissue has been demonstrated for a number of tissues, in particular pancreas and mammary gland. The function of Ep-CAM should be taken into consideration when developing new therapeutic approaches targeting this molecule.

Key words Carcinoma · Cell adhesion · CO17–1A · Epithelium · Ep-CAM · GA733

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Introduction

The monoclonal antibody (mAb) (CO)17–1A was one of the first mAbs generated against an antigen that is often present at the cell surface of human carcinoma cells [1, 2]. Since mAb 17–1A recognizes a tumor-associated antigen expressed by the majority of human epithelial neoplasias, the 17–1A antigen attracted major attention as a target for immunotherapy to combat human carcinomas [3, 4, 5, 6]. As early as the 1990s, hundreds of patients were being treated with the 17–1A mAb, but the treatment applied as various forms was not effective against solid tumors. However, the post-operative treatment of colorectal cancer patients gave promising results, since adjuvant treatment with 17–1A successfully reduced the 7 years mortality among Duke's C colorectal cancer patients with minimal residual disease [5, 6]. In line with these results, the antibody was approved in 1995 for routine clinical use in Germany. Moreover, other immunotherapy strategies targeting the 17–1A antigen are in the process of development, suggesting that in the near future this molecule will be (widely) targeted in new clinical trials to combat carcinomas.

The 40-kDa glycoprotein recognized by 17–1A has been described by various names originating from the name of the respective mAb (e.g. MH99, AUA1, MOC31, 323/A3, KS1/4, GA733, HEA125, etc.) that was raised against the molecule [1, 7, 8, 9, 10, 11, 12]. The corresponding cDNA was independently cloned by a number of groups using different names (e.g. KSA,

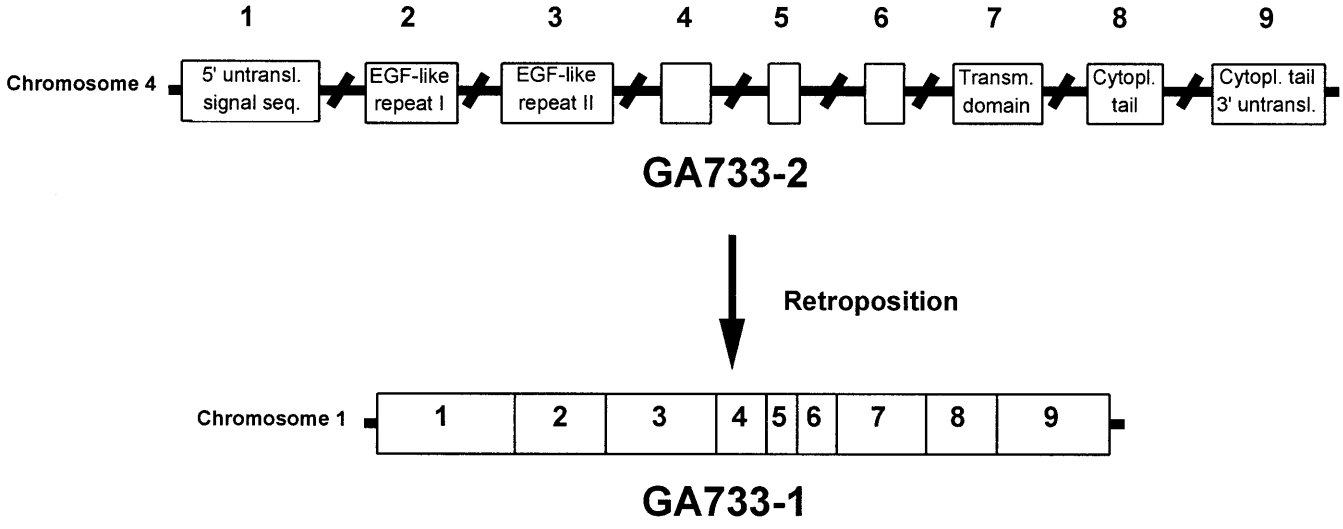

Fig. 1 Structure of the *GA733–2* and *GA733–1* genes (after Linnenbach et al. [19]). The *GA733–2* gene, encoding the human transmembrane glycoprotein Epithelial Cell Adhesion Molecule (*Ep-CAM*), consists of a total of nine exons located on chromosome 4. The retroposition of *GA733–2* mRNA into chromosome 1 resulted in the intronless GA733–1 gene

EGP, EGP40, GA733–2) for the encoded molecule [13, 14, 15, 16]. A few years ago, we demonstrated that the 17–1A antigen can function as a homophilic cell–cell adhesion molecule, and suggested the name Ep-CAM (Epithelial Cell Adhesion Molecule), which reflects its tissue specificity and function [17, 18]. This review discusses the properties of the 17–1A antigen, alias Ep-CAM. Recent findings indicate that this molecule has a major morphoregulatory function, relevant not only to epithelial tissue development, but also carcinogenesis and tumor progression. A better understanding of the biology of the 17–1A antigen may prove useful in developing improved strategies for the treatment and diagnosis of human carcinomas. The name Ep-CAM will be used further in this review irrespective of the designation chosen for this molecule by the original group whose data are discussed.

The gene

Ep-CAM is encoded by the *GA733–2* gene [19]. The human gene with a minimal estimated size of approximately 14 kilobases (kb) is located on chromosome 4. The *GA733–2* locus maps to chromosomal region 4q. The putative *GA733–2* promoter contains consensus binding sites for the *Sp1* and *AP*–1 transcription factors, but no TATA or CAAT boxes were found [19]. Comparison between genomic and cDNA sequences reveals that the *GA733–2* gene consists of nine coding exons as depicted in Fig. 1 [19]. Amino acid sequences encoded by exon 1 correspond to a signal peptide sequence. Exons 2 to 6 encode for sequences that form the extracellular domain. The transmembrane region is encoded by exon 7. Exon 8 encodes a 15-amino-acid portion of the cytoplasmic domain, including a cluster of six positively charged amino acids. Exon 9 encodes the remaining 13 amino acids of the cytoplasmic domain, the stop codon, and the 3'-untranslated region.

The transcribed mRNA is approximately 1.5 kb. The 5'-untranslated regions from independently isolated Ep-CAM mRNAs revealed a variation in the size of this region, containing either 16 or 160 bases [13, 15]. All reported sequences for Ep-CAM mRNAs were identical in their coding part, namely an open reading frame of 942 bases. The 3'-untranslated region contains two possibly important motifs [13]. The first sequence (ATTTA) has been proposed to be a signal for specific degradation of mRNA for cytokines, lymphokines, and some proto-oncogenes [20]. The second sequence (TTATTTAT) has been identified as a consensus sequence in the 3' region of the inflammatory mediators [21]. This suggests that the expression of Ep-CAM may be highly regulated at the mRNA level. We have analysed Ep-CAM mRNAs from a large number of carcinoma cell lines, but found no variations in the splicing of exons encoding the intracellular domain of the molecule (unpublished results). Moreover, no mRNA splice variants that encode different isoforms of Ep-CAM have been reported so far.

The *GA733* gene family

The only molecule known to be homologous to Ep-CAM is the GA733–1 gene product (or EGP-1/Trop-2), which shares 49% homology with the Ep-CAM amino acid sequence. Taking into account conserved substitutions, the two antigens have a similarity of 67% [16]. Ep-CAM and GA733–1 share two highly homologous regions [16]. The first region spans 39 residues of the extracellular domain, and is characterized by 79% identical amino acids and 97% similarity. The second region represents the transmembrane domain. The combined results of molecular cloning, DNA sequencing, amino acid sequence comparison, and chromosome mapping studies indicate

that both exon shuffling and retroposition have been factors in the evolution of the *GA733* gene family [19]. The intronless *GA733–1* gene probably originates from the retroposition of Ep-CAM mRNA into a region on chromosome 1 as depicted in Fig. 1. The retroposition event, which resulted in the *GA733–1* gene, most likely preceded the divergence of avian and mammalian species approximately 300 million years ago [19]. After retroposition, the GA733–1 evolved independently of Ep-CAM, and may be viewed as a molecule that belongs to the GA733 family rather than a protein closely related to Ep-CAM.

The molecule encoded by the *GA733–1* gene is slightly larger than Ep-CAM [19]. The polypeptide backbone contains four N-linked glycosylation sites that result in a 50-kDa glycoprotein. The molecule can be phosphorylated within its cytoplasmic tail at serine 303, which is not present in the Ep-CAM cytoplasmic domain [22]. The conserved tyrosine residue that is present in both molecules has never been reported to be phosphorylated. Phosphorylation of the cytoplasmic serine residue suggests a possible role for GA733–1 as a cell surface receptor involved in signal transduction. Indeed, stimulation of human carcinoma cells with anti-GA733–1 mAbs induces an intracellular calcium signal [23].

At least five mAbs are known that specifically recognize GA733–1, namely RS7, MR23, 162–46.2, MOv 16, T16 [23, 24, 25]. The five mAbs do not reveal cross-reactivity with Ep-CAM epitopes (our unpublished result; [23]). Moreover, no mAb specific for Ep-CAM has been shown to react with the GA733–1 protein.

The GA733–1 molecule is also mainly expressed in epithelial tissue. GA733–1 expression levels are low in tissues with relatively high levels of Ep-CAM expression, such as colon and lung tissue [24]. In contrast, GA733–1 is expressed at relatively high levels in Ep-CAM-negative epithelium. In skin, the GA733–1 molecule is present in supra-basal and spinous layers of the epidermis, with increased expression levels in the more differentiated cells [26]. It is also interesting that a substantial decrease in GA733–1 expression was detected in breast carcinoma cell lines as compared to immortalized normal epithelium (our unpublished results), but the significance of this is unclear. Recently, the *GA733–1* gene was genetically linked to the autosomal recessive disorder Gelatinous Drop-Like corneal Dystrophy (GDLD), since 33 out of 40 disease alleles (83%) in a panel of Japanese families were reported to have deleterious mutations causing truncation of the GA733–1 protein [27]. The function of GA733–1, possibly overlapping with that of Ep-CAM, is unknown and requires further investigation.

Evolutional conservation of Ep-CAM

Using Southern blot analysis, sequences homologous to the human *GA733–1* and *GA733–2* genes were detected in monkey, mouse, hamster, and chicken [19]. Polyclonal antibodies directed against human Ep-CAM detected homologous proteins in mice, rats, and non-human primates [28]. MAbs 17–1A and 323/A3 cross-react with hamster and rhesus monkey Ep-CAM respectively (our unpublished results). Thus, Ep-CAM seems to be highly conserved among high vertebra, which is even better illustrated by the homology between mouse and human Ep-CAM. The nucleotide sequence of mouse Ep-CAM is 80% identical to human Ep-CAM. The homology of the amino acid sequence is even higher, namely 82% [29].

Tissue pattern expression

Expression during embryonic development

Relatively little information is available concerning the expression of Ep-CAM during human embryonic development. Ep-CAM expression is detected in fetal lung, kidney (proximal tubules), liver, pancreas, skin, and germ cells. At different stages of lung development, epithelial cells express Ep-CAM [30]. As early as the embryonic stage (week 7–8 embryo), relatively high levels of Ep-CAM are expressed by the epithelial cells of the primordial lung. In fact, during the remaining period of development (pseudoglandular, canalicular, and alveolar stage), and throughout adult life, epithelial cells of the lung reveal immuno-reactivity for Ep-CAM [30]. The majority of hepatocyte precursors are Ep-CAM positive in the liver of week 8 embryos, but negative in adult liver [31]. During pancreatic development Ep-CAM is co-expressed with N-CAM and E-cadherin in week 18 embryos [32]. Moreover, it was reported that in fetal pancreas the highest level of Ep-CAM expression was detected in developing islet-like cell clusters budding from the ductal epithelium [33]. These cell clusters with up-regulated Ep-CAM expression (during development) are thought to become the early endocrine cells. In fetal skin of 8–12 week embryos, keratinocytes are Ep-CAM positive, and during development of the hair follicle high levels of Ep-CAM expression were detected (our unpublished results). For the formation of the primitive sex cords in humans, Ep-CAM is expressed at the embryonic stages by germ cells during migration at day 10.5 and early gonad assembly at day 12.5 [34]. In human embryos, no Ep-CAM expression was observed in the thymus, but in mice Ep-CAM is expressed in thymus epithelium and thymocytes in adult tissue, and at even higher levels in fetal thymus [35, 36].

In studies using the mAbs GZ1, GZ2, and GZ20, which recognize rat Ep-CAM, the molecule was reported to be expressed during the very early stages of embryogenesis [37, 38, 39]. Immunocytochemistry revealed the expression of Ep-CAM in fertilized oocytes, the two-cell stage, and some cells of the morula. At the blastocyst stage, the zona pellucida showed no Ep-CAM staining, whereas the trophoblast and inner-cell mass were positive. Rat embryos of 8.5 days (at the stage of the three germ layers) revealed positive staining for both the ecto-

Table 1 Distribution of Ep-CAM in normal human adult tissues. The level of Ep-CAM expression by specific cell types within epithelial tissues is indicated by – for no expression, + for low expression levels, ++ for intermediate expression levels, and +++ for high expression levels. Data on distribution and expression levels were summarized from multiple studies using different Ep-CAM-specific monoclonal antibodies including unpublished data from own laboratory

Tissue	+/–	Distribution
Oral cavity	+	Stratified squamous epithelial mucosa –, secretory cells +
Oesophagus	+	Stratified squamous epithelium –, columnar epithelium +/–
Stomach	+	Mucosa +
Duodenum, jejunum, ilium	+	Mucosa ++
Colon, rectum	+	Mucosa +++
Salivary gland	+	Acinar cells +, duct cells +, basal and suprabasal cells +, squamous epithelium –
Pancreas	+	Ductal epithelium ++, acini ++, islets +
Liver	+	Bile ducts ++, Bile canaliculi +/–, Hepatocytes –
Gallbladder	+	Glandular epithelium ++
Trachea	+	Mucosa ++
Bronchi	+	Mucosa ++,
Lung acini	+	Ciliated bronchioli epithelium +, alveolar duct +, alveoli (pneumocytes) +
Skin	+/–	Keratinocytes –, melanocytes –, hair follicle +, sweat gland +, dermis –
Kidney	+	Distal tubules ++, proximal tubules +, collecting ducts ++, Henle's loop +, Bowman's capsule +, Capillary tufts –
Ureter	+	Transitional epithelium +
Bladder	+	Transitional epithelium (urothelium)+
Urethra	+	Transitional epithelium +
Thyroid gland	+	Follicular epithelium ++, C cells +
Parathyroid gland	+	Chief and oxyphil cells ++
Adrenal gland	+	Cortical epithelium +, medullary chromaffin cells –
Pituitary gland	+	Adenohypophyseal cells ++, pituicytes –
Testis	–	Some spermatogonia +
Epididymis	+	Ciliated, basal, and cuboidal cells ++
Prostate, seminal vesicles	+	Secretory, basal, and ductal cells ++
Ovary	+	Oocytes ++, follicular epithelial cells –
Oviduct	+	(Non-)ciliated cells +
Uterus/cervix	+	Endometrium ++, myometrium –, endocervical glands ++, ectocervical squamous epithelium –
Mammary gland	+	Ductal epithelium ++, alveoli +
Thymus	+	Medullary epithelium +, Hassal bodies +, cortical epithelium –
Tonsil	+	Crypt epithelium +
Spleen	–	
Lymph node	–	
Bone-marrow-derived cells	–	
Skeletal muscle	–	
Brain	–	
Vessels	–	
Connective tissue	–	

derm and entoderm, while the mesoderm was negative. At day 11.5, rat embryos expressed Ep-CAM in all epithelia including those of mesodermal origin, such as the Wolffian duct and coelomic epithelium. In contrast, neural and connective tissue revealed no Ep-CAM expression. Embryos of 14.5 days showed expression in all epithelia, except the Müllerian duct. Finally, day 17.5 embryos expressed Ep-CAM in all epithelia including glandular tissue such as pancreas anlage and thyroid, whereas neural tissue, lymphatic organs, muscular tissue, liver parenchyma, and the gonads remained negative [39]. These studies suggest an important role for Ep-CAM in embryonic development. However, to evaluate the importance of Ep-CAM during embryogenesis or the morphogenesis of individual tissue, further studies (especially with tissue-targeted gene disruption) are required.

Expression in normal adult tissues

Based on immunohistochemical data, Ep-CAM is a strictly epithelial molecule in adult humans (Table 1). Ep-CAM is detected at the basolateral cell membrane of all simple (especially glandular), pseudo-stratified, and transitional epithelia. In contrast, normal squamous stratified epithelia are negative for Ep-CAM. In adult human tissues no expression was found in mesenchymal, muscular, and neuro-endocrine tissues [12, 40]. Furthermore, no Ep-CAM expression was detected in cells of lymphoid origin. The level of expression may differ significantly between the individual tissue types. In the gastrointestinal tract, the gastric epithelium expresses very low levels of Ep-CAM. Expression levels are substantially higher in small intestine, and in colon Ep-CAM is probably expressed at the highest levels among all epithelial cell types. Glandular epithelium of the gall-bladder express Ep-CAM [12], but the transitional epithelium (uro-

thelium) of the bladder is only slightly positive [41]. In the lower respiratory tract, the trachea, bronchi, bronchioles, and alveoli are Ep-CAM positive [12]. In adult liver the bile ducts are Ep-CAM positive, whereas hepatocytes are negative [31]. Most epithelial cells of the kidney, such as cells of the proximal tubules, distal tubules, and ducts, express Ep-CAM [12]. In pancreas Ep-CAM expression has been detected in the ductal epithelium, acini, and there is some expression in the islets [12, 33]. In skin, the sweat ducts and the proliferative zone of the hair follicle reveal Ep-CAM staining, whereas keratinocytes and melanocytes are mainly negative [12, 42]. Within the basal layers of the epidermis some Ep-CAM reactivity can be observed in the reserve cells, since mAb MH99 was reported to be reactive with some cells within the basal layer of skin keratinocytes [26]. The glands of the endocrine system (thyroid, parathyroid, pituitary and adrenal glands) contain Ep-CAM-positive epithelium [12]. In mammary glands, the ductal epithelium reveals relatively high levels of Ep-CAM expression as compared to the lower levels in alveolar epithelium [10, 12]. Ep-CAM expression is detected in most epithelial tissues of the female genital tract (ovaries, oviducts, cervix, and uterus). Normal endocervical glandular epithelium (both columnar and reserve cells) reveals high expression levels of Ep-CAM, whereas ectocervical squamous epithelial cells do not express the molecule [43]. Some Ep-CAM expression may be detected in the basal cells of morphologically normal ectocervical tissue, but only in areas bordering lesions of cervical intraepithelial neoplasia [43]. In tissues of the male genital tract, some of the epithelial cells in testis, epididymis, seminal vesicle, and prostate reveal Ep-CAM expression [12].

Table 2 Ep-CAM expression in human malignant neoplasias. Most carcinomas express Ep-CAM, whereas tumors derived from non-epithelial tissues are Ep-CAM negative. The level of Ep-CAM expression by tumor cells is indicated by – for no expression, + for low expression levels, ++ for intermediate expression levels, and +++ for high expression levels. Data on distribution and expression levels were obtained from multiple studies using different Ep-CAM-specific monoclonal antibodies and reflect the pattern of Ep-CAM expression rather than a reactivity of a particular antibody

Type of tumor	Ep-CAM expression
Oral mucosal carcinoma:	
Basal cell carcinoma	++
Squamous cell carcinoma	+
Laryngeal (squamous cell) carcinoma	+
Esophageal (squamous cell) carcinoma	+
Gastric adenocarcinoma	++
Carcinoma of small intestine	+++
Colorectal adenocarcinoma	+++
Pancreatic carcinoma	++
Liver carcinoma:	
Hepatocellular carcinoma	–
Cholangiocarcinoma	++
Biliary duct carcinoma	++
Lung carcinoma	+++
Skin carcinoma:	
Basal cell carcinoma of skin	++
Squamous cell carcinoma of skin	–
Renal cell carcinoma	++
Transitional cell carcinoma of bladder	++
Thyroid carcinoma	++
Prostate carcinoma	++
Ovarian carcinoma	++
Endometrium carcinoma	++
Cervical carcinoma:	
Squamous cell carcinoma of cervix	++
Adenocarcinoma of cervix	+++
Mammary carcinoma	++
Mesotheliomas:	
Non-epithelioid	–
Epithelioid	++
Germ cell tumour	+/–
Wilms' tumor (epithelial component)	+/–
Melanoma	–
Sarcoma	–
Lymphoma	–
Meningioma	–

Correlation with benign and malignant tumor development

Active proliferation in a number of epithelial tissues is associated with increased or de novo Ep-CAM expression [41, 43, 44]. This is especially evident in tissues that normally reveal no or low levels of Ep-CAM expression, such as squamous epithelium. At the early stages of neoplasias of the uterine cervix, de novo expression of Ep-CAM is often observed in areas with atypical, undifferentiated cells of the squamous epithelium [43]. Thus, in cervical intraepithelial neoplasia (CIN) grades I and II, the basal and suprabasal cells are Ep-CAM positive, while grade III lesions reveal up to 100% positive cells in all layers of the squamous epithelium. Moreover, a clear increase in both the number of positive cells and the level of Ep-CAM expression is observed during the progression from CIN I to CIN III. Expression of Ep-CAM in atypical cells of CIN lesions correlated with the disappearance of markers for squamous differentiation and enhanced proliferation. In weak, mild, and severe oral mucosal dysplasias high levels of Ep-CAM expression were detected in basal and suprabasal cells with a clear border between Ep-CAM-positive dysplastic cells and Ep-CAM-negative normal epithelial cells [44].

In glandular epithelium of the gastrointestinal tract, one can observe a clear gradient of decreasing expression of Ep-CAM from crypts to villae [37, 38]. The level of Ep-CAM expression correlates with the proliferative activity of intestinal cells, and inversely correlates with their differentiation [45]. Dysplastic or metaplastic proliferation corresponds to an increase (sometimes to very high levels) in Ep-CAM expression. In gastric epithelium that normally expresses low levels of Ep-CAM, a strong expression of Ep-CAM is observed in proliferative metaplastic lesions, such as intestinal metaplasia (unpublished results). Even in colon, where the epitheli-

um expresses the highest levels of Ep-CAM, the development of polyps is reported to be associated with an increased expression of the molecule. Hepatocytes are Ep-CAM positive during embryonic development (week 8 embryos), but negative in adult liver [31]. However, during liver regeneration processes cells that morphologically resemble precursor stem cells are Ep-CAM positive, but, as they mature into hepatocytes, they again become Ep-CAM negative [31]. Dysplastic lesions of the bladder epithelium (urothelium) reveal increased Ep-CAM expression as compared to normal urothelium [41].

Malignant proliferation is nearly always associated with Ep-CAM expression at some stage of tumor development. Most carcinomas, but no other tumor types, express high levels of Ep-CAM (Table 2). However, Ep-CAM expression in carcinomas can be heterogeneous, and is probably affected by a shift of tumor cell differentiation to either mesenchymal or squamous (in squamous carcinomas) cell phenotypes. It has been reported for dysplastic oral mucosa that well-differentiated squamous cell carcinomas are negative for Ep-CAM, whereas poorly differentiated squamous cell carcinomas are Ep-CAM positive [44]. Most squamous carcinomas are Ep-CAM positive, except for (Ep-CAM negative) squamous carcinoma of the skin. The expression of Ep-CAM distinguishes squamous cell carcinoma of the skin from the Ep-CAM-positive basal cell carcinoma [46]. Varying levels of Ep-CAM expression were detected in the majority of squamous and adenocarcinomas of the uterine cervix [43]. However, as compared to CIN III, some decrease in the expression (both intensity and number of positive cells) is observed in areas of squamous differentiation.

Ep-CAM as a marker protein for differential diagnosis

In human tissue Ep-CAM is expressed only in epithelium and neoplasias derived from epithelia. Therefore, the molecule may be used as a marker to distinguish epithelial neoplasias from neoplasias derived from non-epithelial tissues. Ep-CAM-positive tumors are derived from epithelial cells, whereas Ep-CAM-negative tumors may originate from non-epithelial as well as epithelial tissues. Furthermore, Ep-CAM may be used as a marker to histologically differentiate between epithelial neoplasias. Occasionally, difficulties in the histological differential diagnosis between basal-cell carcinoma (BCC) and squamous-cell carcinoma (SCC) of the skin may arise. Basal squamous cell epithelioma, a tumor combining morphological properties of BCC and SCC, is one common example of these difficulties, but other histological types of BCC may also be erroneously interpreted as SCC [46]. Staining for Ep-CAM demonstrated that all BCCs are diffusely and intensely labelled, whereas none of the SCCs expressed Ep-CAM, irrespective of the histological type or grade of differentiation [46]. In liver neoplasias, Ep-CAM was found to be expressed in almost all cholangiocarcinomas, whereas the majority of hepatocellular carcinomas were Ep-CAM negative, suggesting that the hepatocellular carcinoma originates from a highly differentiated precursor [31]. The results also indicate that Ep-CAM can be used as an additional immunohistochemical marker to distinguish cholangiocarcinoma from hepatocellular carcinoma due to the differential expression in these epithelial tumors. Finally, it was demonstrated that Ep-CAM can be used as a marker to discriminate carcinomas from Ep-CAM-negative mesotheliomas, except for the epithelioid type [47].

It has been demonstrated for SCCs of the head and neck that expression of the classic cell adhesion molecule E-cadherin correlates inversely with lymph node metastasis [48]. It is also reported that E-cadherin functions as an invasion suppressor molecule [49]. Since Ep-CAM functions as an intercellular adhesion molecule, like E-cadherin, it has been claimed that the expression might reduce the metastasizing capability of tumor cells, and correlate with a better prognosis of carcinoma patients [50]. Thus, Ep-CAM may also be useful as a marker for increased malignancy potential in the diagnosis of cancer patients. Indeed, a lack of Ep-CAM expression in the primary tumor of laryngeal carcinomas significantly correlated with nodal metastasis [51]. Furthermore, the expression of Ep-CAM increases from baseline levels in normal oral epithelium to high levels in mild, moderate, and severe dysplasias, but both invasive well-differentiated and invasive poorly differentiated SCC revealed reduced or no Ep-CAM expression [44]. In contrast, Ep-CAM was reported to function as a marker of increased malignant potential in transitional cell carcinoma of the bladder, since the percentage of cells expressing Ep-CAM increases from grade I to grade III [41]. Furthermore, in a large cohort of primary breast tumors, high levels of Ep-CAM expression correlated with larger tumor size and infiltrated lymph nodes [52]. Thus, it seems that Ep-CAM plays a dual role in tumorigenesis, which requires further investigation.

Immunohistochemical detection

The results obtained by immunohistochemical analysis of human tissues with a variety of mAbs against Ep-CAM were approximately identical. All published antibodies react with cryostat tissue sections, and most of them do not lose their reactivity to the antigen after paraformaldehyde (4%) fixation of tissues, but are sensitive to glutaraldehyde or formalin fixation and tissue embedding in paraffin. A simple antigen retrieval procedure (treatment with trypsin/pronase) allows immunohistochemical staining with anti-Ep-CAM antibodies of formalin-fixed, and paraffin-embedded tissues. Some of the mAbs were reported to have reactivity with Ep-CAM on routinely fixed histological material without pre-treatment, but the reactivity was substantially reduced. The areas of relatively low Ep-CAM expression that are well

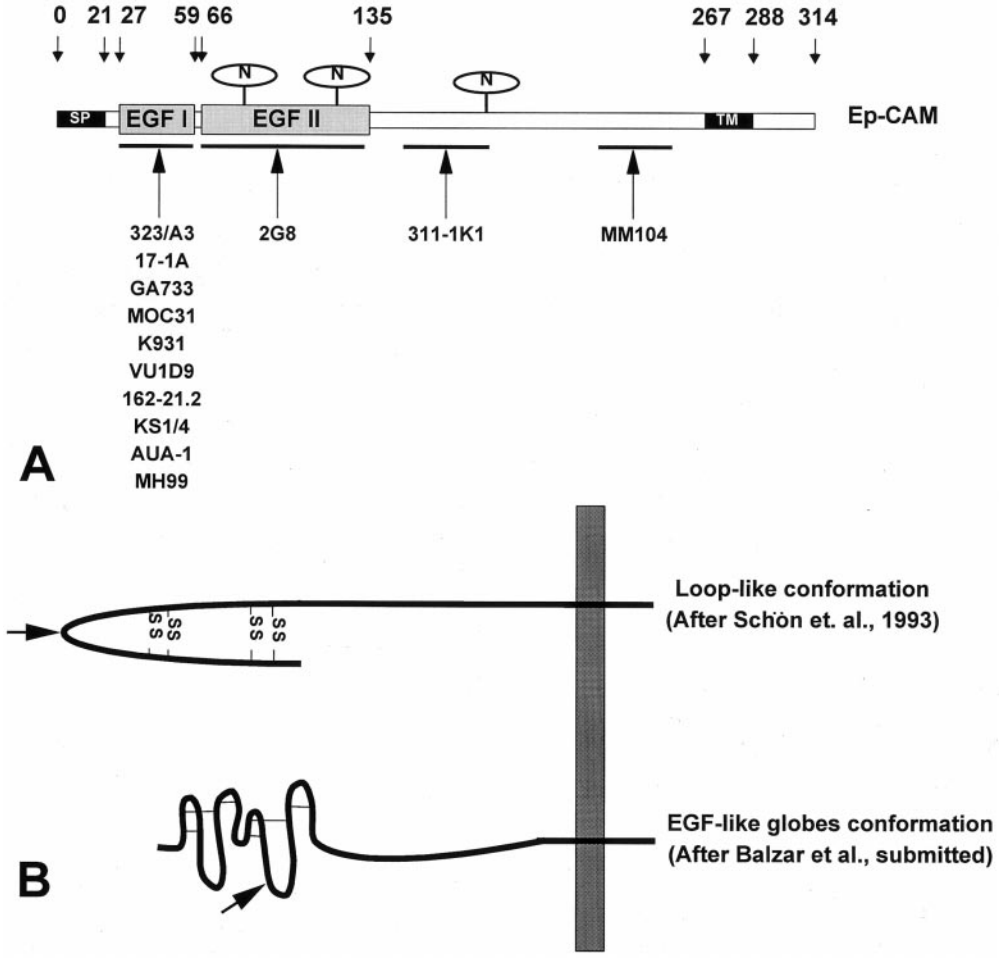

Fig. 2A,B Structure of Ep-CAM. **A** The Ep-CAM polypeptide consists of 314 amino acids. The *numbers* above the *short black arrows* indicate the amino acid residues. The *encircled N* indicates sites for N-glycosylation. *Below* are given the epitopes recognized by the indicated Ep-CAM-specific monoclonal antibodies (*mAbs*). **B** Conformation of the Ep-CAM protein. The di-sulphide bridges formed by cysteine residues within the N-terminal half of the extracellular domain may result in two conformational models for the Ep-CAM molecule: a loop-like conformation (as suggested by Schön et al. [53]), and a conformation based on correct folding of the EGF-like repeats. The new data strongly point to the correctness of the latter model (Balzar et al., submitted). *Black arrow* indicates the potential proteolytic cleavage site at the position of Arg80

detected on frozen sections may be negative in trypsin pre-treated formalin sections, whereas the areas of high Ep-CAM expression are well detected in pre-treated formalin sections (unpublished results). Moreover, histological data obtained with low-affinity mAbs (e.g. 17–1A or AUA-1) may differ from the results obtained with high-affinity mAbs (e.g. 323A3 or GA733), as mainly the areas of high expression levels are detected with low-affinity mAbs in immunohistochemistry.

The Ep-CAM molecule

The largest open reading frame of Ep-CAM encodes for a 314-amino-acid polypeptide (Fig. 2A), which contains a 23-amino-acid leader sequence, a 242-amino-acid extracellular domain with two epidermal-growth-factor-like (EGF-like) repeats within the cysteine-rich N-terminal part, a 23-amino-acid transmembrane domain, and a 26-amino-acid cytoplasmic domain.

The extracellular domain

When mice are immunized with cells derived from human carcinomas, the extracellular domain of the Ep-CAM molecule is one of the most immuno-dominant epitopes at the cell surface. This has resulted in a large number of mAbs being developed against tumor-associated antigens which are specifically reactive with the Ep-CAM extracellular domain. Figure 2A presents a list of monoclonal antibodies directed against epitopes on the Ep-CAM molecule. Studies with mutant Ep-CAM molecules show that all known mAbs have their epitopes located within one of the structural domains of the Ep-CAM extracellular domain (Balzar et al., submitted). Thus, mAbs 17–1A, 323/A3, KS1/4, GA733, MOC31, VU1D9, K931 all react with one of the partially overlapping epitopes in the first EGF-like repeat. Previous cross-inhibition studies showed that these mAbs can be subdivided into three major groups according to epitopes [53]. MAb 2G8 recognizes the second EGF-like repeat, while mAbs MM104, and 311–1K1 react with the cysteine-poor region.

The 265-amino-acid-containing extracellular domain starts at the N-terminus with a signal sequence containing 11 hydrophobic residues. Signal peptidase cleavage probably occurs seven residues after the core sequence, between alanine 23 and glutamine 24 [16]. Sequence analysis of the Ep-CAM extracellular domain shows that the N-terminal cysteine-rich half contains two EGF-like repeats with the motifs $CX_1CX_8CX_7CX_1CX_{10}C$ (position 27–59) and $CX_{32}CX_{10}CX_5CX_1CX_{16}C$ (position 66–135),

followed by a cysteine-poor domain (Fig. 2A). The first and second EGF-like repeats of Ep-CAM are closely related to, respectively, the fourth and fifth EGF-like repeats within the rod-like domain of nidogen (positions 776–809 and 819–889), a laminin-binding extracellular matrix protein [15, 54]. Within the second EGF repeat there is a high homology region between Ep-CAM and nidogen molecules that is not limited by cysteine residues only. The second EGF-like repeat of Ep-CAM also shows homologies to the thyroglobulin type I repeat (96–160; $CX_{23}CX_{10}CX_6CX_1CX_{19}C$) which can also be found in placental protein 12 (150–225; $CX_{29}CX_{10}CX_{10}CX_1CX_{20}C$), and an inhibitor of cysteine proteinases, Equistatin, isolated from the sea anemone *Actinia equina* [55].

In contrast to the fourth repeat of nidogen, the first repeat of Ep-CAM does not contain a potential β-hydrohylation site, which makes Ca^{2+} binding by Ep-CAM highly unlikely. According to their structure and some consensus motifs, the EGF-like repeats can be subdivided into three major types, I, II, and III [56]. The EGF-like repeats of Ep-CAM reveal the closest homology to repeats of types II and III, which are also present in molecules such as nidogen, EGF precursors, the LDL receptor, L-selectin, and PECAM [56, 57]. The high homology between the EGF-like repeats in Ep-CAM and the rod-like domain of nidogen would predict an Ep-CAM extracellular domain structure as depicted in Fig. 2B. This structure differs from a loop-like structure as was previously suggested [53, 58, 59]. However, when the EGF-like repeats of Ep-CAM are expressed individually, they are properly folded and reproduce the epitope profile of the respective repeat within the intact molecule (Balzar et al., submitted). Furthermore, as was demonstrated with extracellular domain deletion mutants, the repeats function as independent modules in adhesive interactions of Ep-CAM molecule (Balzar et al., submitted). This strongly suggests that the modular structure, analogous to the respective region of the nidogen rod-domain, is the conformation of the Ep-CAM molecule (Fig. 2B).

Many transmembrane molecules with EGF-like repeats are involved in cell adhesion or signalling [57]. Structural analysis of Ep-CAM suggested possible involvement of Ep-CAM in either cell–substrate or cell–cell adhesion [15]. Ep-CAM was also frequently proposed to function as a growth factor receptor, but no actual experimental data have ever been presented to support this.

The sequence of the Ep-CAM molecule predicts the presence of three potential N-linked glycosylation sites (Fig. 2A). It is likely that all three sites are used, and that the attached carbohydrates contain mannose oligosaccharide chains [8, 53]. Blocking N-linked glycosylation using tunicamycin results in a single polypeptide chain of approximately 34 kDa [58]. Since most carcinoma cell lines produce multiple Ep-CAM forms of approximately 38, 40, and 42 kDa [17], the polypeptide backbone may be differently glycosylated. Whether the different Ep-CAM forms differ functionally has never been reported. Treatment of cells with O-glycanase does not cause changes in the molecular weight of the Ep-CAM molecule, suggesting that no O-linked glycosylation is involved [58].

The Ep-CAM molecule may exist as two additional post-translationally modified variants, namely a cleaved and a non-cleaved form. Pulse-chase studies have shown that Ep-CAM is synthesized as a 34-kDa protein, which is glycosylated to a 40-kDa glycoprotein. After longer chase periods a 32-kDa Ep-CAM form appears, which is derived from the 40-kDa form by proteolysis [58]. As is depicted in Fig. 2B, at the position arginine 80 of the Ep-CAM amino acid sequence a potential cleavage site is present for trypsin-related proteolytic enzymes [16, 60]. After cleavage, the two fragments remain covalently cross-linked via disulphide bridges under native conditions, whereas reduction of the disulphide bridges generates a 6-kDa and 32-kDa peptide fragment. Epithelial- and carcinoma-derived cell lines reveal different degrees of cleavage (our unpublished results; [58]). Some cell lines contain only the 40-kDa non-cleaved Ep-CAM form, while others reveal both the 32-kDa and 40-kDa Ep-CAM protein. Moreover, the incubation of lysates from cell lines with only the 40-kDa Ep-CAM protein in the presence of lysates from cell lines having both Ep-CAM forms results in the conversion of the 40-kDa non-cleaved protein into a 32-kDa molecule [58]. Although cell-type-specific cleavage might be a mechanism for generating specific differences in cell surface glycoproteins, the significance of cleavage for the function of Ep-CAM has never been demonstrated.

The transmembrane and cytoplasmic domains

The transmembrane region contains 23 hydrophobic amino acid residues, and is followed by a relatively short 26-residue highly charged cytoplasmic domain. The cytoplasmic domain contains the internalization motif NPXY, which has been identified in many cell surface receptors, such as the LDL receptor and mannose 6-phosphate receptor [61]. The tyrosine residue present in the internalization motif is conserved in the Ep-CAM and GA733–1 cytoplasmic tails. Although GA733–1 can be phosphorylated at serine 303, the unique tyrosine residue that is present in the tails of both Ep-CAM and GA733–1 has never been reported to be phosphorylated (our unpublished results; [22]).

Besides the internalization motif, the relatively short cytoplasmic tail of Ep-CAM also contains two α-actinin-binding sites [62]. Motif comparison in the cytoplasmic tails of molecules interacting with α-actinin reveal no clear similarities, except for the arginine- and lysine-rich consensus that can be found in the first Ep-CAM α-actinin-binding site (RKKRMAK), and the α-actinin-binding sites in ICAM-1 (RKIKK) and L-selectin (RRLKKGKKSKR) [62]. The involvement of the cytoplasmic domain for Ep-CAM function is further discussed below.

Ep-CAM-mediated cell–cell adhesion

When Ep-CAM is expressed in L cells (murine fibroblasts incapable of intercellular adhesion), the resulting transfectants are capable of forming aggregates. The Ep-CAM molecules are present at the surfaces of interacting cells, concentrated at the cell–cell boundaries. The transfectants do not interact with parental L cells, which suggests that Ep-CAM is involved in homotypic adhesion [17]. The adhesions mediated by Ep-CAM are relatively weak, as compared to some other adhesion molecules, such as classic cadherins. However, Ep-CAM adhesions are sufficiently strong to suppress the scattering of L cell transfectants in matrigel [17], or invasion of transfectants through reconstructed basement membrane [50]. Based on the inhibition of metastasis of Ep-CAM-transfected tumor cells in mice, as compared to control tumor cells [50], it may be claimed that Ep-CAM expression correlates with a better prognosis for carcinoma patients. Indeed, results of a study investigating markers for the assessment of nodal metastasis in laryngeal carcinomas revealed that a loss of Ep-CAM expression in the primary tumor correlates with nodal metastasis, a high incidence of local recurrence, and a poor prognosis [51].

When transfected in Ep-CAM-negative human epithelial cells, and expressed at average levels observed in carcinoma cell lines, Ep-CAM is capable of mediating aggregation in the absence of calcium [17, 18]. When carcinoma cells are allowed to aggregate in the absence of calcium, the degree of aggregation can be reduced by the presence of mAb 323/A3 specific for Ep-CAM [18]. Furthermore, it has been shown that the aggregation of human fetal pancreatic cells can be blocked with anti-Ep-CAM mAbs KS1/4 and 323/A3 [33].

The cytoplasmic domain is required for Ep-CAM to mediate intercellular adhesion, since L cell transfectants expressing Ep-CAM mutants lacking the complete cytoplasmic domain are not capable of forming aggregates, in contrast to the wild-type Ep-CAM transfectants. However, both L cell transfectants expressing wild-type Ep-CAM or cytoplasmic domain deletion mutants are capable of binding to Ep-CAM-coated solid phase, indicating that the extracellular domain is capable of forming Ep-CAM intercellular adhesions [62]. Because the cytoplasmic domain deletion mutant is not capable of inducing the aggregation of L cell transfectants, this indicates that as long as the counter-receptor Ep-CAM molecule is “fixed”, the extracellular domain is sufficient for adhesion. Thus, deletion of the cytoplasmic domain of the Ep-CAM molecule results in a protein that still has homophilic specificity, but is incapable of forming stable adhesions.

Ep-CAM is capable of associating with the actin cytoskeleton via the cytoplasmic tail. Treatment of Ep-CAM-expressing cells with the actin depolymerizing agent cytochalasin D results in the destruction of Ep-CAM-mediated adhesions and internalization of the Ep-CAM molecule [62]. In contrast, agents disrupting other types of cytoskeleton (intermediate filaments or microtubuli) do not result in the internalization of Ep-CAM. Moreover, Ep-CAM molecules involved in cell–cell interactions may be discriminated from the remaining cellular pool by detergent solubility using the mild zwitterionic detergent CHAPS. It is also demonstrated that Ep-CAM interacts with the actin-based cytoskeleton via direct binding of the cytoplasmic domain to α-actinin [62]. Two domains within the cytoplasmic tail are capable of α-actinin binding, as probably both are required for interactions that secure the formation of cell–cell contact. Whether other molecules are involved in the association of Ep-CAM with the cytoskeleton needs to be further investigated.

In L cells transfectants the opposing intercellular membranes are brought into close proximity at Ep-CAM-mediated cell–cell contacts, but no structures resembling junctional complexes, such as adherens junctions or desmosomes, have been identified at these contacts [63]. In L cells co-transfected with Ep-CAM and E-cadherin both molecules localize at cell–cell contact sites, forming independent adhesions, with no Ep-CAM detectable within morphologically distinguishable cadherin-mediated adherens junctions. In epithelial and carcinoma cell lines Ep-CAM and E-cadherin co-localized almost at the complete lateral membrane. However, no co-localization was observed between Ep-CAM and components of the tight junction (occludin and ZO-1), desmosomes (desmoplakins), or cell-matrix adhesions (β1-integrin) [63]. Ultra-structural analysis of the localization of Ep-CAM in normal colon tissue by immuno-electron microscopy confirmed the results of cell lines. In polarized epithelial cells of normal human colon, Ep-CAM was present at the lateral cell membrane including the adherens junction areas, but was fully excluded from the apical cell membrane, tight junctions, and desmosomes [63].

Function of Ep-CAM in epithelia

Inducing the internalization of Ep-CAM molecules from the cell surface by anti-Ep-CAM mAbs, excluding Ep-CAM from the participation in adhesive interactions of epithelial cells, results in a decrease in the degree of aggregation [18]. Furthermore, disengagement of Ep-CAM-mediated cell–cell interactions by Ep-CAM-specific mAbs in human fetal pancreatic cells results in a significant increase in both insulin and glucagon gene transcription [33]. This suggests that Ep-CAM internalization from the cell surface and/or functional inactivation of Ep-CAM by mAbs may be a signalling event in the differentiation of pancreatic islet cells [33]. In contrast, an increased expression of Ep-CAM, as demonstrated for the different stages of cervical intra-epithelial neoplasia, correlates with increased proliferation and a loss of markers for (terminal) differentiation [43]. In normal and transformed human keratinocytes, the expression of Ep-CAM is also inversely correlated with differentiation [64]. Moreover, in transformed keratinocytes Ep-CAM

directly correlates with cell proliferation [65]. Thus, there is a clear connection between Ep-CAM and signalling cascades, leading to a regulation of proliferation and differentiation.

Increased expression of Ep-CAM in cells interconnected by classic cadherins (E- or N-cadherin) has a negative effect on cadherin-mediated adhesions, whereas Ep-CAM-mediated adhesions become predominant [66]. Overexpression of Ep-CAM in cadherin-positive cells causes no change in the total amount of cellular cadherin, but decreases the association of the cadherin/catenin complex with the cytoskeleton. As Ep-CAM expression increases, the total amount of α-catenin decreases, whereas cellular β-catenin levels remain constant [66]. The adhesion-defective mutant of Ep-CAM lacking the complete cytoplasmic domain has no effect on adhesions mediated by classic cadherins, suggesting that the cytoplasmic domain is involved in the regulation of cadherin-mediated adhesions. Negative effects on cadherin-mediated adhesions by increased expression of Ep-CAM were also observed in L cell transfectants expressing both Ep-CAM and chimeras of E-cadherin, where the truncated cytoplasmic domain of cadherin was replaced by either complete or a fragment of α-catenin (Winter et al., submitted). Although several important molecules present in the cytoplasmic complex of E-cadherin (such as β-catenin and α-actinin) are excluded from the cytoskeleton anchor of the E-cadherin/α-catenin chimeras, the negative effect of Ep-CAM was still observed. This suggests that Ep-CAM affects the link between α-catenin and F-actin by a yet unknown mechanism. The observed co-localization of Ep-CAM and E-cadherin at the lateral membrane in epithelial cells may be important for the modulating effect of Ep-CAM on cadherin-mediated junctions [62].

Increased Ep-CAM expression correlates with proliferation, decreased cadherin-mediated adhesion, and a less differentiated phenotype, suggesting that Ep-CAM regulates the strength of intercellular adhesion, and provides epithelial cells with flexible interconnections necessary for epithelial morphogenesis and tissue maintenance. This has been shown in at least one model, the ontogeny of the human pancreas. Developmentally regulated expression of Ep-CAM plays a morphoregulatory role during the development of pancreatic islets of Langerhans [33]. Blocking of Ep-CAM by mAbs affected the transcription of genes encoding the pancreatic enzymes glucagon and insulin [33]. The opposite, affected differentiation by forced expression of Ep-CAM, was shown for mammary gland epithelium. Human Ep-CAM ectopically expressed in the mammary glands of transgenic mice leads to ductal hyperplasia and affects the differentiation of both lobular and ductal cells (Balzar et al., submitted). In mammary glands of virgin transgenic female mice active secondary branching of ducts and enhanced proliferation (phenomena often observed in mice with forced expression of a growth factor relevant to mammary cell proliferation) was observed. For example, the ectopic expression of heregulin, a member of the neuregulin family of ligands of *erb*B receptor tyrosine kinases involved in signal transduction, induces similar mammary gland morphogenesis [67].

Place of Ep-CAM among other adhesion molecules

Structurally Ep-CAM does not resemble any of the four major families of cell adhesion molecules, namely cadherins, integrins, selectins, and members of the immunoglobulin super-family [68, 69]. The Ep-CAM extracellular domain contains EGF-like repeats, which are also present in selectins [70], and members of the lin-12/notch/GLP-1 transmembrane receptor family [71]. Based on the presence of EGF-like repeats in the extracellular domain, Ep-CAM was proposed to function as a cell adhesion molecule [15], or a cell surface receptor capable of signal transduction [25]. Despite the fact that the molecule is capable of mediating homophilic adhesive interactions, there is not sufficient evidence that Ep-CAM-mediated adhesions are required for epithelial cell support. Other molecules, such as receptor protein tyrosine phosphatases [72], molecules involved in defining the cell fate of the lin-12/notch/GLP-1 receptor family [71], and molecules involved in juxtacrine signalling, are capable of adhesive interactions as well. The border between intercellular adhesion and juxtacrine signalling, as two different functions, is becoming less clear, since many "classic" adhesion molecules (e.g. N-CAM and E-cadherin) were demonstrated to function as signalling morphoregulators [73]. Figure 3 shows a comparison of Ep-CAM with other cell surface receptors participating in either adhesion or juxtacrine signalling, indicating that some of the receptors share structural similarities with Ep-CAM. A secreted form, typical of most juxtacrine signalling molecules, is not reported for Ep-CAM. The recently reported data concerning the organization of Ep-CAM-mediated adhesions suggest that it more resembles a typical adhesion molecule, being connected to actin microfilaments [62]. However, the exact role of Ep-CAM in epithelial cell functioning remains to be further investigated.

Immunotherapy targeting Ep-CAM

The generation of mAbs for immunopathological diagnosis of cancer resulted in the discovery of antigens expressed by tumor cells [2, 74]. Many of these antibodies were specific for cell surface antigens of the tumor cells. Ep-CAM was one of the most immunogenic proteins to which antibodies were generated in mice immunized with carcinoma cells. Since Ep-CAM localizes at the cell surface of most carcinomas, the molecule is an attractive target for immunotherapy. Ep-CAM-directed therapy against carcinomas started with treatment using unconjugated mAbs, which has resulted in some anti-tumor effects [3, 4, 75]. However, after a decade of mAb therapy on solid tumors in patients, it can

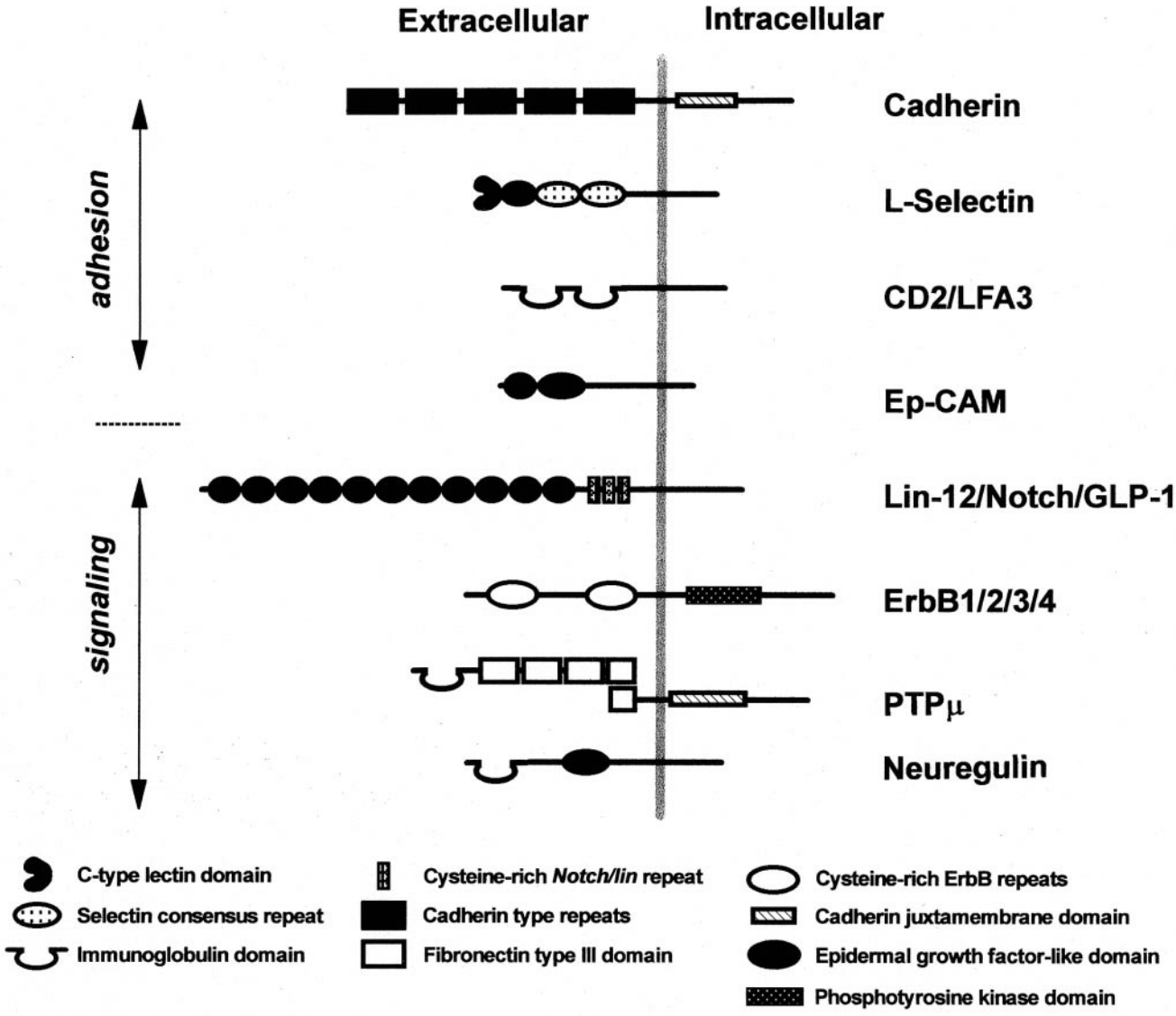

Fig. 3 Schematic domain structure of Ep-CAM in comparison to other cell surface proteins involved in either cell–cell adhesion or transmembrane/juxtacrine signalling

be concluded that naked mAbs generally lack the efficacy to eradicate solid established tumor masses [76]. This lack of efficacy is believed to be caused by very poor localization of mAbs in larger tumors. The capacity of mAbs to eliminate single tumor cells in carcinoma patients may prevent the outgrowth of metastasis and could therefore be crucial for the survival of patients. Indeed, when Dukes C colorectal carcinoma patients with surgically resected solid tumor were treated for minimal residual disease with the Ep-CAM-specific mAb 17–1A, this resulted in a 30% increase in the 7 years survival of 17–1A-treated patients as compared to non-treated patients [6]. Since mAb 17–1A is a low-affinity antibody, better immunotherapy results may be obtained using high-affinity mAbs [77]. However, it has been demonstrated that treatment with high-affinity mAbs, such as GA733 and 323/A3, may cause damage to Ep-CAM-positive normal epithelial tissues resulting in toxicity problems [78].

Recently, other strategies targeting Ep-CAM have been developed for the treatment of carcinomas, although most of them are only tested in pre-clinical studies. To provide better mAb tumor retention, high-affinity recombinant phage antibodies directed against Ep-CAM were developed [79]. Recombinant bispecific single-chain antibodies specific for both Ep-CAM and the CD3/T cell receptor complex demonstrated (approximately 1000-fold) higher specific cytotoxicity against tumor cells in vitro [80, 81]. Therefore, these bispecific single chain antibodies may result in improved therapy of minimal residual disease by retargeting activated T cells to micrometastatic carcinoma cells [80]. Activation of Ep-CAM-specific T cells was also achieved both in vitro and in vivo by peptide vaccination [82] or DNA vaccination (our unpublished results). To overcome problems with respect to major-histocompatibility-complex (MHC-) restricted target recognition by T cells, and down-regulation of MHC molecules expression by tumor

cells, chimeric T cell receptors specific for Ep-CAM were generated that were able to lyse tumor cells in vitro (Van Ratingen et al., submitted). Expression of these chimeric T cell receptors in cytotoxic T lymphocytes from patients by retroviral transduction might be valuable for T-cell-based immunotherapy of carcinoma patients.

Since Ep-CAM has high expression levels in some normal human adult tissues (e.g. colon), one can expect toxicity as a side-effect of a therapeutic approach. Indeed, infusion of carcinoma patients with high-affinity mAbs against Ep-CAM (e.g. GA733) was accompanied by mild and short-lasting gastrointestinal toxicity [78]. Thus, appropriate pre-clinical models are required to study the efficacy and toxicity of newly developed strategies. Transgenic mice expressing human Ep-CAM in epithelial tissues might serve as such a model and have recently been generated (Balzar et al., submitted).

Concluding remarks

Many classic cell adhesion molecules, i.e. integrins or selectins, have recently been identified as components of signalling cascades. Moreover, signalling from various cell adhesion molecules is regarded as a major mechanism of controlling processes such as proliferation, differentiation, and (programmed) cell death [83, 84]. The function of CAMs is not only to mechanically attach cells to adjacent cells and substrates, but also to respond to changes in the environment and adjust the biological response of the cell through outside-in signalling. Since the adhesions mediated by Ep-CAM are relatively weak, as compared to cadherin-mediated adhesions or desmosomes, one may question their relevance to maintaining the epithelial tissue architecture. On the other hand, Ep-CAM does resemble "classic" adhesion molecules, since it associates with the cytoskeleton and forms independent adhesions [62, 63]. Although no true junctions are observed for Ep-CAM, the molecule is clearly capable of moving adjacent cell membranes together at the areas of reciprocal homotypic interactions.

The ability of Ep-CAM to regulate cadherin-mediated adhesions, tissue morphogenesis and the transcription of genes (as was found for the pancreatic enzymes glucagon and insulin) indicates that the molecule is involved in signal transduction regulating epithelial morphogenesis.

The (over-) expression of Ep-CAM correlates with both benign and malignant (hyper-) proliferation of epithelial cells. However, the exact role of Ep-CAM in tumor development remains unclear. Ep-CAM-mediated cell–cell adhesions prevent cell scattering, suggesting that the molecule might prevent metastasis. However, the negative effect of Ep-CAM on cadherin-mediated adhesions may actually promote invasion and metastasis from carcinoma nodules. Thus, the dualistic role of Ep-CAM in tumor development requires further investigation.

Since Ep-CAM is expressed by most carcinomas, the molecule has attracted the attention in the field of cancer immunotherapy. Two important issues must be addressed for major improvements in Ep-CAM-targeted immunotherapy. First, Ep-CAM expression is heterogeneous in carcinomas, which may cause partial eradication of the tumor after treatment. Second, since Ep-CAM is an epithelial differentiation antigen, and not a tumor-specific antigen, immunotherapy may have severe side-effects causing toxicity to normal tissues. An increased interest in the biological properties of the Ep-CAM molecule (as can be observed from recent publications) will ultimately contribute to a better understanding of the function of Ep-CAM in development, maintenance, and tumorigenesis of epithelial tissues. Moreover, it will also contribute to development of new therapeutic strategies employing this antigen as a target.

Acknowledgement This research was supported by the Dutch Cancer Foundation (grants RUL 94–762 and 95–1107) and a research grant from Centocor, Malvern, Pa., USA.

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