The Emerging Role of EpCAM in Cancer and Stem Cell Signaling

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Abstract

Initially discovered as a dominant antigen on colon carcinomas, the epithelial cell adhesion molecule (EpCAM) was considered a mere cell adhesion molecule and reliable surface-binding site for therapeutic antibodies. Recent findings can better explain the relevance of EpCAM's high-level expression on human cancers and cancer propagating cells, and its negative prognostic potential for survival of patients with certain cancers. EpCAM has oncogenic potential and is activated by release of its intracellular domain, which can signal into the cell nucleus by engagement of elements of the *wnt* pathway. [Cancer Res 2009;69(14):5627–9]

Structure of EpCAM

Epithelial cell adhesion molecule (EpCAM) was described 30 years ago as a dominant antigen in human colon carcinoma tissue (1). EpCAM is a glycosylated, 30- to 40-kDa type I membrane protein, which is expressed in a variety of human epithelial tissues, cancers, and progenitor and stem cells. EpCAM is comprised of an extracellular domain with epidermal growth factor (EGF)- and thyroglobulin repeat-like domains, a single transmembrane domain, and a short 26-amino acid intracellular domain called EpICD (Fig. 1). EpCAM in normal cells is predominantly located in intercellular spaces where epithelial cells form very tight junctions. It has, therefore, been speculated that EpCAM on normal epithelia is sequestered and, therefore, much less accessible to antibodies than EpCAM in cancer tissue, where it is homogeneously distributed on the cancer cell surface.

EpCAM as Oncogenic Signal Transducer

Systematic analysis of EpCAM expression for intensity and frequency showed that EpCAM is expressed on essentially all human adenocarcinoma, on certain squamous cell carcinoma, on retinoblastoma, and on hepatocellular carcinoma (2, 3). Importantly, EpCAM is part of the signature of cancer-propagating cells in numerous solid tumors and of normal progenitor and stem cells (4, 5). It was, however, not until 2004, before a causal role for EpCAM in proliferation, migration, and mitogenic signal transduction was unraveled (6, 7). *De novo* expression of EpCAM in human or rodent cells enhanced anchorage- and serum growth factor-independent proliferation and the expression of various target genes, including *c-myc* and cyclins. In breast cancer cell lines, EpCAM silencing by RNA interference led to a reduction in proliferation, migratory, and invasive capacity (6). Transcription of the *epcam* gene in these cells was inhibited upon binding of wild-type p53 to a consensus site located within intron 4 (8).

Activation of EpCAM by Proteolysis

Loss of EpCAM staining at the tip of budding colorectal cancer cells was observed upon staining with antibodies specific for EpCAM's extracellular domain (EpEX; ref. 9), and might relate to the recently uncovered mode of activation of EpCAM signaling via regulated intramembrane proteolysis (RIP) and shedding of EpEX (10). Sequential cleavage of EpCAM by tumor-necrosis-factor alpha converting enzyme (TACE/ADAM17) and a gamma-secretase complex containing presenilin 2 (PS-2) results in release of EpEX into the culture medium, and release of EpICD into the cytoplasm (Fig. 1). EpICD becomes part of a large nuclear complex containing transcriptional regulators β-catenin and Lef, which are both components of the *wnt* pathway. Treatment of cells with recombinant EpEX resulted in EpCAM cleavage (10), implying that EpEX functions as a soluble ligand and agonist for EpCAM-expressing cells, possibly providing an auto- and paracrine activation signal. Cell-to-cell contact has been identified as an initial trigger for EpCAM activation (10). Interaction of EpCAM with members of the tetraspanin web (11) might be a means to regulate the availability of the extracellular domain of EpCAM for homotypic aggregation and/or interactions with yet unknown ligands. Overexpression of proteases involved in EpCAM cleavage, especially TACE, in malignant tissue, may be a further means to control and promote EpCAM signaling. Finally, a subcellular redistribution of EpCAM from a baso-lateral location in normal tissue to a dispersed pattern on cancer cells could also be involved in a differential activation of EpCAM. This may explain why tissue samples from colonic mucosa, in contrast to colon carcinoma, did not reveal nuclear EpICD staining despite expression of substantial levels of membrane-associated EpCAM (10).

FHL2 as Cytosolic Interaction Partner for EpICD

Four and one-half LIM domains protein 2 (FHL2) was identified as a cytosolic interaction partner of EpICD (10). Deletion mutants of FHL2 identified the fourth LIM domain as necessary for binding EpCAM. LIM domains 2 and 3 of FHL2 are binding β-catenin/p300 (12), which would be consistent with a nonoverlapping use of LIM domains. FHL2 is mandatory for signal transduction by EpCAM because both the up-regulation of the target genes *c-myc* and *e-fabp* and EpCAM-initiated proliferation were reduced by FHL2-specific RNA interference. FHL2 further regulates localization and activity of TACE and PS-2 (12). Hence, FHL2 has the potential to serve as a scaffolding protein for various signaling proteins used by EpCAM. By the proposed interference with E-cadherin-mediated cell adhesion via the PI3 kinase pathway (13), EpCAM may increase the level of its soluble adaptor β-catenin, which may be a means to

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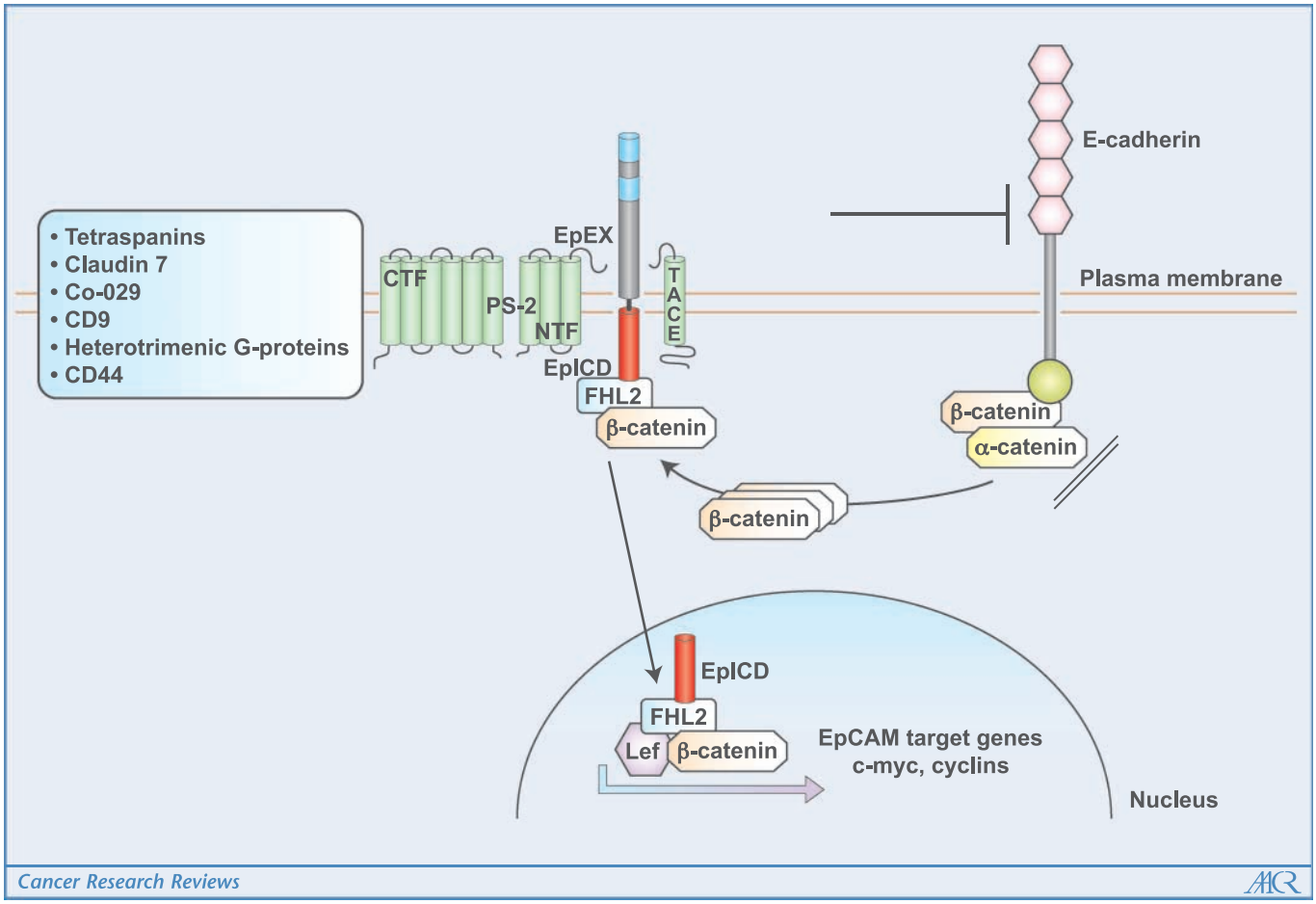

Figure 1. Schematic of signaling pathways of EpCAM. Upon cleavage by TACE/PS-2, EpICD translocates into the nucleus in a multiprotein complex. Together with FHL2, β-catenin, and Lef-1, EpICD contacts DNA at Lef-1 consensus sites. Owing to its ability to inhibit E-cadherin–mediated adhesion, EpCAM provides itself with β-catenin as a central interacting protein.

enhance its nuclear signaling by the *wnt* pathway (Fig. 1). It is, however, unknown how EpCAM engages the PI3 kinase pathway.

EpCAM's Nuclear Signaling Complex

Biochemical approaches indentified a 650-kDa EpICD-containing nuclear complex specifically binding to Lef-1 DNA consensus sites, which was no longer formed in cells pretreated with inhibitors of TACE and PS-2 (10). Transcriptional activity of Lef-1 in colon carcinoma cell lines was substantially diminished when EpCAM expression was knocked down by siRNA, as were induction of *c-myc* and cell proliferation. Expression of EpICD was capable of counteracting these deficiencies, and could alone enhance *c-myc* transcription and cell proliferation. A number of EpCAM-regulated target genes have been identified including *c-myc* and *cyclins* (7), and additional genes involved in cell growth and proliferation, cell cycle, and cell death (14).

Expression of EpICD or EpCAM Is Tumorigenic

A tumorigenic activity of full-length EpCAM and EpICD was observed in a SCID mouse tumor model (10). HEK293 cells expressing either EpICD or full-length EpCAM generated large tumors in 100% of animals, whereas only one out of eight injected mice developed a small tumor with control transfected HEK293 cells. There was a trend that cells expressing full-length EpCAM induced the formation of larger tumors than cells expressing the short-lived EpICD peptide. Noteworthy, the murine homolog of EpCAM led to decreased growth, colony formation, and invasiveness of murine colorectal carcinoma cells, whereas overexpressed human EpCAM solely impaired invasiveness (15). Hence, differences obviously exist across species in the oncogenic potential and effects on invasiveness of EpCAM, which may depend on the experimental system (6, 15). Signaling by murine EpCAM is, as yet, unexplored and deserves thorough future research. Although expression of human EpCAM is evidently associated with increased proliferation, the effects of EpCAM expression on tumor cell migration and invasiveness seem to be more complex.

EpCAM and Stem Cell Phenotype

The *wnt* signal transduction pathway, components of which are used for both EpCAM signaling and regulation of the *epcam* gene (16), is of high significance for normal and malignant stem cells (17). A major characteristic of stem cells is self-renewal in conjunction with signals that retain cells in an undifferentiated state, which is fundamental for their pluripotency and limitless proliferation. Key signals for achieving this particular phenotype are provided by components of the *wnt* pathway, LIF/STAT3 and c-Myc, and by transcription factors nanog, oct3/4, Klf4, and sox2 (18). The newly discovered signaling properties of activated

EpCAM in conjunction with its frequent expression on normal and cancer stem cells and their progeny suggests that EpICD can provide *wnt*-like signals in normal cancer and cancer stem cells. This is supported by experiments knocking down EpCAM expression in murine embryonic stem cells using siRNA, and by its forced overexpression. EpCAM reduction resulted in diminished proliferation and expression of stem cell markers, whereas enforced expression of EpCAM allowed for the stabilization of Oct3/4 expression even under differentiating conditions (Gires O. Unpublished data).

It is tempting to speculate that the high-level expression of EpCAM on cancer-initiating and on normal stem cells and its positive autoregulation may ensure that the protein can provide a sustained proliferative signal to such cells. Furthermore, a role of EpCAM as a morphoregulatory protein in normal developing tissue may be reused in carcinogenesis in that cancer stem cells benefit from activated human EpCAM for proliferation, self-renewal, and anchorage-independent growth and invasiveness.

Future Perspectives

With the identification of EpCAM as a mitogenic signaling molecule, its sole role as homotypic cell adhesion molecule is in need of substantial revision. Apparently, EpCAM has a dual role as cell adhesion molecule and receptor involved in the regulation of gene transcription and cell proliferation, as has been described for E-cadherin and other proteins (19). Full-length EpCAM should, hence, be viewed as a precursor for its mitogenic signaling moiety EpICD. Preliminary data from immunohistochemical analysis of human tissues suggest that the EpCAM precursor is rather stable within the membrane of normal epithelial tissue, but is prone to cleavage in cancer tissue (10). In cultured cancer cell lines, the EpCAM precursor is also swiftly activated by added EpEX or within zones of cell-to-cell contact.

Protein partners of EpCAM could be crucial for regulation of RIP. For instance, claudin-7 recruits EpCAM to tetraspanin-enriched microdomains and, thereby, controls EpCAM functions in tumorigenesis (20). EpCAM overexpression could be a means to outnumber potentially inhibitory partners. Regulation of EpCAM activation by its various associated proteins is an area of important forthcoming research.

Future studies of EpCAM activation and signaling will be greatly aided by antibodies detecting nuclear EpICD. Whereas there is a large database on EpCAM staining for many cancer and normal tissues, these studies notoriously detected the extracellular domain of EpCAM, which could either represent the EpCAM precursor or cell-bound EpEX, or both. In no case was the state of EpCAM activation evident from such studies. Staining of cells for the nuclear presence of EpICD, the expression of EpCAM target genes, such as *c-myc*, and for proliferation marker Ki67 may complement future functional analyses, and eventually explain the discrepant prognostic value of EpCAM expression seen between various malignant diseases.

EpCAM is intensely used as a therapeutic target for antibody-based approaches. Future development of EpCAM-directed therapeutics may profit from newly identified functions of EpCAM as mitogenic signal transducer in various ways. An important insight is that EpCAM is apparently needed to maintain distinct cancer cell attributes (6, 7, 10) and, potentially, the cancer stem cell phenotype as well. This function can reduce the risk of immune escape by loss of EpCAM target expression from cancer cells. EpCAM-directed therapies may be selective for those cancer cells with the strongest negative impact on prognosis and for cancer-propagating subsets of malignant cells.

EpCAM signaling itself may be a target for therapeutic intervention. Whereas inhibitors of TACE and presenilins will not be specific for EpCAM, their use for cancer therapy may receive an additional development rationale by inhibiting EpCAM signaling. The protease inhibitors may increase levels of intact EpCAM, which serves as the extracellular anchor for anti-EpCAM antibodies with immunological activity or bearing toxic payloads. Another promise of EpCAM as therapeutic target lies in its expression on cancer stem cells. Should existing or future EpCAM-directed therapies prove to be efficacious in eradicating EpCAM-expressing cancer stem cells, they could have high potential to more effectively treat solid tumors or remove residual tumor cells left after standard therapies, which bear a high risk for causing relapse and lethal metastasis. This possibility is currently being assessed *in vitro*, in animal models, and in ongoing clinical trials with EpCAM-directed immunotherapies.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

Acknowledgments

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