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EpCAM: Another Surface-to-Nucleus Missile

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The epithelial-specific cell adhesion molecule (EpCAM) modulates cell adhesion and proliferation. Its overexpression correlates with tumor cell proliferation, and EpCAM is a therapeutic target. In the February issue of *Nature Cell Biology*, Maetzel et al. demonstrate that proliferative responses to EpCAM require regulated intramembrane proteolysis and a nucleocytoplasmic intracellular domain fragment.

Receptor-activated proteins acting singly (STATs) or in a cascade (Ras/MAP kinase) are well-recognized surface-to-nucleus signaling pathways. However, the capacity of surface receptors to directly translocate to the nucleus and affect cellular functions is less accepted. While a significant number of cell surface receptors have been reported to translocate to the nucleus, there are only a few examples (Notch, APP, ErbB4, Ryk) in which the translocation is ligand dependent and has been convincingly shown to alter nuclear function (Carpenter and Liao, 2008; Lyu et al., 2008; Ma et al., 2008). There is, however, preliminary evidence for several other candidates.

Establishment of nuclear function is important, as the translocations, which may involve a small fraction of the total receptor pool, can otherwise be dismissed as trafficking side effects. In the examples cited above, the mechanism of surface-to-nucleus translocation involves a two-step proteolytic processing pathway. The initial step is ligand-activated cleavage of the ectodomain by an ADAM metalloprotease. While the exact biochemical mechanism that initiates ectodomain cleavage is not clear, the cell surface-associated fragment, containing a few ectodomain residues, the transmembrane domain, and the intracellular domain (ICD), is subsequently cleaved within the transmembrane domain by γ-secretase to release the ICD fragment into the cytoplasm. The soluble ICD fragment, which may be metabolically unstable, is then translocated to the nucleus. In the process of nuclear translocation, Notch, APP, and ErbB4 ICDs are known to associate with transcription factors and thereby influence gene expression and cellular responses. To date, none of these ICDs has been shown to directly recognize specific DNA sequences.

Now, Maetzel and colleagues (2009) have added another cell surface molecule with significant implications in cancer biology to this list. Epithelial-specific cell adhesion molecule (EpCAM/CD326) is a type I transmembrane glycoprotein with an ectodomain, one transmembrane domain, and a cytoplasmic domain of 26 residues (Baeuerle and Gires, 2007; Trzpis et al., 2007). It is specifically expressed in epithelial tissue, is overexpressed in some cancers, and is a homotypic adhesion protein that can antagonize cadherin-mediated cell-cell adhesion (Litvinov et al., 1994, 1997). In view of these properties, EpCAM is a therapeutic target, and there are several anti-EpCAM agents in clinical trials (Baeuerle and Gires, 2007).

Mechanistically, inducible expression of EpCAM has been demonstrated to provoke expression of c-*myc* and the Myc target genes *cyclin A* and *cyclin E* (Munz et al., 2004). Cells expressing EpCAM proliferate more rapidly, grow in an anchorage-independent manner, and have a reduced requirement for growth factors. Consistent with the growth-promoting role of EpCAM, knockdown of endogenous EpCAM in tumor cells decreases cell proliferation and migration (Munz et al., 2004; Osta et al., 2004). However, relatively little is known about the intracellular trafficking of EpCAM or the means by which it communicates with the nucleus.

Maetzel et al. have now taken a major step toward elucidating the mechanism by which EpCAM affects nuclear function. The authors demonstrate that ADAM17 (TACE) and γ-secretase sequentially cleave EpCAM, producing a soluble ectodomain fragment and a 5 kDa ICD fragment, respectively. The application of relatively selective inhibitors implicated the above-mentioned proteases, as did coprecipitation experiments that detected ADAM17 and presenilin 2 (the protease component of the γ-secretase complex) in association with EpCAM. The ICD fragment was detected in the cytoplasm and nucleus of experimental cells, but not when protease inhibitors were present.

In the experiments presented, cells were allowed to grow as islands, permitting significant cell-cell contact and presumably homotypic interactions that provoke EpCAM cleavage. In support of this, the authors show that addition of soluble EpCAM ectodomain to single cells stimulates formation of the ICD fragment. Examination of human normal and tumor colonic tissue sections revealed EpCAM ICD in the latter, but not in the former. On the basis of these data, the cleavage of EpCAM parallels that of a variety of other cell surface adhesion molecules, such as cadherin and CD44 (Carpenter and Liao, 2008). However, in many cases, including cadherin and CD44, cleavage is provoked by a nonspecific agent, such as TPA or ionomycin. In contrast, ICD formation from Notch, ErbB4, APP, and EpCAM can be stimulated by biologically relevant ligands. In the case of Ryk, ICD formation is constitutive, while nuclear localization of ICD requires Wnt, a Ryk ligand (Lyu et al., 2008).

The question of the biological significance of the EpCAM ICD fragment formation was addressed via several approaches. First, the ICD fragment was exogenously expressed, and cellular responses were measured. Second, the capacity of TACE and/or γ-secretase pharmacologic inhibitors or siRNAs to block EpCAM-dependent responses was tested. To assess specificity issues in these latter experiments, the ICD fragment

was expressed in inhibitor-treated cells to determine whether the ICD could overcome inhibitor abrogation of cell responses. Also, experiments were performed in which endogenous EpCAM expression was knocked down using siRNA and the resulting decrease in cell proliferation was reversed by exogenous expression of the ICD fragment. These lines of evidence all support the conclusion that formation of the ICD is required for EpCAM to stimulate cell proliferation.

The mechanism by which the ICD modulates cell proliferation is proposed to center on its association with "four and a half LIM domain" protein 2 (FHL2), a nucleocytoplasmic protein that interacts with a large number of proteins, including β-catenin, and that can function as a cotranscriptional activator in several systems (Johannessen et al., 2006). The EpCAM ICD was detected in the nucleus and cytoplasm as speckles, and the authors report its presence in the nucleus as a 650 kDa complex that also contains FHL2, Lef1, and β-catenin. These same proteins were also detected in electrophoretic mobility shift assays using a *Lef1* consensus sequence probe, and formation of this complex was blocked by TACE and γ-secretase inhibitors. The authors show that expression of the ICD fragment in the absence of EpCAM can induce c-*myc* expression and propose that formation of the ICD complex with the scaffolding protein FHL2 plus Lef1 and β-catenin accounts for the capacity of EpCAM to stimulate c-*myc* expression and cell proliferation. Lef1/TCF is known to be a major regulator of c-*myc* expression.

The oncogenic potential of the ICD fragment was demonstrated using a mouse xenograft model, in which HEK293 cells stably expressing EpCAM or the ICD fragment produced nearly equivalent tumors. As previously mentioned, gastrointestinal tumor sections frequently exhibit nuclear EpCAM ICD, while normal tissue sections do not. The authors suggest that the known upregulation of TACE and FHL2 in tumors could serve as a reasonable explanation for this observation.

These data provide substantial evidence that the positive influence of EpCAM on cell proliferation can be accounted for by liberation of its ICD from the plasma membrane. It remains to be examined whether this mechanism may be operative in other EpCAM-mediated cellular responses, such as morphogenesis, differentiation, and inflammation (Trzpis et al., 2007).

In terms of oncogenesis and ongoing trials with EpCAM-targeted agents, it is likely that absence of the ICD fragment would constitute a biomarker for effectiveness. Also, it is possible, as Maetzel et al. suggest, that combinatorial therapy in which anti-EpCAM together with TACE and/or γ-secretase inhibitors are employed could be a more effective therapeutic approach than the use of anti-EpCAM agents alone. However, this would likely entail a decrease in therapeutic specificity given the broad spectrum of TACE and γ-secretase substrates.

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