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Epsins' novel role in cancer cell invasion

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The epsin family of endocytic adaptors has been found to be upregulated in cancer; however the relevance of these findings to this pathological condition is unclear. We have recently demonstrated that epsins are required for cell migration. In fact, epsin overexpression promotes cancer cell invasion. Further, and in agreement with our previous findings, we also observed that overexpression of epsins led to epithelial cell migration beyond colony boundaries. Additionally, our results show that epsin-3 is the most potent paralog enhancing cell migration and invasion. Interestingly, epsin-3 expression is not widespread but highly restricted to migratory keratinocytes and aggressive carcinomas. Upon further investigation, we also identified epsin-3 as being expressed in pancreatic cancer cells. These findings suggest that upregulation of the *EPN3* gene is specifically associated with invasive, aggressive cancers. We predict that investigation of these links between the endocytic machinery and mechanisms involved in tumor dissemination will contribute to the development of novel anti-metastatic and anti-cancer strategies.

Key words: endocytosis, cancer, cell invasion, cell migration, signaling

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The Epsin Family of Endocytic Adaptors Promote Cancer Cell Invasion

It is widely accepted that functional abnormalities in the endocytic machinery can lead to the onset of malignant transformation. In its most straightforward interpretation, lack of function of endocytic proteins would lead to deficient endocytosis and therefore to prolonged signaling from activated receptors. Interestingly, downregulation of the expression levels of endocytic proteins such as Dab2, Numb and POB1 have been observed in several cancers including ovarian, prostate and breast cancer.[1-5] Another mechanism by which abnormal endocytic protein function can lead to carcinogenesis is through the generation of aberrant fusion proteins.[6] For example, chromosomal translocations involving the *CALM* (Clathrin Assembly Lymphoid Myeloid leukemia) and *AF10* (ALL1 Fused 10) genes produce a fusion protein implicated in acute leukemia.[7]

Nevertheless, there are several examples of endocytic proteins upregulated in cancer. For example, elevated levels of epsins have been reported to be augmented in skin, breast and lung cancer.[8-10] Additionally, intersectin has been shown to induce fibroblast transformation in vitro.[11] Interestingly, both endocytic proteins have been directly implicated in the activation of Rho GTPase signaling pathways. Specifically, whereas the intersectin-L isoform has intrinsic Cdc42 GEF activity, epsins bind and inhibit the function of GAPs for Cdc42 and Rac1.[12] Although it is not completely clear if amplified RhoGTPase signaling is sufficient to induce malignant transformation, it is predicted to enhance the dissemination of cancer. Indeed, we have demonstrated that the epsin family of endocytic adaptors is required for cell migration[13] and that this function depends on the interaction of these proteins with the Cdc42/Rac1 GAP and Ral effector RalBP1.[13] Further, our studies indicate that epsin-RalBP1 complex formation is required for proper Rac1 signaling.[13]

RalBP1 has been observed to be highly upregulated in several invasive cancers including bladder, lung, prostate and

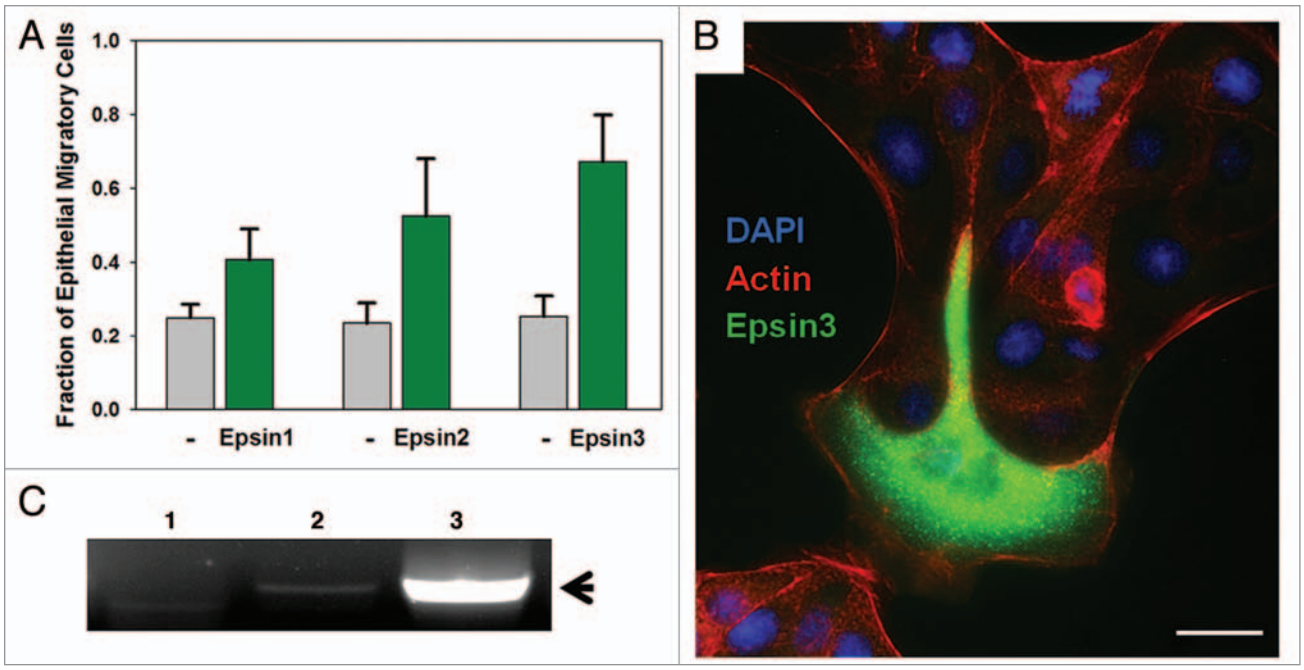

Figure 1. Epsin overexpression induces migratory behavior in epithelial cells. MDCK cells were transiently transfected with GFP-Epsin1, 2 and 3. After transfection, cells were trypsinized and seeded on glass coverslips for 24 hr at low density to promote the formation of colonies containing approximately 50 cells. The coverslips were then fixed, co-stained with rhodamine-phalloidin and DAPI, and imaged by epifluorescence microscopy. (A) Fraction of cells at the colony periphery acquiring migratory behavior was quantified in three independent experiments. Results for epsin-transfectants and untransfected (-) cells are indicated as the Mean ± SEM. (B) Example of epithelial cells transfected with GFP-epsin-3 displaying migratory behavior. Scale bar: 20 microns. (C) RNA prepared from HeLa (1), Panc-1 (2) and BxPC-3 (3) cell lines was used as template for RT-PCR with human epsin-3 specific primers. Arrow points to epsin-3 cDNA specific fragment.

skin cancer[14,15] and implicated in cancer cell migration, spreading and survival.[16,17] It should be noted that epsins, particularly epsin-3, are upregulated in breast and skin cancer.[9,10] Importantly, either epsin or RalBP1 overexpression lead to enhanced cell invasion through the basement membrane.[13] This observation suggests that enhanced expression of these endocytic proteins contribute to cancer aggressiveness by promoting cell invasion. In agreement with this prediction, we have observed morphological changes in MDCK epithelial cells upon overexpression of epsin-2 and epsin-3 which indicate enhanced cell migration (**Fig. 1**). Specifically, epsin-transfected cells repeatedly extend lamellipodia beyond the colony boundaries in a way which closely resembles epithelial leader cell migration[18] but they also can be found migrating out of epithelial colonies entirely (**Fig. 1B**). Interestingly, a strikingly similar transition in MDCK behavior has been observed previously upon overexpression of the Arf6 GEF ARNO.[19] ARNO overexpression causes the extension of broad lammelipodia and enhanced cell migration which can be attributed to enhanced activation of both Arf6 and Rac1.[19] Our previous findings show that the epsin family of adaptors is also signaling to promote Arf6 and Rac1 activation, suggesting that these independent results are obtained by the activation of similar GTPase signaling pathways.[13]

We consistently observed that epsin-3 was the most potent paralog for inducing enhancement of cell invasion[13] and MDCK migration (**Fig. 1**). Interestingly, epsin-3 has a limited expression pattern, essentially restricted to migratory cells and basal carcinomas.[9] In fact, epsin-3 expression is highly upregulated in breast cancer cell lines.[9,10] Further, we have also identified epsin-3 as being expressed in mouse pancreatic cancer models[13] and in invasive human pancreatic cell lines such as BxPC-3 (**Fig. 1C**). These findings suggest that upregulation of the *EPN3* gene is specifically associated with invasive cancer. Our laboratory is currently engaged in further investigations required to prove or disprove this hypothesis.

Perspectives

Additional epsin-dependent mechanisms for the enhancement of cancer cell invasion. Although our data indicate that the ability of epsin to affect cell invasion is mediated by its interaction with RalBP1 and the resulting RhoGTPase activation,[13] we cannot discard additional contributions by other mechanisms. Indeed, endocytosis itself has been proposed to play an important role during cell migration. Thus, defects in the function of endocytic proteins such as Dab2, ARH, Numb, AP2 and clathrin, have also been linked to abnormal cell migration due to defective integrin endocytosis.[20-23]

Additionally, epsin has been directly and specifically connected to the activation of the Notch signaling pathway[24,25] which is known to be involved in cell migration/invasion.[26] In Drosophila, epsin is the only endocytic adaptor necessary for activation of Notch signaling in signal sending cells, likely due to its special ability to internalize ubiquitinated Notch-ligands.[24,25] Further, this Notch-signaling activation

function has been shown to be conserved in worms and mice.[27,28] Nevertheless, this juxtacrine cell-to-cell mechanism is unlikely to be involved in the epsin-mediated enhancement of fibrosarcoma cell migration and invasion.[13] The epsin-3's prevalent effects over other paralogs' cannot be explained by this mechanism. Specifically, since Notch-ligand internalization is a ubiquitin-dependent process,[29] all epsin paralogs (which bear functional ubiquitin-interacting motifs) are predicted to be equally effective in promoting cell invasion enhancement. However, it is possible that an epsin-induced, Notch-dependent mechanism operates in the context of multi-cellular environments, such as pancreatic acini.

Nevertheless, the contributions of epsin-mediated enhancement of cancer cell invasion due to endocytosis in general, and of Notch-ligands in particular, still needs to be assessed.

Cell sensitivity to anti-cancer drugs. Metastatic cells are usually associated with enhanced resistance to chemotherapy.[30] Therefore, factors or pathways that contribute to migratory behavior are of high interest for therapeutic purposes. Given our recent findings, epsins rightfully join the list of potential targets for anti-metastatic and anti-cancer strategies, which already includes their interaction partner RalBP1. In fact, it is tempting to speculate that in addition to other proposed mechanisms,[17] RalBP1's ability to promote cancer cell survival is related to its capability of inducing migratory behavior. Therefore, function impairment of endocytic proteins crucial to cell invasion (such as epsins and RalBP1) represents an exciting new direction for developing effective cancer therapeutics.

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