A New Central Scaffold for Metastasis: Parsing HEF1/Cas-L/NEDD9

Geraldine M. O’Neill,1 2 Sachiko Sea,3 Ilya G. Serebriiskii,4 Stuart R. Lessin,4 and Erica A. Golemis1

1Oncology Research Unit, The Children’s Hospital at Westmead, NSW, Australia; 2Discipline of Paediatrics and Child Health, The University of Sydney, NSW, Australia; 3Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; and 4Fox Chase Cancer Center, Philadelphia, Pennsylvania

Abstract
Greater understanding of metastasis is required to improve cancer treatment outcomes. Recently, changes in expression of the scaffold protein HEF1/CAS-L/NEDD9 were found to be a potent prometastatic stimulus in melanoma and other cancers. Mechanistic studies suggest diverse cellular roles of HEF1 and highlight its importance in the response to extracellular cues that drive invasion and metastasis. As a metastatic “hub” for signaling in cancer, HEF1 may provide a useful target for drug discovery efforts. [Cancer Res 2007;67(19):8975–9]

Introduction
In recent years, our understanding of the metastatic process has evolved significantly. It is now appreciated that the formation of distant metastases requires functionally distinct events, including (a) invasion of a tumor through basement membrane and stroma, (b) tumor cell intravasation, (c) survival of a tumor in the bloodstream, (d) homing of the tumor to a specific site (frequently, although not invariably), and (e) extravasation and colony formation. This complexity implies either that the combined action of multiple proteins is necessary for metastasis, or that proteins which can promote metastasis are multifunctional.

The identification of tumor-encoded genes, whose elevated expression marks transition to metastasis and which are required for establishment of tumors at distant sites, will yield important diagnostic and therapeutic benefits. Many research groups have sought to define such genes, and in the past year, signaling activity and overexpression of HEF1/Cas-L/NEDD9 (hereafter designated HEF1; refs. 1–3) has been shown to be required for invasion by glioblastomas (4) and strongly linked to promotion of melanoma metastasis (5). Importantly, elevated HEF1 protein expression was detected at focal adhesions during interphase in adherent cells and at centrosomes and other parts of the mitotic apparatus during G2-M. HEF1 lacks any known enzymatic function but contains many functional modules for protein interaction, leading to its classification as a scaffolding protein (2, 3, 7, 8). Validated interaction sequences include (Fig. 1A) an SH3 domain, at least 15 SH2 domain-binding sites, and an evolutionarily well-conserved carboxy-terminal domain of unknown structure. Proteins that functionally and/or physically interact with HEF1 include many with direct roles in promoting tumor invasion, as discussed below. Vertebrate HEF1 has two paralogues, p130Cas/BCAR1 and Efs/Sin, which conserve domain structure and many, but not all, functional interactions (6, 8): together, HEF1, p130Cas, and Efs are called members of the Cas family.

Biological Activities of HEF1 that Favor Metastasis
The HEF1 gene is conserved in all vertebrates and localizes at chromosome 6p25-24 in humans and chromosome 13 A3.3 in mice. The HEF1 protein is predominantly cytoplasmic, concentrating at focal adhesions during interphase in adherent cells and at centrosomes and other parts of the mitotic apparatus during G2-M. HEF1 may provide a useful target for drug discovery efforts.

10Oncology Research Unit, The Children’s Hospital at Westmead, NSW, Australia; 2Discipline of Paediatrics and Child Health, The University of Sydney, NSW, Australia; 3Department of Hematology and Oncology, Graduate School of Medicine, University of Tokyo, Tokyo, Japan; and 4Fox Chase Cancer Center, Philadelphia, Pennsylvania

Requests for reprint: Erica A. Golemis, Fox Chase Cancer Center, 333 Cottman Avenue, Philadelphia, PA 19111. Phone: 215-728-2860; Fax: 215-728-3616; E-mail: EA_Golemis@ffccc.edu.

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domains, the most important of which are Crk and Crk-L. A Cas-
Crk complex has been described as a “master switch” for cell 
migration; through Crk-L and DOCK180, HEF1 activates Rac and 
other components of the cell migration machinery (reviewed in 
ref. 8).

\((b)\) HEF1 activates machinery for tumor growth, invasion, and 
homing. Tumor progression depends on the activation of essential 
effector kinases that mediate proliferation and survival in an 
expanding hypoxic tumor mass. Tumor invasion depends on the 
increased expression and activity of extracellular proteases that
HEF1/Cas-L/NEDD9 in Metastasis

degree or remodel basement membranes to allow cellular movement while activating latent growth factors that promote tumor progression. Related to this, HEF1 overexpression may induce molecules involved in tissue remodeling and invasion (12). HEF1 overexpression also induces the activation of extracellular signal-regulated kinase (ERK), p38, and c-Jun-NH2 kinase (JNK) kinases through interactions with intermediary signaling effectors (reviewed in ref. 6). For example, overexpressed HEF1 transcriptionally induces ErbB2/HER2/neu, a growth factor receptor at the top of the Ras > Raf > MAP/ERK kinase > ERK pathway, whereas the HEF1 interactor FAK directly binds SHC, which influences activation of Ras through its binding partners, epidermal growth factor receptor and growth factor receptor binding protein 2.

HEF1 also binds proteins of the AND-34/CHAT family (13), which activate JNK and ERK by signaling through Rap1 (14). Activated Rap1 is also an important intermediate in “inside-out” cell signaling, in which internally derived signals activate integrin-ligand binding in response to upstream cues that typically involve chemokine stimulation. This process, much studied in consideration of the migration and “homing” of lymphoid cells, is now appreciated as playing a role in tumor invasion and targeting of metastases. Lymphoid cells from HEF1 null mice, or with depleted HEF1, are greatly impaired for chemokine response, migration, and homing, accompanied by failed activation of CHAT-H and Rap1 (14, 15). Critically, these defects involve the CXCR4-CXCL12 targeting system, which is important not only for targeting of lymphocytes to secondary organs, but is also a major contributor to tumor metastasis (16). By inference, CXCR4 signaling is expected to be hyperactivated in cells with overexpressed HEF1.

(c) HEF1 conditions transforming growth factor-β (TGF-β) responses. One of the enigmas of tumor progression is how tumor interpretation of TGF-β signals modulates over time. Extrinsically TGF-β inhibits the growth of early tumors; however, TGF-β promotes the growth of later stage invasive tumors (17), down-regulating E-cadherin and promoting mesenchymal transformation. Intriguingly, HEF1 binds directly to TGF-β pathway effectors and inhibitors, including multiple SMADs (e.g., ref. 18 and discussed in ref. 6). Via these interactions, HEF1 induces negative feedback for aspects of TGF-β-dependent signaling. Intriguingly, the TGF-β pathway signaling molecule SMAD7 has very recently been shown to inhibit melanoma metastasis to bone (19); HEF1 overexpression would be predicted to limit SMAD7 activity, thus promoting metastasis.

(d) HEF1 activates RhoA and Aurora A, providing early counter pressures for tumor growth. Given the extensive biology linking HEF1 to invasion signaling pathways, recent observations that this protein also regulates cell cycle progression through mitosis were unexpected (6, 10, 11). In MCF-7 cells and other epithelial cell lines, HEF1 functions at two discrete points during cytokinesis. At the centrosome, HEF1 interacts with and activates Aurora A kinase during mitotic entry. Overexpressed HEF1 hyperactivates Aurora A, inducing failure of cytokinesis. Separately, HEF1 positively regulates RhoA activation, and elevated HEF1 expression leads to abnormally persistent RhoA activity throughout cytokinesis, preventing normal cellular reattachment to surrounding matrix and providing a second stimulus for deficient cytokinesis. HEF1-overexpressing cells exhibiting defective cytokinesis then arrest in G2 and subsequently undergo apoptosis at high frequency, implying the triggering of cell division checkpoints.

The recognition that HEF1 overexpression triggers cell division checkpoints and apoptosis may be particularly important in understanding why HEF1 overexpression is associated with later (rather than early) stages of tumor progression. In the Tyr–rtTA+/Tet-RAS–INK4a/Arf−/− melanoma mouse model, both Rb-dependent and p53-dependent cell division checkpoints have been disabled, and concurrently, constitutive Ras overexpression provides a strong stimulus toward continued proliferation. Such prior changes may be essential for cells to tolerate proapoptotic effects of sustained HEF1 overexpression. In this context, it is interesting that metastatic melanomas are often characterized by genomic rearrangements and aneuploidy and manifest a high level of apoptosis relatively to premetastatic tumors.

(e) HEF1 and Aurora A regulate ciliary disassembly. Cilia are small organelles that protrude from the surface of many mammalian cell types and act as cellular “antennas,” with growth factor receptors localized at cilia sensing extracellular cues to regulate cell growth. Defects in ciliary structural integrity or associated signaling induce numerous developmental syndromes, are a primary cause of polycystic kidney disease, and have been, in the past year, strongly linked to cancer development (reviewed in ref. 20). Very recently, HEF1 activation of Aurora A at the ciliary basal body was shown to trigger a ciliary resorption pathway involving the tubulin deacetylase HDAC6 as an effector protein (21). This unexpected finding suggests a totally new mechanism by which overexpression of HEF1 can influence the growth properties of metastatic cancers: much more work is necessary to understand the importance of this observation.

What Causes HEF1 Up-regulation before Melanoma Metastasis?

In normal and transformed cells, HEF1 is dynamically regulated in response to both intracellular and extracellular signals. Molecular mechanisms important for control of HEF1 expression

Figure 1. A, schematic of the structure of the HEF1/NEDD9/CasL protein. Human HEF1 is 834 amino acids; key functional domains include an amino terminal SH3 domains which binds FAK, a “substrate domain” (SD) containing multiple embedded SH2 binding sites analogous to the mechanosensing domain of p130Cas recently described by Sawada et al., a likely four-helix bundle based on molecular modeling of primary sequence of HEF1 against the crystal coordinates for the conserved region of p130Cas (results not shown), and an evolutionarily conserved C-terminal domain which binds Src family kinases and other proteins (indicated with a “?” as details of structure remain unknown). B, HEF1 (bright yellow, with “H”) is a component of the integrin-dependent Src-FAK-Crk migration signaling cascade, influences cellular homing through CHAT/CXCR4, engages in crosstalk with the Ras pathway, is an intermediate in TGF–β–dependent signaling, activates the centrosomal Aurora-A/Jub/a/TPX2 machinery governing mitotic entry and cytokinesis, and activates Aurora-A/HDAC6 at the basal body to initiate ciliary disassembly. C, curated online resources (based on experimentally well-validated protein interaction data) indicate numerous direct HEF1 interactions with cancer-related signaling pathways. Blue lines, protein interactions; many HEF1 partners also take part in extensive self-interactions within functional clusters that are likely augmented by increased HEF1 levels. Clusters are particularly relevant to FAK/Src/integrin (rose), CHAT/Rap1 (green), Aurora A (blue), TGF–β–SMADs (gold), and all others (yellow). All HEF1-only interactions present in online databases (blue lines) and common interactions of HEF1 and BCAR1/p130Cas (dashed pink lines, for contrast) are distinguished; interactions between all other proteins, including functional and physical interactions (green). For clarity, only interactions relevant to the discussion in this review are shown. Data on protein–protein interactions were collected in Cytoscape (http://cytoscape.org/), combining data from a Bionet plug-in (http://enr.bio.nyu.edu/cytoscape/bionetbuilder/), and ENML String (string.embl.de/), with each retrieving information from several databases, including DIP (http://dip.doe-mbi.ucla.edu/), BIND (http://www.bind.ca/), KEGG (http://www.genome.jp.kegg/), Prolinks (http://mysqsl5.ncbi.nlm.nih.gov/cgi-bin/funnelcontrol/pronav), HPRD (http://www.hprd.org/), and The BioGrid (http://www.thebiogrid.org/).
Prospect for Therapeutic Exploitation of HEF1

At present, HEF1 is an attractive biomarker of metastatic melanomas. Whether this will be true for other cancers remains to be established; for instance, while HEF1 promotes metastatic behavior in glioblastomas (4), reduced levels of HEF1 transcript characterize an MDA-MB-231 breast cancer cell line selected by serial in vivo passages for efficient metastasis to the lung in mice (9). This may represent tumor type–specific HEF1 action. Alternatively, given that the stoichiometry of HEF1 and its binding partners is likely to be critical to the signaling function of HEF1, it is possible that these data simply reflect an inhibition of HEF1 function — achieved either by loss of gene product or by altered protein stoichiometry resulting from overexpression. Future delineation of different possibilities will be important for determining HEF1 function in metastatic cancer. No serious attempt has as yet been made to target HEF1 for drug development; as a scaffolding protein with no assigned catalytic function, drug selection strategies are clearly more challenging than for enzymes. However, several points suggest a HEF1-directed targeting strategy is feasible. A peptide aptamer screen has identified discrete peptides that bind and stabilize the HEF1 protein from degradation, implying that it may be possible to identify agents that destabilize the protein (28). Significantly, although HEF1−/− mice manifest some developmental and migration defects, these animals are viable and fertile (15), indicating that loss of HEF1 function can probably be well tolerated in adults. Drugs targeting FAK, Src, BCR-ABL, and TGF-β already in the clinic may be particularly effective in tumors overexpressing HEF1 through limiting HEF1 phosphorylation. Finally, early evidence suggests a potential role for HEF1 in other pathologic conditions, including stroke, rheumatoid arthritis, and human T-cell lymphotrophic virus-1 infection, as well as leukemias and lymphomas (reviewed in refs. 6, 7). A decade after its first description, HEF1 is poised to yield exciting insights into the process of metastasis and may provide an important new target in chemotherapy.

Note added in proof


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References


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