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

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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 potential 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 blood stream, (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) have 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 essential for the metastatic properties of the involved tumors both in vitro and in vivo (5). As HEF1 is a scaffolding protein, its action involves regulated assembly of protein complexes. Consequently, the effect of altered HEF1 expression is dependent on the relative stoichiometry and availability of other complex constituents. Hence, moderate overexpression may drive the assembly of functional complexes, causing constitutive activation of downstream effector pathways. Conversely, excessive HEF1 overexpression may be equivalent to loss of HEF1 expression, if either condition induces complex disruption. It is perhaps significant that one study has identified reduced HEF1 expression as part of a signature for metastatic breast cancers (discussed further below; ref. 9). Further, both overexpression and deletion of HEF1 cause mitotic defects in cultured cells (10, 11). Whereas the exact mechanism of HEF1 action in metastasis requires further investigation, to date, studies of HEF1 overexpression, deletion, and genetic deletion have revealed the following metastasis-relevant properties.

(a) HEF1 positively regulates the Src-FAK-Crk “migratory switch.” The initial reports identifying HEF1 established that this protein interacts directly with FAK, Src, and Crk (2, 3). FAK is commonly constitutively activated in melanomas and an important target of cancer drug development. The consensus of work by many groups suggests a mechanism in which cell attachment triggers the interaction of Src, HEF1, and FAK; overexpression or mutational activation of one of these proteins can also drive complex formation. These interactions enhance the activation of Src and FAK and lead to extensive tyrosine phosphorylation of HEF1, creating binding sites for effector proteins with SH2

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 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 (Fig. 1B and C) 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.

Significantly elevated levels of HEF1 mRNA (10-fold to 70-fold) and protein exist in >35% of the metastatic melanomas produced by “escaper” tumors in Tyr−/−Tat−/−Ras+;Ink4a/Arf−/− mutant mice, as well as in a similar percentage of human metastatic melanomas (5). Importantly, elevated HEF1 protein expression was essential for the metastatic properties of the involved tumors both in vitro and in vivo (5). As HEF1 is a scaffolding protein, its action involves regulated assembly of protein complexes. Consequently, the effect of altered HEF1 expression is dependent on the relative stoichiometry and availability of other complex constituents. Hence, moderate overexpression may drive the assembly of functional complexes, causing constitutive activation of downstream effector pathways. Conversely, excessive HEF1 overexpression may be equivalent to loss of HEF1 expression, if either condition induces complex disruption. It is perhaps significant that one study has identified reduced HEF1 expression as part of a signature for metastatic breast cancers (discussed further below; ref. 9). Further, both overexpression and deletion of HEF1 cause mitotic defects in cultured cells (10, 11). Whereas the exact mechanism of HEF1 action in metastasis requires further investigation, to date, studies of HEF1 overexpression, deletion, and genetic deletion have revealed the following metastasis-relevant properties.

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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...
degrade 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 mimics 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. Extrinsic 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−/−rt1A+/+;tet-HAS-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 relative to premetastatic tumors.

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 include those that are associated with the TGF-β pathway (17). First, HEF1 is up-regulated by the TGF-β pathway, which regulates RhoA and Aurora A, providing early counter pressures for tumor growth. Second, HEF1 is up-regulated by the TGF-β pathway, which is associated with the RhoA pathway (17). Third, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Fourth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Fifth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Sixth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Seventh, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Eighth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Ninth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Tenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Eleventh, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Twelfth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Thirteenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Fourteenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Fifteenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Sixteenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Seventeenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Eighteenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Nineteenth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth. Twentieth, HEF1 is up-regulated by the TGF-β pathway, which activates RhoA and Aurora A, providing early counter pressures for tumor growth.
and scaffolding function include transcriptional activation, phosphorylation, and both proteasome-mediated and caspase-mediated proteolysis (reviewed in detail in ref. 6). Although up-regulation of the HEF1 mRNA in metastasis has been shown in some cases to arise from chromosomal amplification (5), this up-regulation more commonly occurs at the level of mRNA transcription. Given that the HEF1 mRNA is down-regulated during nervous system development (ref. 1 and discussed in ref. 6), it is interesting to speculate that HEF1 overexpression in melanoma may reflect aberrant reactivation of a developmental program.

A growing number of transcriptional pathways have been reported to regulate HEF1; intriguingly, some of these are particularly relevant to metastasis. For example, hypoxia is now appreciated as creating conditions conducive for metastasis, and particularly relevant to metastasis. For example, hypoxia is now reported to regulate HEF1; intriguingly, some of these are activation of a developmental program.

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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 stochiometry 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 stochiometry resulting from overexpression. Future delineation of these 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 lymphotropic 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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