

## How Cell Shape Regulates Cancer Cell Division

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The overall interest of the laboratory is to better understand how the molecular machinery that controls cell attachment influences the ability of cells to divide, and how this machinery differs in normal versus cancerous cells. Through understanding these processes, we hope to improve our knowledge of the essential lesions underlying tumor metastasis, thereby allowing therapeutic targeting of these lesions.



### **HEF1: Building a new signaling pathway directly linking adhesion and division.**

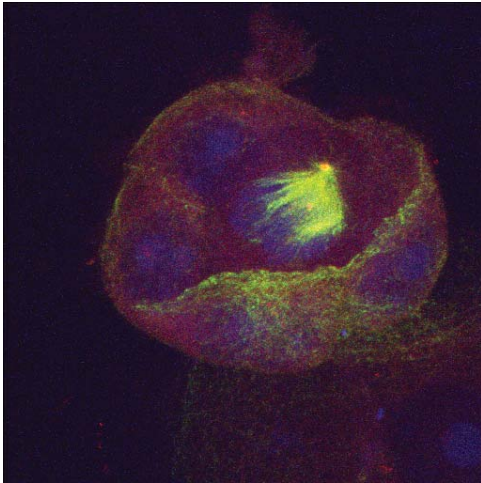
Pugacheva, Dadke, Bluestein, Moyer, Gao, in collaboration with Nicolas,<sup>§</sup> Canutescu,<sup>§</sup> Longmore,<sup>a</sup> Gladson<sup>b</sup>

The adaptor protein HEF1 belongs to the family of Cas proteins, consisting of three defined members p130Cas, HEF1/Cas-L and Efs/Sin1. Our work in progress has identified a fourth potential family member, HEPL, but its ongoing characterization remains at an early stage. The Cas proteins have a well-defined function in the integrin-dependent signaling cascade, transducing signals from the integrin receptor to downstream effector molecules. Recently, we have shown that HEF1 is directly involved in the control of mitosis (1), elucidating a novel mechanism for adhesion-dependent regulation of cell division in normal cells versus in tumors.

HEF1 is localized at two distinct places in the cell. One pool of HEF1 associates with focal adhesion sites on the basal cell surface. Upon integrin engagement, this pool of HEF1 becomes activated through phosphorylation by

FAK and Src kinases, transmitting adhesion signals to the cytoskeleton and nucleus, in turn activating effectors such as Crk, Ras and the JNK1/2 kinases. We have now established that a second pool of HEF1 resides at the centrosome and mitotic spindle. While a subpopulation of HEF1 is constantly localized at centrosome, the amount of HEF1 in this structure increases dramatically at the G2/M boundary of cell cycle, as focal adhesions disassemble. Upon formation of the mitotic spindle, the major pool of HEF1 moves along the spindle microtubules, accumulating towards the mid-zone at the end of mitosis.

We have found that overexpression of HEF1 causes accumulation of cells with supernumerary centrosomes, arising as the consequence of defective cytokinesis (Figure 1). We have shown that this defective cytokinesis occurs because HEF1 overexpression induces hyperactivation of Aurora A (AurA) kinase, and RhoA GTPase. HEF1 directly interacts with AurAs and stimulates activation of AurA at the beginning of mitosis. HEF1 is also an AurA substrate: phosphorylation of HEF1 by AurA upon



**Figure 1.** MCF7 (human breast adenocarcinoma) cells treated for 48h with siRNA to HEF1 were immunostained with antibodies against  $\gamma$ -tubulin (red), and  $\alpha$ -tubulin (green). DNA was stained with TOTO3 (blue). (Interphase cells shown have decreased adhesion, and mitotic cell has a monopolar spindle. Confocal microscopy was performed using a Radiance 2000 laser scanning confocal microscope coupled to a Nikon Eclipse E800 upright microscope in the Microscopy Facility. Scale bar, 10  $\mu$ m.

mitotic entry causes it to dissociate from the AurA complex.

HEF1 also sustains mitotic activation of RhoA, an important stimulator of mitotic cell rounding. HEF1 directly interacts with a RhoA activating protein, Ect2 (a GDP-GTP exchange factor), and overexpression of this complex causes constitutive activation of RhoA, which is normally inactivated at the end of mitosis to allow cytokinesis and cell abscission. Depletion of HEF1 in human cell lines, using siRNA technology, causes accumulation of cells in the G1 phase of cell cycle, and is characterized by centriole splitting. We have shown that the Nek2 kinase, which promotes centrosome disjunction and migration to opposite poles of cell, was prematurely activated in HEF1-depleted cells. These results taken together suggest HEF1 and potentially other basal adhesion proteins are directly involved into control of cell cycle progression.

***In vivo* studies of Cas proteins.** Tikhmyanova, Dadke, in collaboration with Godwin,<sup>§</sup> Wu,<sup>§</sup> Nicolas,<sup>§</sup> Roegiers<sup>§</sup>

The data that we and others have accumulated on HEF1 and the Cas protein family suggests

that deregulation of these proteins may contribute to cancer development, by affecting the processes of apoptosis, migration (metastasis), and genomic instability. Our ongoing work characterizes the expression of HEF1, p130Cas, and other proteins with which they are functionally associated (such as AurA) in primary tumors and normal tissue of the breast and ovary. Preliminary studies indicate that Cas levels vary in tumors in a manner that may correlate with tumor prognosis. Besides AurA, we are focusing on potential cross-regulation between the Cas proteins and E-cadherin, a biomarker of epithelial cell differentiation, which is commonly down-regulated in aggressive tumors.

Study of Cas proteins in animal models is complicated by the fact that there are 3 paralogous members of the family in mammals. However, *Drosophila melanogaster* has only one Cas gene, which makes it a convenient genetic system to study CAS function. We are in the process of knocking out fly Cas, by inducing imprecise P-element excision in a fly stock in which a P-element is inserted immediately 5' of the Cas gene. In addition, we are generating an RNAi-producing Snapback construct targeted to Cas. Injection of embryos with this construct, strain selection, and appropriate follow-up matings will allow us to deplete Cas in specific somatic tissues in *Drosophila*. We will characterize the phenotypes of the knockouts, and use them for genetic experiments with other fly strains to test interactions predicted in cell culture experiments.

**Mapping and analysis of Shc-Cas interactions.** Izumchenko, Serebriiskii, in collaboration with Wolfson<sup>c</sup>

The Shc family of proteins (ShcA, ShcB, and ShcC) are candidate regulators of the shift from normal to pathological brain cell growth in both adult and childhood brain cancers. The Shc proteins are multidomain adaptor proteins, with structural features to allow protein interactions with multiple partners. Although structurally similar, some evidence suggests that the three Shc proteins may assemble non-identical protein complexes, with different cellular functions. Recent work has determined that the ratio of Shc paralogs produced in normal brain tissue versus in brain tumors is totally subverted. Some, but not all, of the Shc proteins have also been shown to contribute to other

types of tumors, such as ovarian tumors. The Shc proteins take part in activation of mitogenic MAPK signaling, pro-survival signaling (through PI3K, Akt, and NF-kappaB), and in signaling involving focal adhesion kinase (FAK) and Src that impacts both cell motility and cell survival. These Shc-associated signaling molecules have been shown to interact directly or indirectly with Cas family members, making Cas proteins prime candidates to mediate Shc functions.

We wish to better understand the role of ShcC versus ShcA signaling in cancer development, with particular emphasis on the role of Cas proteins in this process. As a tool to organize existing data about Shc protein interactions, we have adapted the Cytoscape bioinformatics toolset to assemble a map of potential protein partners for each of the Shc proteins. ShcA has been documented as physically interacting with more than 70 other proteins. These fall into a number of functionally related groups, including proteins related to receptor tyrosine kinase signaling, to anti-apoptotic response, and to cytoskeletal control. In contrast, although ShcC is functionally connected to many pathways, few physical interactions have been reported for this protein. Pursuing the hypothesis that Shc proteins may directly communicate with the cellular attachment apparatus, we have also determined that at least ShcC forms complexes with HEF1, which has recently been described as a regulator of brain cell migration (2). We have assembled a series of constructs expressing truncated derivatives of ShcC, ShcA, HEF1, and p130Cas, and have begun to perform a series of co-transfections and co-immunoprecipitations to allow structure-function analysis. We are now comparing the interaction profiles of HEF1, p130Cas, ShcA, and ShcC. Our goal is to compare the ability of ShcA and ShcC to interact with a panel of defined interacting signaling proteins known to be important for ShcA or ShcC promotion of tumor invasion, based on the hypothesis that Cas proteins are important for Shc-dependent cell transformation.

**The HEI10 cell cycle modulator negatively regulates cell motility.** Singh, Gao, in collaboration with Lessin,<sup>§</sup> Wu,<sup>§</sup> Nicolas<sup>§</sup>

HEI10 is a RING finger protein that regulates the passage through G2 in both yeast and in vertebrates. Previously, we have shown that it

functions as an E3 ubiquitin ligase to promote the proteolysis of cyclin B, such that over-expression of HEI10 extends the G2 phase of cell cycle. HEI10 is itself subject to cell-cycle-controlled changes in expression and localization, and is an *in vitro* substrate for cyclin B/cdc2, implying feedback between the HEI10 protein and the cell cycle machinery. (Toby et al., *Mol. Cell. Biol.* 23:2109, 2003). With collaborators, we have also determined that HEI10 interacts with Merlin, encoded by the tumor suppressor neurofibromatosis 2 (NF2), alteration of which is known to be associated with a number of tumors of neuroectodermal lineage, and to induce both cell proliferation and migration. Given that HEI10 was also reported to be abundant in aggressive melanomas (Smith *et. al.*, *Cancer Biol. Ther.* 3:104, 2004), we wished to delineate the functional role of HEI10 with following aims: 1) Is HEI10 more abundant in aggressive versus non-aggressive tumors, and if so, is this difference specific to melanoma, and 2) Does direct manipulation of HEI10 cause changes in cell migration and attachment?

We have analyzed numerous primary tumor cell lines of diverse stages, as well as multiple cancerous and primary cell lines, using real time RT-PCR to measure HEI10 mRNA levels. Based on this analysis, we conclude that HEI10 is not specifically upregulated in malignant melanomas, contradicting earlier reports. However, we have found that depletion of HEI10 by RNA interference increases cell motility and cell invasion. Using a Boyden chamber assay, this was demonstrated in both the U2OS (osteosarcoma) and MCF7 (breast adenocarcinoma) cell lines. Separately, HEI10-depleted U2OS cells traveled faster than control cells in a plate-based wound-healing assay. The motility of HEI10-depleted cells increased within 2–3 days of siRNA treatment, preceding a subsequent inhibition of cell proliferation observed at 4 days following depletion, suggesting complex action.

We are currently establishing the molecular mechanism for the increased motility phenotype in HEI10 depleted cells. Pilot studies did not demonstrate HEI10-dependent changes in the expression or activity of canonical promotility factors such as Pak1, and small Rho-class GTPases. Intriguingly, a recent publication has shown that increased activity of cyclin B and Cdk1 can promote increased motility in

prostate cancer cell lines, modulating actin cytoskeletal organization through phosphorylation of caldesmon (Manes et al., *J. Cell Biol.* 161: 817, 2003). Since we have already shown cyclin B is a substrate in a HE110-induced ubiquitin proteasome pathway, we investigated this possible mechanism for the increased motility of HE110-depleted cells, and we have confirmed levels of cyclin B and Cdk1 are significantly elevated. We are working to refine this proposed model for HE110 function.

### **The role of extracellular matrix in regulating tumor response to therapeutic agents.**

Serebriiskii, Lamb, in collaboration with Cukierman,<sup>§</sup> Godwin<sup>§</sup>

Our studies described above, and many examples in the scientific literature, have demonstrated numerous connections between proteins that mediate cell communications to the extracellular environment and core processes of cell cycle and cell survival controls. A practical implication of this work is that extracellular environment may not only influence the ability of tumors to progress and become metastatic, but may also influence the internal response of the tumor cells to therapeutic

agents. If so, better understanding the process may significantly improve preclinical drug development, by allowing the creation of cell culture screening systems for new drugs that more closely simulating the *in vivo* environment in which a tumor will be treated.

The Cukierman group is expert in the development of three-dimensional extracellular matrix-based cell culture systems. In collaborative experiments, we have used 12 different human tumor cell lines, plating these in parallel within NIH-3T3 fibroblast-derived matrices or on tissue culture plastic, then measuring a series of growth parameters including proliferation, shape, and survival following treatment with a panel of cytotoxic or protein-targeted drugs. We have found that tumor cell lines display discrete individual profiles of response to matrix: the ability of matrix to strongly influence cell proliferation does not correspond to ability to influence shape, and vice versa. Importantly, the effectiveness of some but not all drugs in limiting cell proliferation was influenced by the growth of tumor cells on matrix. Ongoing work will address the underlying mechanistic differences important for matrix-based drug response, with particular emphasis on ovarian tumor models.

### **Publications**

1. Pugacheva, E.N., Golemis, E.A. HEF1 regulates centrosomal maturation and spindle formation through control of the Aurora A kinase. *Nature Cell Biol.* 7:937-946, 2005.
2. Natarajan, M., Stewart, J.E. Jr., Golemis, E.A., Pugacheva, E.N., Alexandropoulos, K., Grammer J.R., Gladson, C.L. HEF1 is a necessary and specific downstream effector of FAK that promotes the migration of glioblastoma cells. *Oncogene* 25:1721-1732, 2006. [Epub Nov. 15, 2005]

Golemis, E.A., Adams, P.D. (Editors) Protein-Protein Interactions, Second Edition: a Molecular Cloning Manual. Cold Spring Harbor Laboratory Press, New York, 938 pages, 2005.

Izumchenko, E., Wolfson, M., Golemis, E.A., Serebriiskii, I.G. Yeast hybrid approaches. *In Yeast Gene Analysis*, edited by M. Stark and I. Stansfield, Elsevier Press (in press).

Jin, T., Howard, A.J., Golemis, E.A., Wang, Y., Zhang, Y.-Z. Overproduction, purification, crystallization and preliminary X-ray diffraction studies of the human spliceosomal protein TXNL4B. *Acta Cryst.* **F61**:282-284, 2005.

Jin, T., Howard, A., Golemis, E.A., Wang, Y., Zhang, Y.-Z. Overproduction, purification, crystallization and preliminary X-ray diffraction studies of the human transcription repressor ERH. *Acta Cryst.* **F61**:531-533, 2005.

Khazak, V., Golemis, E.A., Weber, L. Development of a yeast two-hybrid screen for selection of human Ras-Raf protein interaction inhibitors. *In Chemical Genomics: Reviews and Protocols*, edited by E.D. Zanders. *Methods Mol. Biol.* **310**:253-271, 2005.

Khazak, V., Kato-Stankiewicz, J., Tamanai, F., Golemis, E.A. Yeast screens for inhibitors of Ras-Raf interaction, and characterization of MCP inhibitors of Ras-Raf interaction. Chapter 62, Regulators and Effectors of Small GTPases. *Methods Enzym.* Part H, edited by C. Der (in press).

Pugacheva, E.N., Golemis E.A. Building a better web: progress in the concept and methodology of protein interaction studies, pp. 13-35. *In Protein-Protein Interactions*, Second Edition: a Molecular Cloning Manual, 938 pages, edited by E.A. Golemis and P.D. Adams. Cold Spring Harbor Laboratory Press, New York, 2005.

Serebriiskii, I.G., Fang, R., Latypova, E., Hopkins, R., Vinson, C., Joung, J.K., Golemis, E.A. A combined yeast/bacterial two-hybrid system: development and evaluation. *Mol. Cell. Proteomics* 4:819-826, 2005.

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Fox Chase infusion room staff members win the dog-owner look-alike contest at the Center's 7th Annual "Paws for the Cause" dog walk.