Acknowledgments

Supported by the German Cancer Society (Deutsche Krebshilfe, Max Eder Program, Bonn, Germany) (O.S.), NIH grant R01 CA112199 (F.M.-B.), and NIH R01 CA112390 (L.M.E.). The authors apologize to those whose important contributions to the field could not be cited due to the restriction on the number of references.

Selected reading

Atkins, M.B., Hidalgo, M., Stadler, W.M., Logan, T.F., Dutcher, J.P., Hudes, G.R., Park, Y., Liou, S.H., Marshall, B., Boni, J.P., et al. (2004). J. Clin. Oncol. *22*, 909–918.

Fan, F., Wey, J.S., McCarty, M.F., Belcheva, A., Liu, W., Bauer, T.W., Somcio, R.J., Wu, Y., Hooper, A., Hicklin, D.J., and Ellis, L.M. (2005). Oncogene *24*, 2647–2653.

Gerber, H.P., McMurtrey, A., Kowalski, J., Yan, M., Keyt, B.A., Dixit, V., and Ferrara, N. (1998). J. Biol. Chem. *273*, 30336–30343.

Guba, M., von Breitenbuch, P., Steinbauer, M., Koehl, G., Flegel, S., Hornung, M., Bruns, C.J., Zuelke, C., Farkas, S., Anthuber, M., et al. (2002). Nat. Med. *8*, 128–135.

Guba, M., Koehl, G.E., Neppl, E., Doenecke, A., Steinbauer, M., Schlitt, H.J., Jauch, K.W., and Geissler, E.K. (2005). Transpl. Int. *18*, 89–94.

Hicklin, D.J., and Ellis, L.M. (2005). J. Clin. Oncol. *23*, 1011–1027.

Hresko, R.C., and Mueckler, M. (2005). J. Biol. Chem. *280*, 40406–40416.

Hudes, G., Carducci, M., Tomczak, P., Dutcher, J., Figlin, R., Kappoor, A., Staroslawska, E., O'Toole, T., Park, Y., and Moore, L. (2006). Proc. Am. Soc. Clin. Oncol. *24*, 2s, abstract LBA4.

Hurwitz, H., Fehrenbacher, L., Novotny, W., Cartwright, T., Hainsworth, J., Heim, W., Berlin, J., Baron, A., Griffing, S., Holmgren, E., et al. (2004). N. Engl. J. Med. *350*, 2335–2342.

Jain, R.K. (2005). Science *307*, 58–62.

Motzer, R.J., Hutson, T.E., Tomczak, P., Michaelson, M.D., Bukowski, R.M., Rixie, O., Oudard, S., Kim, S.T., Baum, C.M., and Figlin, R.A. (2006). Proc. Am. Soc. Clin. Oncol. *24*, 2s.

O'Reilly, K.E., Rojo, F., She, Q.B., Solit, D., Mills, G.B., Smith, D., Lane, H., Hofmann, F., Hicklin, D.J., Ludwig, D.L., et al. (2006). Cancer Res. *66*, 1500–1508.

Phung, T.L., Ziv, K., Dabydeen, D., Eyiah-Mensah, G., Riveros, M., Perruzzi, C., Sun, J., Monahan-Earley, R.A., Shiojima, I., Nagy, J.A., et al. (2006). Cancer Cell, this issue.

Sarbassov, D.D., Guertin, D.A., Ali, S.M., and Sabatini, D.M. (2005). Science *307*, 1098–1101.

Sarbassov, D.D., Ali, S.M., Sengupta, S., Sheen, J.H., Hsu, P.P., Bagley, A.F., Markhard, A.L., and Sabatini, D.M. (2006). Mol. Cell *22*, 159–168.

Shi, Y., Yan, H., Frost, P., Gera, J., and Lichtenstein, A. (2005). Mol. Cancer Ther. *4*, 1533–1540.

DOI 10.1016/j.ccr.2006.07.013

Microcephalin guards against small brains, genetic instability, and cancer

Through its roles in cell cycle control and DNA damage response, microcephalin (also known as BRIT1 or MCPH1) has been implicated in fundamental biological processes, including regulation of brain size and maintenance of genomic integrity. Two new reports in *Nature Cell Biology* and this issue of *Cancer Cell* provide further insights into the functions of microcephalin in DNA damage checkpoints and timing of mitosis. Depletion or disease-associated mutations of microcephalin resulted in centrosomal abnormalities and chromosomal instability. These findings and aberrantly reduced expression in human carcinomas identify microcephalin as a candidate novel tumor suppressor.

Microcephalin is the product of the first identified gene among several loci whose mutations cause an autosomal recessive disorder known as primary microcephaly (Jackson et al., 2002), a condition characterized by a markedly reduced brain size and mental retardation (Woods et al., 2005). Microcephaly of patients with microcephalin mutations reflects defects in neurogenesis, due to deficient mitosis in neural precursor cells. The mitosis-regulatory function of microcephalin prevents premature chromosome condensation (PCC) and may provide a timing device for mitotic onset (Trimborn et al., 2004). Interestingly, evolutionary analysis indicates that some changes in the microcephalin gene have been positively selected for during human and great ape evolution and contributed to the most striking differences between humans and apes: brain size and cognitive ability (Woods et al., 2005).

Another clue about the biological role of microcephalin was suggested by the presence of three Brca1 carboxy-terminal (BRCT) domains in the protein. BRCT domains are peptide and phosphopeptide binding modules present in a range of proteins involved in DNA damage response, including checkpoint control and DNA repair (Kastan and Bartek, 2004). Indeed, microcephalin turned out to be required for proper execution of the intra-S phase and the G2/M checkpoints in response to ionizing radiation, and it colocalized in the so-called irradiation-induced nuclear foci with other DNA damage response proteins such as phosphorylated histone H2AX (γH2AX), thereby identifying microcephalin as a component of the DNA damage response network (Xu et al., 2004; Lin et al., 2005).

Those initial studies suggested that microcephalin could play at least two roles in cell physiology, in regulation of unperturbed mitotic cell cycles, and in response to genotoxic stress. Two recent, complementary reports now shed more light on these emerging cellular roles, and functional consequences of diverse mutations of microcephalin/MCPH1/BRIT1 found in primary microcephaly (Alderton et al., 2006) or, for the first time, in cancer (Rai et al., 2006).

First, the team headed by Penny Jeggo and Mark O'Driscoll (Alderton et al., 2006) approached this issue inspired by their previous discovery that hypomorphic mutations in ATR and defects in ATR kinase signaling, one of the two central pathways within the DNA damage machinery (Kastan and Bartek, 2004), caused Seckel syndrome, another disease characterized by microcephaly (O'Driscoll et al., 2003). The authors compared responses to ultraviolet light and a repli-

cation inhibitor, hydroxyurea, in cell lines harboring different truncating mutations in microcephalin, derived from microcephaly patients, versus the ATR-deficient Seckel syndrome cells. Alderton et al. (2006) conclude that the role of microcephalin in the S and G2/M checkpoints, including the control of Cdc25A phosphatase turnover that is targeted by these checkpoints (Kastan and Bartek, 2004), is shared by the ATR-Seckel syndrome cells. Also, nuclear fragmentation and supernumerary mitotic centrosomes were present in either microcephalin- or ATR-defective cells. Alderton et al. also found a physical interaction of microcephalin with Chk1, the kinase that is activated by ATR in response to replication stress and UV light, and they propose that microcephalin may operate in the ATR pathway downstream of Chk1. Thus, the fact that ATR and microcephalin may function in the same pathway could explain those cellular phenotypes and some clinical features that are shared by the Seckel syndrome and primary microcephaly patients.

On the other hand, there were also features unique to microcephalin-deficient cells: aberrantly low levels of the tyrosine 15-phosphorylated, inactive form of the major mitosis-promoting kinase Cdk1, and much more pronounced PCC, a hallmark of premature mitotic onset. Alderton et al. (2006) proposed that the role of microcephalin in the regulation of mitotic entry is independent of ATR, and that the striking phenomenon of PCC in the absence of functional microcephalin reflects aberrantly high Cdk1-cyclin B kinase activity.

The other study, published in this issue of *Cancer Cell* (Rai et al., 2006), focuses on the role of microcephalin in response to DNA damage and the potential involvement of microcephalin in tumorigenesis. The starting point of the work led by Shiaw-Yih Lin was the previously identified link between BRIT1/microcephalin and radiation-induced checkpoints (Xu et al., 2004; Lin et al., 2005), and the established notion in the field that many components of the DNA damage response network have properties of tumor suppressors (Kastan and Bartek, 2004). In the first set of experiments, Rai et al. (2006) showed that, in cells exposed to ionizing radiation, the microcephalin/BRIT1 protein localizes to sites of DNA damage marked by accumulation of other established DNA damage response proteins, such as the MDC1 and 53BP1 checkpoint mediators, NBS1, or phosphorylated ATM, the key signaling kinase in response to DNA double-strand breaks (Kastan and Bartek, 2004). In addition, the authors conclude that, in cells depleted of microcephalin/BRIT1 by treatment with siRNA, radiation-induced foci formation by the above-mentioned upstream components of the DNA damage response cascade is inhibited, as is their interaction with chromatin. Analogous experiments with UV irradiation indicated that microcephalin/BRIT1 was required for proper formation of ATR, RPA, and Rad17 foci and for UV-induced phosphorylation of RPA and Rad17 (Rai et al., 2006), suggesting that microcephalin is a proximal factor in the hierarchical web of the DNA damage pathways.

Arguably, the most exciting outcome of the work by Lin and colleagues is the evidence for a potential role of microcephalin/BRIT1 in protection against cancer, a notion supported by the following observations. (1) Depletion of microcephalin/BRIT1 resulted in elevated frequency of chromosomal abnormalities. (2) Both gene copy number and expression of microcephalin/BRIT1 (at both the mRNA and protein levels) were aberrantly reduced in several breast cancer cell lines and in human epithelial tumors of the ovary and prostate compared to corresponding normal tissues. (3) In one of ten breast cancer specimens, a small deletion, resulting in a premature stop codon, was found in microcephalin/BRIT1. This truncation eliminates the two C-terminal BRCT domains of microcephalin/BRIT1, and functional analysis showed this to be a loss-of-function defect. In addition, the mutant was present in a hemizygous state in the tumor, with no detectable wild-type transcript from the other allele (Rai et al., 2006). Collectively, these results strongly suggest that microcephalin/BRIT1 is a candidate novel tumor suppressor in multiple cell lineages.

While the two reports offer intriguing data that further implicate microcephalin as an important player in the genome maintenance machinery and a likely tumor suppressor, many questions and a puzzling discrepancy were also raised by these studies. The latter relates to the fact that, whereas Alderton et al. (2006) place microcephalin rather downstream in the ATR-Chk1-Cdc25A checkpoint cascade, Rai et al. (2006) propose that it plays an upstream role in responses to both ionizing and UV radiation. Whether these differences might reflect different mutations and knockdown protocols used in the two studies, for example, needs to be established by future work.

The obvious open questions relate particularly to the mechanistic basis of microcephalin action in the DNA damage pathways. What is the exact role of microcephalin in the interplay between the Chk1 kinase and its substrate, Cdc25A? Also, how does microcephalin impact on Tyr15-phosphorylation of Cdk1, and hence on the timing of mitotic entry and chromosome condensation? For example, could the striking effect of microcephalin on mitotic timing be related to its interaction with Chk1, and the fact that both microcephalin (Alderton et al., 2006) and Chk1 (Krämer et al., 2004) reside on centrosomes, and centrosomal Chk1 regulates the Tyr15-phosphorylation status of Cdk1 and the onset of mitosis (Krämer et al., 2004)? This scenario would also fit the concept that the mitotic role of microcephalin operates in normal cell cycles and is distinct from its role(s) in response to genotoxic stress. Similarly, how would microcephalin achieve in molecular terms its apparent upstream effects on the DNA damage response cascades reported by Rai et al. (2006)?

Finally, in terms of the link with tumorigenesis, it will be exciting to develop a mouse gene knockout model, and also find out if either or both the mitosis timing function and the role in the genome integrity maintenance contribute to the tumor suppressor properties of microcephalin. Given the recently discovered role of the DNA damage response machinery as an inducible barrier against cancer progression in early lesions (Gorgoulis et al., 2005; Bartkova et al., 2005), microcephalin defects such as those reported by Rai et al. (2006) for the breast, ovarian, and prostate tumors could significantly contribute to inactivation of this defense mechanism in human cancer pathogenesis.

Acknowledgments

The author is supported by grants from the Danish Cancer Society, the Danish National Research Fund, and the European Commission (integrated projects Active p53, Mutant p53, and DNA Repair). I apologize to researchers whose work is not cited due to restrictions on the number of references.

Jiri Bartek[1,*]

[1]Institute of Cancer Biology and Centre for Genotoxic Stress Research, Strandboulevarden 49, DK-2100 Copenhagen, Denmark
*E-mail: jb@cancer.dk

Selected reading

Alderton, G.K., Galbiati, L., Griffith, E., Surinya, K.H., Neitzel, H., Jackson, A.P., Jeggo, P.A., and O'Driscoll, M. (2006). Nat. Cell Biol. *8*, 725–733.

Bartkova, J., Horejsi, Z., Koed, K., Kramer, A., Tort, F., Zieger, K., Guldberg, P., Sehested, M., Nesland, J.M., Lukas, C., et al. (2005). Nature *434*, 864–870.

Gorgoulis, V.G., Vassiliou, L.V., Karakaidos, P., Zacharatos, P., Kotsinas, A., Liloglou, T., Venere, M., Ditullio, R.A., Jr., Kastrinakis, N.G., Levy, B., et al. (2005). Nature *434*, 907–913.

Jackson, A.P., McHale, D.P., Campbell, D.A., Jafri, H., Rashid, Y., Mannan, J., Karbani, G., Corry, P., Levene, M.I., Mueller, R.F., et al. (2002). Am. J. Hum. Genet. *63*, 541–546.

Kastan, M.B., and Bartek, J. (2004). Nature *432*, 316–323.

Krämer, A., Mailand, N., Lukas, C., Syljuäsen, R.G., Wilkinson, C.J., Nigg, E.A., Bartek, J., and Lukas, J. (2004). Nat. Cell Biol. *6*, 884–891.

Lin, S.Y., Rai, R., Li, K., Xu, Z.X., and Elledge, S.J. (2005). Proc. Natl. Acad. Sci. USA *102*, 15105–15109.

O'Driscoll, M., Ruiz-Perez, V.L., Woods, C.G., Jeggo, P.A., and Goodship, J.A. (2003). Nat. Genet. *33*, 497–501.

Rai, R., Dai, H., Multani, A.S., Kaiyi, L., Chin, K., Gray, J., Lahad, J.P., Liang, J., Mills, G.B., Meric-Bernstam, F., and Lin, S.-Y. (2006). Cancer Cell, this issue.

Trimborn, M., Bell, S.M., Felix, C., Rashid, Y., Jafri, H., Griffiths, P.D., Neumann, L.M., Krebs, A., Reis, A., Sperling, K., et al. (2004). Am. J. Hum. Genet. *75*, 261–266.

Woods, C.G., Bond, J., and Enard, W. (2005). Am. J. Hum. Genet. *76*, 717–728.

Xu, X., Lee, J., and Stern, D.F. (2004). J. Biol. Chem. *279*, 34091–34094.

DOI 10.1016/j.ccr.2006.07.014

Co-opted integrin signaling in ErbB2-induced mammary tumor progression

Although almost two decades of study point to a central role for aberrant ErbB2 activation in breast cancer, many cellular and biochemical mechanisms underlying ErbB2-induced tumor initiation and progression remain to be resolved. A study by Guo et al. published recently in *Cell* indicates that the signaling function of β4 integrin actively contributes to the initiation, growth, and invasion of ErbB2-induced mammary tumors in transgenic mice by promoting the activation of c-Jun and STAT3. These observations offer novel mechanistic insight into ErbB2 action and highlight the notion that ErbB2 co-opts the functions of other signaling proteins to elicit tumor progression.

Overexpression of the ErbB2 (HER2/neu) receptor tyrosine kinase is observed in a variety of solid tumor types, including 20%–30% of breast tumors. Overexpression correlates with poor patient prognosis and resistance to some therapies. In vitro, expression of a constitutively active point mutant of ErbB2 is sufficient to mediate the transformation of cultured cells, suggesting that aberrant kinase activation is sufficient to initiate tumorigenic processes. Likewise, expression of an activated form in the mammary epithelium of transgenic mice gives rise to the rapid emergence of invasive tumors, pointing to a central role for ErbB2 activation in breast cancer malignancy. Coupled with reports that ErbB2 overexpression is sufficient to activate its tyrosine kinase activity, such observations have prompted the development of strategies to interfere with ErbB2 activity in breast tumors. Indeed, the humanized anti-ErbB2 monoclonal antibody Herceptin has been in clinical use for over a half dozen years, and other therapies are under development.

While it is clear that aberrant ErbB2 activation actively contributes to the genesis and progression of breast tumors, cellular and biochemical mechanisms underlying ErbB2-mediated proliferation and invasion remain to be fully elucidated. Of particular interest are the mechanisms connecting activated ErbB2 to the breakdown of mammary epithelial cell-cell interactions. ErbB2 activation dissolves interepithelial cell interactions mediated by tight junctions or by adherens junctions through E-cadherin, leading to a loss of cell polarity and the initiation of invasion. An insightful study by Guo et al. (2006) points to a key role for cellular signaling mediated by β4 integrin in ErbB2-mediated proliferation and invasiveness of breast tumor cells and underscores an unappreciated role for STAT3 signaling in mediating the loss of mammary epithelial adhesion.

Hemidesmosomal α6β4 integrin contributes to the anchoring of mammary epithelial cells to the basement membrane through its intracellular interactions with the cytoskeleton and extracellular interactions with the matrix component laminin-5. Several studies suggest that the large intracellular domain of the β4 subunit is also involved in cellular signaling. For example, expression of β4 integrin in β4-deficient cultured breast cancer cells augments cellular invasive properties (Shaw et al., 1997). This effect requires the β4 intracellular domain and is suppressed by inhibitors of PI3 kinase activity, suggesting that β4 signaling to the PI3 kinase pathway augments the invasiveness of these cells. Moreover, blocking antibodies to either α6 or β4 integrin subunits suppress the formation of apoptosis-resistant acinar structures in Matrigel by mammary epithelial cells (Weaver et al., 2002), suggesting a role for β4-mediated cellular polarity in mediating antiapoptotic signaling. Finally, β4 has been demonstrated to physically interact with ErbB2 in some cultured breast tumor cells, and the two proteins synergize in promoting cellular proliferation and invasion (Falcioni et al., 1997). Taken together, these and other in vitro studies strongly point to a potential role for β4 signaling in promoting breast tumor progression.

To examine the role of β4 integrin signaling in ErbB2-induced mammary tumors in vivo, Guo et al. employed a knockin mouse where expression of the endogenous *β4* gene was replaced with a variant (called 1355T) lacking the carboxy terminal ~450 amino acids. This form is capable of interacting with laminin-5 and the keratin cytoskeleton, thus maintaining the ability