New Insights into RSK Activation and Hematopoietic Cancer

Ana Cuadrado[1] and Angel R. Nebreda[1,*]
[1]CNIO (Spanish National Cancer Center), Melchor Fernández Almagro, 3, E-28029 Madrid, Spain
*Correspondence: anebreda@cnio.es
DOI 10.1016/j.ccr.2007.08.017

The tyrosine kinase receptor FGFR3 is thought to play a role in hematopoietic malignancies. A new study in this issue of *Cancer Cell* identifies the serine/threonine kinase RSK2 as a key substrate of FGFR3 in human t(4;14)-positive multiple myeloma (MM) cells. Constitutively active FGFR3 directly phosphorylates RSK2 on Tyr529, which primes RSK2 for activation by the kinases ERK1 and ERK2 (ERK1/2). In turn, RSK2 activity plays an important role in the survival of FGFR3-expressing MM cells.

The elucidation of signal transduction mechanisms that underlie tumor initiation and progression should help to improve diagnosis as well as to identify potential therapeutic targets for rational cancer treatment. MM is a common hematological malignancy in elderly patients that affects terminally differentiated plasma B cells. Molecular and cytogenetic data indicate that translocations involving 14q32 into the immunoglobulin heavy (IgH) chain switch region are frequent in human MM cells. For example, about 15% of MM patients have the t(4;14) translocation that involves FGFR3, one of the four tyrosine kinase receptors that engage fibroblast growth factor (Chesi et al., 1997). In some cases, the translocated *FGFR3* gene contains the activating mutation K650E and is referred to as FGFR3 TDII. Dysregulated FGFR3 is also associated with other hematological malignancies such as peripheral T cell lymphomas, which express the constitutively active TEL-FGFR3 fusion formed by the transcription factor TEL and the kinase domain of FGFR3. Studies with cell lines and murine models support a role for FGFR3 in hematopoietic cell transformation.

Characterization of the FGFR3-activated signaling pathways in hematopoietic malignancies is important to understand the pathogenesis of MM and to gain insights into potential therapeutic strategies. Interestingly, activating FGFR3 mutations do not occur in human MM cells harboring activating mutations of the GTPases K-RAS or N-RAS, suggesting that FGFR3 and RAS could contribute to MM progression via the same or similar signaling pathways. Accordingly, leukemogenic FGFR3 TDII and TEL-FGFR3 variants have been shown to activate the ERK1/2 kinase cascade, which is also one of the effectors of RAS whose dysregulation has been associated with cell transformation and cancer.

In this issue of *Cancer Cell*, Kang et al. (2007) report that the ERK1/2-activated 90 kDa ribosomal S6 kinase RSK2 is a key mediator of FGFR3 signaling in hematopoietic transformation. The RSK family includes four serine/threonine protein kinases that play important roles in many processes, including cell survival and proliferation (Hauge and Frodin, 2006). All RSK family members have similar overall structure, with two nonidentical, tandem kinase domains (referred to as NTK and CTK) separated by a linker region. RSK activation requires the sequential phosphorylation of four residues by ERK1/2, by RSK itself, and by PDK1, a process that is initiated by the binding of ERK1/2 to a docking site located at the C-terminal end of the RSK protein (Figure 1).

In an effort to understand FGFR3 signaling in hematopoietic malignancies, Kang et al. performed a mass spectrometry-based phospho-proteomic analysis in murine Ba/F3 cells expressing leukemogenic TEL-FGFR3, which bypasses the requirement of IL-3 for proliferation. In this study, constitutively active TEL-FGFR3 was found to induce the phosphorylation of RSK2 on Tyr488 and Tyr529. Further characterization showed that phosphorylation of Tyr529, but not of Tyr488, was important for RSK2 activation downstream of FGFR3. Moreover, recombinant FGFR3 was able to directly phosphorylate Tyr529 of RSK2 in vitro.

How does Tyr529 phosphorylation contribute to RSK2 activation? The results by Kang et al. indicate that Tyr529 phosphorylation enhances the interaction between RSK2 and ERK1/2, which in turn should facilitate the activation of RSK2. This conclusion is unexpected because there was no previous evidence that posttranslational modification of RSK2 is necessary for the binding of ERK1/2 to its C-terminal docking site, although RSK autophosphorylation at Ser737 is known to contribute to its inactivation by decreasing its affinity for ERK1/2. In agreement with this, the inactive (unphosphorylated) forms of ERK1/2 and RSK can be coimmunoprecipitated from cells that have not been stimulated with mitogens (Gavin and Nebreda, 1999; Roux et al., 2003). The structural basis for how Tyr529 phosphorylation can boost binding of ERK1/2 to the docking site of RSK2 located about 200 amino acids away remains to be elucidated.

Whether tyrosine phosphorylation of RSK2 is a specific requirement of FGFR3 signaling in hematopoietic cells or it might represent a more general mechanism for RSK2 activation is not clear yet. Intriguingly, Kang et

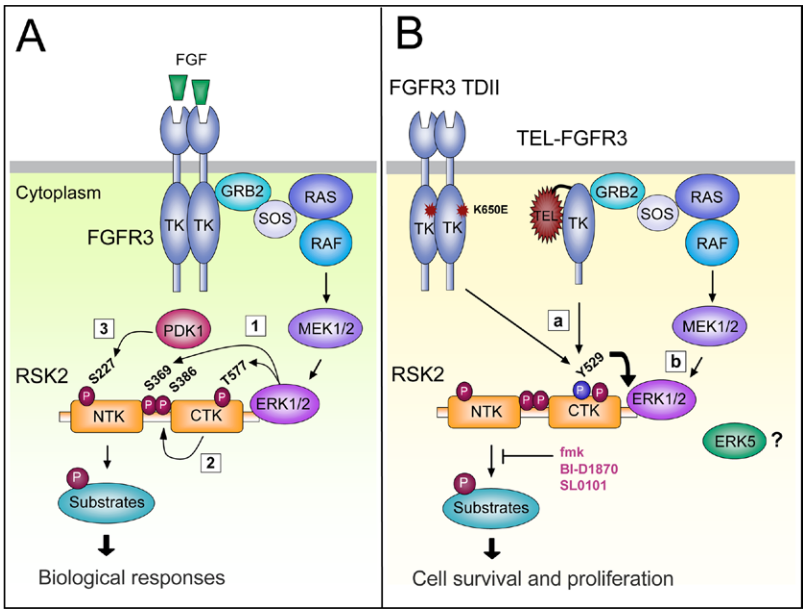

Figure 1. Molecular Mechanisms of FGFR3-Induced RSK2 Activation

(A) Tyrosine kinase receptors, such as FGFR3, trigger the activation of ERK1/2 by a pathway that is initiated by oligomerization and autophosphorylation of the receptor upon ligand binding. This is followed by recruitment of the GRB2-SOS complex and the sequential activation of RAS and the kinases RAF and MEK1 and MEK2 (MEK1/2), which directly phosphorylate and activate ERK1/2. In turn, RSK2 activation requires the binding of ERK1/2 to a docking site sequence located at its C terminus. ERK1/2 phosphorylates at least two residues, Ser369 in the linker region and Thr577 in the activation loop of CTK (1). Phosphorylation of Thr577 activates CTK, which then phosphorylates Ser386 in the linker region and generates a docking site for PDK1 (2). Finally, PDK1 phosphorylates Ser227 in the activation loop of NTK (3), resulting in fully active RSK2 that phosphorylates its downstream targets via NTK.

(B) In hematopoietic cancer cells, constitutively active TEL-FGFR3 and FGFR3 TDII (see text) can induce RSK2 activation by a two-step mechanism. FGFR3 phosphorylates RSK2 at Tyr529 (a), facilitating the binding of ERK1/2 to the C-terminal docking site of RSK2. In addition, FGFR3 triggers the classical ERK1/2 activation pathway that leads to the phosphorylation and activation of RSK2 (b), as explained above. ERK5, another member of the mitogen-activated kinase family, might play a role in FGFR3-induced RSK2 activation during leukemogenesis. Small-molecule RSK inhibitors are indicated.

al. mention in passing that RSK2 can be phosphorylated on Tyr529 upon EGF stimulation of 293T cells, suggesting that other tyrosine kinases, in addition to FGFR3, could contribute to RSK activation via direct tyrosine phosphorylation. It should be noted that Tyr529 is conserved in the four mammalian RSK family members as well as in *Xenopus* and *Drosophila* RSK proteins.

At the functional level, Kang et al. addressed the role of RSK2 in hematopoietic transformation. They found that FGFR3-induced activation of RSK2 was required for the survival of both murine Ba/F3 cells expressing leukemogenic FGFR3 mutants and human t(4;14)-positive MM cells that express FGFR3. Curiously, RSK1 did not appear to significantly contribute to FGFR3-induced cell survival, suggesting a specific requirement for RSK2 but not RSK1 in mediating oncogenic FGFR3 signaling. Of note, it has been recently shown that ectopic activation of the MEK5/ERK5 pathway in HEK293 cells activates RSK2 but not RSK1 (Cude et al., 2007), and ERK5 has also been implicated in the regulation of MM cell survival (Carvajal-Vergara et al., 2005). Whether the ERK5 pathway might be involved in the FGFR3-induced activation of RSK2 in MM cells deserves further study. It also remains to be established what targets of RSK2 account for its contribution to MM cell survival.

The requirement for RSK2 in survival of human t(4;14)-positive MM cells is noteworthy in light of the recent development of very specific RSK inhibitors (Cohen et al., 2007; Sapkota et al., 2007). Fmk is a RSK inhibitor whose specificity is based on the presence of a cysteine residue and a threonine residue at particular positions in the ATP-binding site of CTK, which are only present in the human kinases RSK1, RSK2, and RSK4. Kang et al. found that treatment with fmk led to increased apoptosis in several human t(4;14)-positive MM cell lines as well as in primary FGFR3-expressing MM cells ($CD138^+$) from a t(4;14)-positive patient. Importantly, the survival of $CD138^-$ cells from the same patient or $CD138^+$ cells from a t(4;14)-negative patient was not affected by fmk. These results are in agreement with a previous report showing that RSK inhibition impaired the proliferation of breast cancer cells, while having little effect on nontumoral cells (Smith et al., 2005). Moreover, the triple knockout of RSK1, RSK2, and RSK3 does not appear to affect mouse viability (Dumont et al., 2005), further suggesting that specific RSK inhibitors might not have serious side effects in organism homeostasis. Future work should extend these studies and address the therapeutic window of small-molecule RSK inhibitors.

Finally, Kang et al. report that fmk can also induce apoptosis in the t(4;14)-negative MM cell line RPMI8226, which lacks FGFR3 expression but contains active RSK2, probably induced by the expression of oncogenic RAS. This result suggests a wider range of possible therapeutic applications for RSK inhibitors in cancer treatment beyond FGFR3-positive MM.

REFERENCES

Carvajal-Vergara, X., Tabera, S., Montero, J.C., Esparis-Ogando, A., Lopez-Perez, R., Mateo, G., Gutierrez, N., Parmo-Cabanas, M., Teixido, J., San Miguel, J.F., and Pandiella, A. (2005). Blood *105*, 4492–4499.

Chesi, M., Nardini, E., Brents, L.A., Schrock, E., Ried, T., Kuehl, W.M., and Bergsagel, P.L. (1997). Nat. Genet. *16*, 260–264.

Cohen, M.S., Hadjivassiliou, H., and Taunton, J. (2007). Nat. Chem. Biol. *3*, 156–160.

Cude, K., Wang, Y., Choi, H.J., Hsuan, S.L., Zhang, H., Wang, C.Y., and Xia, Z. (2007). J. Cell Biol. *177*, 253–264.

Dumont, J., Umbhauer, M., Rassinier, P., Hanauer, A., and Verlhac, M.H. (2005). J. Cell Biol. *169*, 227–231.

Gavin, A.C., and Nebreda, A.R. (1999). Curr. Biol. *9*, 281–284.

Hauge, C., and Frodin, M. (2006). J. Cell Sci. *119*, 3021–3023.

Kang, S., Dong, S., Gu, T.-L., Guo, A., Cohen, M.S., Lonial, S., Khoury, H.J., Fabbro, D., Gilliland, D.G., Bergsagel, P.L., et al. (2007). Cancer Cell, this issue.

Roux, P.P., Richards, S.A., and Blenis, J. (2003). Mol. Cell. Biol. *23*, 4796–4804.

Sapkota, G.P., Cummings, L., Newell, F.S., Armstrong, C., Bain, J., Frodin, M., Grauert, M., Hoffmann, M., Schnapp, G., Steegmaier, M., et al. (2007). Biochem. J. *401*, 29–38.

Smith, J.A., Poteet-Smith, C.E., Xu, Y., Errington, T.M., Hecht, S.M., and Lannigan, D.A. (2005). Cancer Res. *65*, 1027–1034.

The Endless Complexity of Lymphocyte Differentiation and Lymphomagenesis: IRF-4 Downregulates BCL6 Expression

Izidore S. Lossos[1,2,*]
[1]Sylvester Comprehensive Cancer Center, Division of Hematology-Oncology, Department of Medicine
[2]Department of Molecular and Cellular Pharmacology
University of Miami, Miami, FL 33136, USA
*Correspondence: ilossos@med.miami.edu
DOI 10.1016/j.ccr.2007.08.012

The *BCL6* gene is a key factor necessary for formation of germinal centers and is implicated in pathogenesis of diffuse large B cell lymphoma (DLBCL). In this issue of *Cancer Cell*, Saito and colleagues explore regulation of *BCL6* gene expression by CD40-NF-κB signaling pathway and show that the IRF4 transcriptional factor, induced by the NF-κB canonical pathway, directly downregulates *BCL6* expression. The authors further demonstrate that this negative regulatory mechanism may be disturbed in DLBCLs harboring *BCL6* gene translocations or mutations. These finding suggest that IRF4 may function as a key regulator of germinal center reaction and a guardian of lymphomagenesis.

In response to antigen encounter, uncommitted naive B cells are activated and undergo a complex maturational process yielding phenotypically distinct subpopulations, which form highly organized germinal centers (GC) in lymphoid organs. Within the GC, B cells undergo high rate proliferation and affinity maturation, are selected by antigen, switch toward advanced isotypes, and finally differentiate into either memory B cells or plasma cells. The maturation process leading to the generation of GC lymphocytes, and their subsequent differentiation to memory and plasma cells is characterized by tightly regulated suppression or increased expression of specific genes, resulting in distinctive gene expression signatures characterizing individual ontogeny stages of the lymphocytes. Currently, known key players at the GC stage include *BCL6* and activation-induced cytidine deaminase (*AID*), the former necessary for GC formation while the latter is essential for class switch recombination (CSR) and somatic mutations of the immunoglobulin genes. Toward the completion of GC reaction and terminal differentiation, the expression of these genes is downregulated, while genes necessary for plasma cell formation, such as *IRF4* (also known as MUM-1), *Prdm1*, encoding Blimp-1, and *XBP-1* are expressed. Aberrations in this orchestrated process may cause deregulated gene expression, leading to cell transformation and lymphomagenesis.

BCL6 is a proto-oncogene encoding a POZ/Zinc finger sequence-specific transcriptional repressor, which is distinctively expressed in GC B cells. In the GC, BCL6 exerts antiapoptotic effects and favors sustained proliferation of B cells by modulating the transcription of genes involved in cell cycle regulation, proliferation, activation, CSR, and differentiation (Shaffer et al., 2000). The expression of *BCL6* in the GC lymphocytes is tightly regulated at both the transcriptional and the protein levels. *BCL6* gene is negatively self-regulated by binding of BCL6 to specific binding sequences located in its first exon (Wang et al., 2002). Activation of STAT5 may either upregulate or suppress *BCL6* expression, and p53 may increase *BCL6* expression