p38 links RAS to GATA2

Christian Baumgartner & Manuela Baccarini

More than 30% of all human cancers contain activating mutations of the small G-protein RAS. As a result of this, RAS has been intensely studied and many efforts have been made to identify pathways that sustain RAS-driven transformation [1]. Recent studies have indicated that the transcription factor GATA2 is one of these partners in crime, but a mechanistic link between RAS and GATA2 had not been identified [2]. A paper in this issue of *EMBO reports* closes this gap showing that GATA2 can be activated by p38 in RAS-transformed cells [3].

See also: **KR Katsumura *et al*** (September 2014)

RAS are small G-nucleotide binding proteins that cycle between a GDP- and a GTP-bound state. The GTP-bound state is induced by extracellular stimuli such as growth factors, and in this conformation RAS can bind several effector molecules through which it promotes cell proliferation and survival but also migration and alteration of cell metabolism. RAS was the first oncogene discovered and is the one most frequently mutated in human cancer. Oncogenic mutations disrupt the GDP-/GTP-bound cycle, locking RAS in the active state. They mostly affect K- and NRAS, whereas HRAS shows a lower mutation rate and is most frequently detected in solid tumors, especially those of epithelial origin such as colon and lung cancers [1]. NRAS and KRAS are also mutated in 30% of acute leukemias and can therefore be considered oncogenic driving forces in the initiation and progression of leukemogenic processes [4]. In contrast, HRAS mutations are found in a developmental disorder with high cancer risk called Costello syndrome [1].

A number of mouse models have been developed to elucidate the role of Ras and its

effectors in oncogenesis. Using these tools, it has been shown that the Raf/Mek/Erk cascade has a prominent role in proliferation and differentiation, while the PI3K pathway and the Ral-GDS pathway are rather linked to survival. Ultimately, oncogenic Ras leads to an imbalance in gene expression profiles by increasing the activity of several transcription factors including NF- κ B, CREB, Ets-1, AP-1, and c-Myc. Many of these transcription factors are either directly or indirectly activated by Erk via phosphorylation. Both oncogenic RAS [1] and the transcription factors it ultimately activates have long been considered undruggable [5]. Thus, the molecular therapy of RAS-driven tumors has focused on the inhibition of its immediate downstream effector molecules such as Raf or PI3K, which, being kinases, are more easily targeted by inhibitors.

Recently, a requirement for the transcription factor GATA2 in K-Ras-driven transformation has been identified by the Downward laboratory. Non-small cell lung cancer cells and colon cancer cells harboring KRAS mutations are addicted to GATA2 expression for survival; consistently, GATA2 is essential for the onset, progression and maintenance of K-Ras^{G12D}-driven lung tumors in the mouse [2]. Surprisingly, however, activation of the GATA2 network in lung cancer cells does not depend on KRAS, suggesting that the two pathways are independent and that GATA2 activation confers a selective advantage to KRAS-transformed cells by relieving oncogene-induced stress (Fig 1, right panel).

Katsumura *et al* [3] have now discovered a mechanistic link between RAS and GATA2. Acting downstream of RAS, the MAPK p38 is shown to phosphorylate a number of residues in GATA2, of which S192 is the most critical, resulting in activation of GATA2 transcriptional activity. The data provide the first clear demonstration of a specific phosphorylation event targeting GATA2, identify

the kinase responsible for it, and clarify the role of this modification in GATA2 activation. By showing that RAS can actively stimulate GATA2 activity through the activation of a downstream signaling pathway, these results advance our understanding of how RAS and GATA2 interact in hematopoietic and endothelial cells compared to tumor cells of epithelial origin (Fig 1).

The finding that GATA2 was involved in RAS-driven epithelial transformation was unexpected, as GATA2 was known to have a pivotal role in the hematopoietic system. GATA2 is expressed in hematopoietic stem cells (HSC) and precursors. Its expression decreases with differentiation, suggesting that GATA2 is necessary for maintaining pluripotency. In line with this, GATA2 haploinsufficiency compromises proliferation and survival of HSC, and promotes hematopoietic differentiation. On the other hand, overexpression of GATA2 increases quiescence and reduces the proliferation of human HSC. GATA2 has been found to be overexpressed in AML, while loss-of-function mutations have been causally linked to immunodeficiency associated with myelodysplastic syndromes (MDS). Clearly, maintaining a specific level of GATA2 activity is necessary for normal hematopoiesis [6].

It was by studying one of the GATA2 mutants, GATA2 T354M, that Katsumura *et al* discovered the phosphorylation of GATA2 by p38. The T354M mutant shows reduced chromatin occupancy and transcriptional activity, and was found to be hyperphosphorylated. Notably, other non-chromatin binding mutants such as GATA2 C349A or GATA2 Δ 355T, which is a MDS-associated mutant, also show multi-site phosphorylation [3]. This prompts the question of whether the enzyme(s) that dephosphorylate wild-type GATA2 might be chromatin-associated. Since phosphorylation of GATA2 is associated with its

Figure 1. GATA2 and Ras-driven transformation.

Ras mutations are highly transforming. Recently, multiple connections between activated Ras and GATA2-mediated gene expression networks have been unveiled. In this issue, Katsumura *et al* [3] show that Ras activates GATA2 via the ROS-p38 axis. Since all pathway components are expressed in HSC, this “vertical” axis may participate in the control of hematopoietic development, as already shown for the p38-GATA3 pathway, and potentially in Ras-driven leukemogenesis (left). In Ras-driven epithelial tumors, GATA2 transcriptional activity is required for malignant transformation but is “orthogonal” to Ras [2]. GATA2 regulates the proteasome, Rho, and the IL-1 pathway (right). Therefore, depending on the cellular context, upstream regulators of GATA2 (e.g. p38) or downstream mediators of GATA2 activity—such as the proteasome and Rho-dependent kinases (ROCK)—are promising candidates for co-targeting strategies in RAS-driven malignancies.

colocalization with foci containing phosphorylated RNA polymerase II, it is conceivable that dephosphorylation of GATA2 by chromatin-associated phosphatases might release GATA2 from chromatin, allowing for both the termination of GATA2-mediated transcription and the recycling of the transcription factor to be re-activated by new incoming stimuli. In this model, phosphatases would keep a certain stoichiometric ratio between active and non-active transcription factor pools relevant for the proper function of genetic networks.

Like GATA2, both RAS and p38 have roles in hematopoietic development and in leukemia. N-Ras^{G12D} was shown to promote proliferation of most HSC, while at the same time enhancing self-renewal of a small subset of HSC [7]. p38 is active in a subset of HSC with elevated reactive oxygen

species (ROS) and appears to mediate the reduced self-renewal capacity characteristic of this population [8]. Notably, one of the targets of p38 in this context is another GATA transcription factor, GATA3, recently demonstrated to promote HSC cycling and restrict their long-term reconstitutive potential downstream of p38 in stress-induced hematopoiesis [9].

Viewed in light of these findings, it is tempting to speculate that GATA2 might function to increase the proliferative capacity of the subset of HSC with elevated ROS levels and p38 activity. Since oncogenic RAS is also capable of inducing ROS production [10], constitutive p38 activation in RAS-transformed cells might continuously stimulate GATA2 target gene expression, thereby inducing HSC proliferation and blocking differentiation (Fig 1, left panel).

The quest for small-molecule inhibitors of RAS downstream effectors has been on for decades, and we now have compounds that efficiently inhibit PI3K, RAF, and MEK. Paradoxically, in RAS-transformed cells, some of these inhibitors can lead to re-activation of the ERK pathway and/or relieve the feedback on parallel pathways [1]. This experience has shown how complex and intimately intertwined the networks downstream of RAS are, and it has made clear that we need a better understanding of RAS signaling if we want to block it for therapeutic purposes. The papers by Katsumura *et al* [3] and Kumar *et al* [2] show that the GATA2 network can be directly activated by RAS and can confer a selective advantage to RAS-transformed cells (Fig 1). Although the mechanisms differ, both papers suggest that targeting GATA2 networks, either upstream by blocking the kinase that activates the transcription factor itself, or downstream by inhibiting the pathways emanating from transcriptional targets of GATA2, may be a new strategy for the inhibition of RAS-driven tumorigenesis.

References

1. Stephen AG, Esposito D, Bagni RK *et al* (2014) *Cancer Cell* 25: 272–281
2. Kumar MS, Hancock DC, Molina-Arcas M *et al* (2012) *Cell* 149: 642–655
3. Katsumura KR, Yang C, Boyer ME *et al* (2014) *EMBO Rep* 15: 938–947
4. Chung E, Kondo M (2011) *Immunol Res* 49: 248–268
5. Yan C, Higgins PJ (2013) *Biochim Biophys Acta* 1835: 76–85
6. Vicente C, Conchillo A, Garcia-Sanchez MA *et al* (2012) *Crit Rev Oncol Hematol* 82: 1–17
7. Li Q, Bohin N, Wen T *et al* (2013) *Nature* 504: 143–147
8. Jang YY, Sharkis SJ (2007) *Blood* 110: 3056–3063
9. Frelin C, Herrington R, Janmohamed S *et al* (2013) *Nat Immunol* 14: 1037–1044
10. Ogrunc M, Di Micco R, Lontos M *et al* (2014) *Cell Death Differ* 21: 998–1012