Raf-1 and Squamous Cell Carcinoma: Rok-ing the Boat

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Squamous cell carcinoma (SCC) is the second most common form of nonmelanoma skin cancer. In this issue of *Cancer Cell*, Ehrenreiter et al. unveil a critical role for the Raf-1/Rok-α interaction in the pathogenesis of SCCs, thus paving the way for the development of therapeutic modalities to treat this malignancy.

Aberrant Ras signaling has been causally linked to a broad spectrum of human neoplasms. Raf kinase, the first Ras effector to be identified, has been widely implicated in Ras-driven tumorigenesis. As the apical kinase in the Raf/Mek/ERK cascade, the contribution of Raf to the tumorigenic process has been commonly attributed to its capacity to couple deregulated Ras activity to unrestrained ERK signaling. In this issue, the study by Baccarini and her colleagues (Ehrenreiter et al., 2009) reveals an unexpected twist to the involvement of Raf-1 in Ras tumorigenesis. By exploiting Ras-driven SCC models, the authors have demonstrated a critical role for Raf-1 in the initiation and maintenance of SCC through a mechanism that depends on the inhibition of the RhoGTPase target Rok-α rather than the activation of ERK.

The Ras pathway is viewed as a central player in SCC since the majority of these tumors display elevated levels of active GTP-bound Ras (Ridky and Khavari, 2004). Additionally, in mouse experimental models, activating mutations of Ras have been shown to be sufficient to induce SCC-like tumors (Tarutani et al., 2003). To explore the function of Raf in Ras-driven SCC, the authors induced the ablation of Raf-1 in two mouse models: (1) the DMBA/TPA chemical carcinogenesis model in which H-Ras is activated by a codon 61 mutation mutation, and (2) K5-SOS-F transgenic mice, in which Ras activation is achieved via the expression of a constitutively active form of the Ras guanine nucleotide exchange factor SOS under the control of the full-length cytokeratin 5 (K5) promoter. The latter model mimics SCCs more closely because Ras is mutated in only a small percentage (~20%) of SCC but is often activated as a consequence of the overexpression/activation of upstream receptor tyrosine kinases (Ridky and Khavari, 2004). Strikingly, in both models, Raf-1 ablation leads to inhibition of tumor initiation. Moreover, Raf-1 appears to be required for tumor maintenance, as ablation of Raf-1 after tumor formation results in a pronounced tumor regression.

How does Raf-1 loss-of-function cause to the inhibition of tumor initiation and regression of established tumors? Tumorigenesis is often thought to arise as a consequence of an imbalance between programs that control cell proliferation and differentiation. However, little is known about the determinants that coordinate changes in the execution of these programs during neoplastic conversion. In the setting of Ras-driven SCCs, the tumors that develop following the ablation of one copy of Raf-1 feature an increase in the levels of the differentiation marker cytokeratin 10 and a decrease in the expression of the dedifferentiation marker Integrin β1. Moreover, tumor regression following Raf-1 ablation is coincident with an increase in differentiated cells. In both settings however, the increase in the number of differentiated cells is accompanied by a decrease in the population of proliferating cells, making it difficult to discern whether Raf-1 exerts its effect on SCC tumor initiation and maintenance by disrupting differentiation or enhancing proliferation. To address this question, the authors have resorted to an established in vitro keratinocyte cell culture system in which differentiation and proliferation can be selectively manipulated. They have found that whereas keratinocytes derived from K5-SOS-F mice fail to differentiate, the ablation of Raf-1 abrogates this effect without notable changes in the proliferative capacity of the cells. While it is clear that the in vitro system used may have certain limitations, the most straightforward interpretation of these findings is that the dependence of Ras-driven SCC tumors on Raf-1 is linked predominantly to perturbation in keratinocyte differentiation (Figure 1).

Next, Ehrenreiter et al. set out to define the mechanisms by which Raf-1 interferes with keratinocyte differentiation. Naturally, their initial attention was drawn toward the most common Raf effector pathway, the MEK-ERK cascade. Somewhat counterintuitively, they discovered that Raf-1 deficiency does not compromise ERK activity, indicating that, in the context of skin homeostasis, Raf-1 function is dispensable for ERK activation. In retrospect though, these observations could have been expected given an earlier study showing that loss of Raf-1 function in keratinocytes is inconsequential for ERK activity (Ehrenreiter et al., 2005). In that study, it was also established that Raf-1 binds to and inhibits Rok-α. Building on this work, the authors now demonstrate that this inhibitory interaction is critical for Ras-driven SCC, as Raf-1 ablation induces Rok-α hyperactivation, increased differentiation, and consequently, restrained tumor growth (Figure 1). Furthermore, they report that pharmacological inhibition of Rok-α is sufficient to reverse the restraining effects of Raf-1 ablation on tumor growth, providing proof that Raf-1-mediated Rok-α inhibition is an essential element of the molecular network that controls SCC development and maintenance.

Rok-α signaling proceeds through the phosphorylation of multiple targets, each of which could, in principle, be responsible

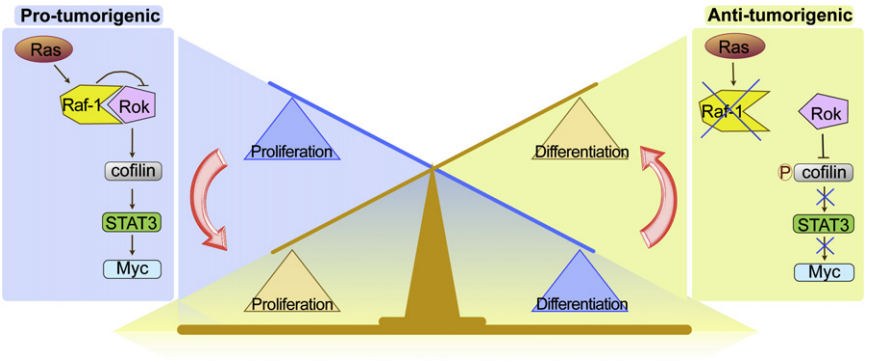

Figure 1. Raf-1 Promotes Ras-Induced Skin Carcinogenesis by Perturbing the Balance between Keratinocyte Proliferation and Differentiation

Raf-1 binds to and inhibits Rok-α. This inhibition leads to heightened levels of unphosphorylated (active) cofilin that, through a mechanism yet to be understood, stimulates STAT3 phosphorylation and Myc accumulation. The engagement of this proposed signaling cascade culminates with the suppression of differentiation, enhanced proliferation, and tumor promotion. When Raf-1 is ablated, Rok-α is free to phosphorylate and inactivate cofilin, and the pathway is turned off. As a result, differentiation predominates over proliferation and tumor growth is halted.

for the observed dependence of SCC tumors on the Raf-1/Rok-α axis (Bishop and Hall, 2000). The authors have opted to focus on one of these targets, LIM kinase, and its substrate cofilin. LIM kinase-mediated phosphorylation of cofilin is inhibitory, whereas the unphosphorylated form is active. Active cofilin has been shown to enhance STAT3 phosphorylation, which, in turn, induces Myc expression (Honma et al., 2006). Since both STAT3 phosphorylation and Myc accumulation have been detected in human SCC (Seethala et al., 2008) and have been shown to promote epidermal tumorigenesis in animal models, it would seem reasonable to postulate that the inhibition of Rok-α by Raf-1 would lead to a decrease in cofilin phosphorylation and a corresponding increase in STAT3 phosphorylation and Myc accumulation. In support of this postulate, the authors demonstrate increased cofilin phosphorylation and reduced STAT3 phosphorylation and Myc expression following Raf-1 ablation (Figure 1). Pharmacological Rok-α inhibition produces the opposite effect: namely, decreased cofilin phosphorylation and increased STAT3 phosphorylation and Myc expression. To date, the cofilin/STAT3/Myc pathway has not been implicated in tumorigenesis. Hence, the findings by Ehrenreiter et al. constitute the first evidence for a potential role for this pathway in skin tumorigenesis. Nonetheless, given that at this point the contribution of other Rok-α effectors has not been rigorously explored, it might be premature to bet exclusively on the Rok-α/cofilin connection.

Active Raf-1 binds to and inhibits Rok-α function in both normal and cancer cells. However, as this study demonstrates, the strict dependence on this inhibitory interaction is exhibited only by tumorigenic skin cells that harbor an upregulated Ras pathway. This behavior is akin to "nononcogene addiction," an emerging concept in cancer biology used to describe the heightened sensitivity of the cancer cell to the inactivation of normal pathways (Luo et al., 2009). The underlying premise of this concept is that cancer cells become addicted to a given cellular function because this function serves to counteract deleterious signals. By extension, it might be argued that SCC tumors are addicted to the restraining effect of Raf-1 on Rok-α because, in the presence of high Ras signaling, Rok-α activity confers a growth disadvantage. Sorting out the molecular basis for this vulnerability promises to be a challenge given the multitude of pathways that are engaged in response to unchecked Ras signaling. Furthermore, some of these pathways impinge directly on Rok-α function through Raf-1-independent mechanisms (Mavria et al., 2006), suggesting that Raf-1 might not act alone, but rather as a component of a broader circuitry that regulates Rok-α activity.

There is no doubt that Ehrenreiter et al. have just scratched the surface of defining the cellular processes that control SCC initiation and progression. Nonetheless, by demonstrating that Ras-driven SCCs are addicted to Raf-1-mediated Rok-α inhibition, they have uncovered a unique window of opportunity for therapeutic targeting. A drug designed to selectively interfere with Raf-1/Rok-α interaction is likely to re-engage the disrupted differentiation programs and, hence, may exhibit antitumorigenic effects. Finally, the work by Ehrenreiter et al. also calls for a careful evaluation of the clinical use of Rok inhibitors. As clearly demonstrated, depending on the pathological context, the outcomes could be dire.

REFERENCES

Bishop, A.L., and Hall, A. (2000). Biochem. J. *348*, 241–255.

Ehrenreiter, K., Kern, F., Velamoor, V., Meissl, K., Galabova-Kovacs, G., Sibilia, M., and Baccarini, M. (2009). Cancer Cell *16*, this issue, 149–160.

Ehrenreiter, K., Piazzolla, D., Velamoor, V., Sobczak, I., Small, J.V., Takeda, J., Leung, T., and Baccarini, M. (2005). J. Cell Biol. *168*, 955–964.

Honma, M., Benitah, S.A., and Watt, F.M. (2006). Mol. Biol. Cell *17*, 1888–1896.

Luo, J., Solimini, N.L., and Elledge, S.J. (2009). Cell *136*, 823–837.

Mavria, G., Vercoulen, Y., Yeo, M., Paterson, H., Karasarides, M., Marais, R., Bird, D., and Marshall, C.J. (2006). Cancer Cell *9*, 33–44.

Ridky, T.W., and Khavari, P.A. (2004). Cell Cycle *3*, 621–624.

Seethala, R.R., Gooding, W.E., Handler, P.N., Collins, B., Zhang, Q., Siegfried, J.M., and Grandis, J.R. (2008). Clin. Cancer Res. *14*, 1303–1309.

Tarutani, M., Cai, T., Dajee, M., and Khavari, P.A. (2003). Cancer Res. *63*, 319–323.