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New Avenue to Inhibit Ras Signaling

Xosé R. Bustelo

Inhibition of Ras-stimulating enzymes is a possible avenue to treat Ras-driven diseases. In this issue of Chemistry & Biology, Evelyn and coworkers report an inhibitor for one such enzyme, Sos1, capable of impairing wild-type Ras signaling in cells.

H-, K-, and N-Ras toggle between ''off'' (GDP-bound) and ''on'' (GTP-bound) states during signal transduction. This cycle is controlled by guanine exchange factors (GEFs) and GTPase-activating proteins (GAPs) that catalyze the stimulation (Figure 1A, point a) and inactivation (Figure 1A, point b) of Ras proteins, respectively. GTP-bound Ras proteins bind to phosphatidylinositol 3-kinase a (PI3Ka), Raf kinases, and other effectors, leading to signaling diversification and amplification events in stimulated cells. This regulation is broken by mutations that generate oncogenic proteins deficient in GTP hydrolysis, overexpression of wild-type Ras (RasWT) proteins, inactivation of GAP-encoding genes, or deregulated upstream signaling elements (Figure 1A). Due to their frequent occurrence in human tumors, most drug discovery efforts have focused on mutant Ras (RasMUT) proteins and key downstream elements (Stephen et al., 2014). However, for pathologies exhibiting high amounts of RasWT signaling, targeting wild-type GTPases could be a therapeutically interesting option. The spectrum of RasWT-dependent diseases might be even larger than currently appreciated, because recent reports have unveiled key roles for H- and N-RasWT in RasMUT- driven tumorigenesis. Such functions include the generation of outbursts of Raf and PI3Ka signaling in mitogen-stimulated cancer cells (Figure 1A, point c) (Young et al., 2013), the dampening of K-RasMUT signals to avoid activation of the DNA damage response (Figure 1A, point d) (Grabocka et al., 2014), and, possibly the engagement of nonoverlapping effectors (Figure 1A, point e) (Stephen et al., 2014). RasWT proteins are also implicated in signaling compensatory effects elicited by anti-Ras therapies, such as the stimulation of Ras pathways caused by the loss of the MEK-mediated inhibition of the EGF receptor typically observed when using MEK inhibitors (Figure 1A, point f) (Young et al., 2013). An inference from these studies is that inhibitors of the Ras GDP/ GTP exchange reaction can represent, either singlehandedly or combined with other treatments, a potential therapeutic avenue for diseases featuring deregulated Ras activity. Up to now, the search for such inhibitors has been focused on Ras-binding compounds promoting GTPase conformations incompatible with GEF interactions. This approach has led to the isolation of compound families that recognize pockets located in the vicinity of the two Ras switch domains, the regions that mediate the Ras-GEF interaction. However, these molecules lack commercial interest because of low potency and inappropriate pharmacochemical features (Wang et al., 2012). Given that these molecules have to interact at 1:1 ratios with Ras proteins to promote noticeable inhibitory effects, it is unlikely that this strategy will yield drugs with good therapeutic indexes. A more efficient alternative might be the use of drugs against the catalytic activity of Sos1, the Ras GEF in charge of stimulating RasWT proteins downstream of most receptors, RasMUT GTPases, and oncogenic protein tyrosine kinases (Jeng et al., 2012; Qian et al., 2000). This GEF is regulated by both receptormediated plasma membrane tethering steps (Figure 1A, point g) and an allosteric effect induced by the binding of active Ras molecules to a regulatory Sos1 domain (Figure 1A, h points) (Cherfils and Zeghouf, 2013). In this issue of Chemistry & Biology, Evelyn et al. (2014) report the in silico screen-based isolation of an inhibitor (NSC658497) directed against a structural pocket located in the Sos1 catalytic domain (Figure 1B, site a). This site was a good pick in hindsight, because the binding of chemicals to a second Sos1 pocket causes increased catalytic rates (Burns et al., 2014) (Figure 1B, site b). As expected, NSC658497 blocks the interaction of Sos1 with RasWT proteins, Sos1 enzyme activity, and Ras signaling in both mitogen-stimulated and hyperactive Sos1 mutant-expressing cells. Although not tested, the in vivo effects of the inhibitor are probably mediated by the concurrent inactivation of the highly related Sos2 protein. This compound cannot bind to versions of the Sos1 catalytic domain carrying mutations in the putative drug-binding site, thus confirming its mechanism of action. In contrast to earlier data using Sos1- and RasWT-depleted cells (Grabocka et al., 2014; Jeng et al., 2012), Evelyn et al. (2014) find no overt effect of NSC658497 in the fitness of K-RasMUT-expressing cancer cells. This suggests that the inhibitor may not have enough potency to block the K-RasMUT-mediated allosteric activation of Sos1 (Jeng et al., 2012) or, alternatively, that the K-RasMUT cell lines utilized in the present study stimulate RasWT proteins using GEF-independent mechanisms. To clarify this discordance, it would be important to investigate the effect of NSC658497 in the Sos1-and RasWT-dependent cancer cell lines used in previous studies (Grabocka et al., 2014; Jeng et al., 2012), expand these analyses to larger numbers of RasMUT-expressing cancer cells, and verify whether the K-RasMUT-expressing cell lines used in the present work are actually Sos1-dependent. Other therapeuticallyinteresting studies to carry out in the near future include the analysis of NSC658497 in protein tyrosine kinasedependent transformation processes (Qian et al., 2000), its synergism with currently available anti-Ras pathway drugs (Young et al., 2013), and validation in animal models. Tackling these issues will help foresee the pharmacological potential and spectrum of applications of this inhibitor and subsequent derivatives. The therapeutic viability of this avenue has yet to be explored. The potency of NSC658497 is low in cells, indicating that further optimization steps will be needed. The eventual applicability of these compounds will also depend on in vivo pharmacokinetic behavior, therapeutic efficacy, toxicity, and side effects directly derived from the inactivation of Sos proteins in healthy tissues. The latter problem requires special attention here, because genetic analyses indicate that the concurrent inactivation of Sos1 and Sos2 causes lethal effects in adult mice (Baltanás et al., 2013).

These problems should not occur when using Sos1-specific inhibitors, as inferred by the viability shown by mice lacking Sos1 in postnatal periods (Baltanás et al., 2013). Regardless of these potential caveats, this work highlights the feasibility of isolating Ras GEF-specific inhibitors,
opens the door to new therapeutic opportunities in Sosdependent diseases, and provides a valuable tool to further address Sos function in cells.

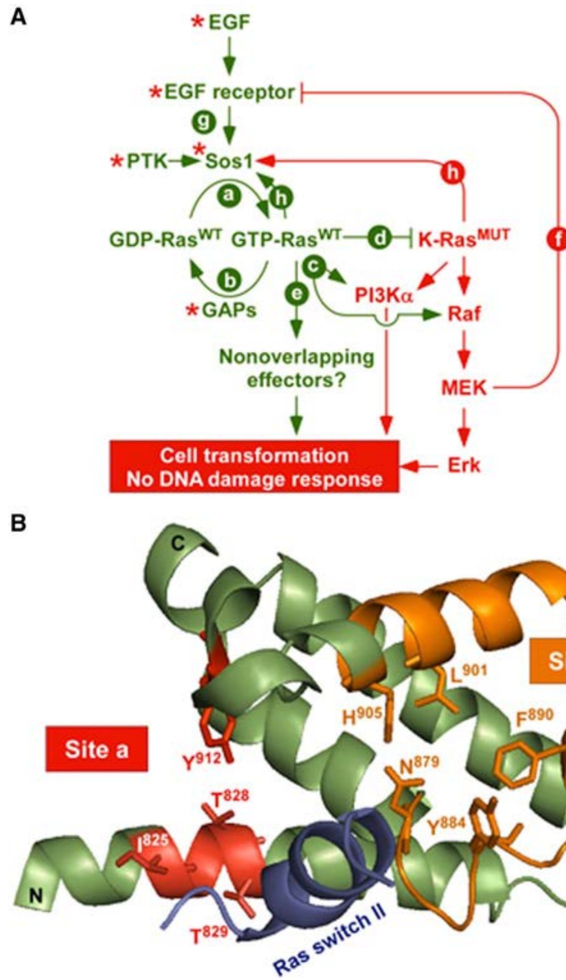

Figure 1. Therapeutic Targeting of Sos1 in Ras-Dependent Diseases

(A) Ras regulatory cycle and Sos1-mediated crosstalk established between Ras^{WT}- (green) and K-Ras^{MUT}-regulated (red) pathways. Red asterisks indi- cate genetic alterations that lead to the spurious activation of Ras^{WT} proteins. Other signaling crosstalk are not shown for the sake of simplicity. PTK, protein tyrosine kinase (membrane anchored or cytosolic).

(B) Binding sites of inhibitory (site a, red) and stimulatory (site b, brown) com- pounds of Sos1 enzyme activity in one of the subregions of the Sos1 catalytic site. Sos1 residues potentially involved in the interaction with these com- pounds are shown in stick form and labeled. The Ras switch II region is shown in blue. The Sos1 aH helix that gets into a cleft between the switch regions and that is critical for the exchange reaction is not shown. N and C, N- and C- termini of the Sos1 fragment shown.

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