RalGDS comes of age

Ras proteins send signals through multiple effector pathways. The Raf/MEK/MAPK and PI 3′ kinase pathways are well-validated Ras effectors in human cancers, but many other candidate pathways could be equally important. RalGDS is such a candidate: in a new paper from Chris Marshall's group, an important role for RalGDS in Ras transformation in vivo has been established for the first time. Mice lacking RalGDS are defective in tumor formation, possibly because of increased apoptosis in Ras-driven tumors. The hunt for a clear role for RalGDS activation in human cancer is on.

The early years of Ras research witnessed an intense and competitive race to discover which enzyme Ras regulates. The discovery in 1993 that Ras binds directly to Raf kinase suggested briefly that the race was over. However, Ras biology clearly differed from Raf, and the later discovery that Ras also binds and activates PI 3′ kinase accounted for some of these properties. Furthermore, Ras effectors were shown to act synergistically, implying that the full transforming power of Ras depends on simultaneous activation of interactive downstream pathways. But this was just the beginning! Ras was shown to bind to AF6/Canoe, RalGDS, and a host of unknowns, with binding properties as compelling as effectors whose biology was better known (Repasky et al., 2004). Figure 1 represents our current view of Ras signaling, greatly oversimplified (Rodriguez-Viciana et al., 2004).

How can we ever learn which of these effector pathways is relevant to Ras biology in vivo, and, more specifically, in human cancer?

Analysis of mutations in human tumors gives strong clues and validates both Raf and PI 3 kinase as crucial Ras effectors (see below). A complimentary approach is also proving informative: roles of candidate downstream effectors interrogated using knockout mice. For example, cyclin D1 (way downstream) is necessary for Ras transformation (Robles et al., 1998; Yu et al., 2001), as is TIAM-1 (Malliri et al., 2002) and PLC-ε (Bai et al., 2004), both possible direct effectors.

RalGDS can now be added to the list of biologically relevant effectors. In a paper from the lab of long-time Ras pioneer Chris Marshall, González-García and coworkers (González-García et al., 2005) show that mice lacking RalGDS show reduced tumor incidence, size, and progression, in a skin cancer model in which mutant H-ras is the primary driver. This important discovery will direct more attention to this pathway, including, one hopes, a search for somatic mutations in human cancers that put RalGDS into the top tier of Ras effectors. Furthermore, RalGDS and the Ral pathway, like cyclin D1, Tiam-1, and PLC-ε, now appear to be excellent targets for therapeutic intervention, at least from a biologist's perspective. Each is necessary for efficient Ras transformation, and each is dispensable in normal tissue. Unfortunately, these proteins may not be "druggable" with current technology, but that is a different story.

RalGDS was discovered as a Ras partner in 1994, in two-hybrid screens performed in the labs of Steve Martin, Jim Bischoff, and Rusty Williams. At the time, it was astonishing that regulation of related small GTPases should be so directly connected, but subsequent studies from Hans Bos, Yoshimi Takai, Larry Feig, Mike White, Alan Hall, and many others have established this as a central theme in signal transduction. RalGDS is one of several known Ras-regulated guanine-nucleotide exchange factors, or GEFs, that function by activating Ral A and B GTPases (Wolthuis and Bos, 1999). Ral interacts with effectors such as Sec5, Filamin, RalBP1, and ZONAB, and very likely many other proteins yet to be identified. Through these interactions, Ral proteins regulate endocytosis, exocytosis, and actin organization, and control gene expression through transcription factors such as fos, jun, AFX,

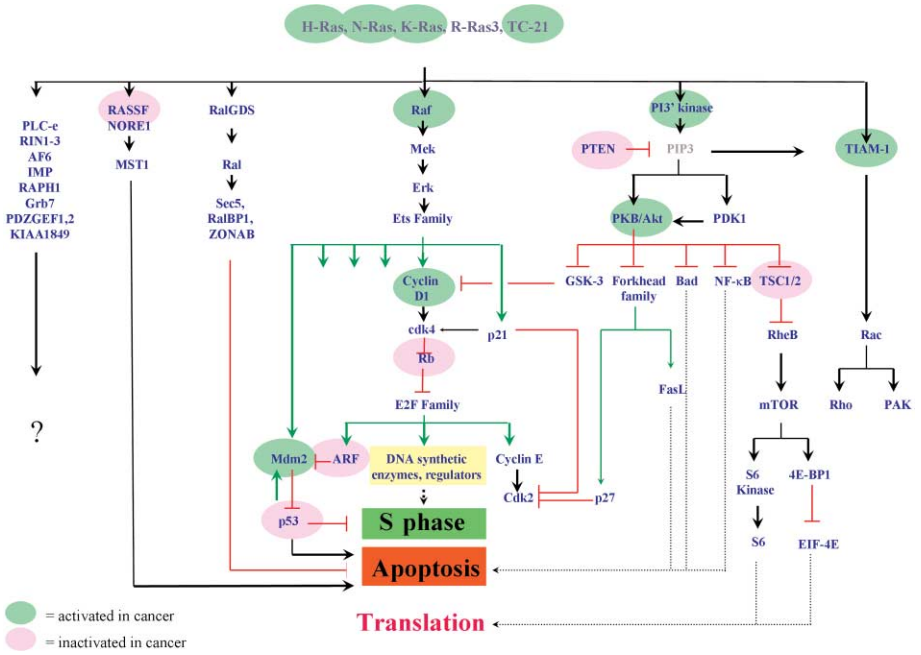

Figure 1. Pathways downstream of Ras

Members of the Ras family bind and activate multiple downstream effectors. Binding is GTP-dependent and engages the highly conserved effector binding region of Ras and its close relatives. Direct activating events are shown in black arrows, trasncriptional events in green. This figure is greatly oversimplified, but ilustrates the complexity of downstream signaling and the fact that downstream pathways are entangled at many levels.

and Zonab. Importantly, as suggested before (Chien and White, 2003) and confirmed by González-García et al. in mouse models in vivo, Ral may also play a critical role in regulating survival of tumor cells.

Caution is needed, however, when extrapolating observations from mouse tumor model systems to human cancer. There is direct evidence that the contribution of Ras effector pathways to cellular transformation may differ between mouse and human cells (Hamad et al., 2002; Rangarajan et al., 2004). Also, different cell types have different requirements for Ras effector pathways for malignant transformation. This has been observed in human cells in culture (Rangarajan et al., 2004), as well as in mouse tumor models. Cyclin D1-deficient mice, for example, are totally resistant to breast cancers induced by Ras, but only impaired in Ras-induced skin tumorigenesis (Robles et al., 1998; Yu et al., 2001). On the other hand, MEFs derived from cyclin $D^{-/-}$ mice can be fully transformed by Ras. These differences in cyclin D1 dependence between mouse mammary cells, keratinocytes, and fibroblasts may relate to the ability of Ras to differentially induce expression of the related cyclins D2 and D3 in a cell type-dependant manner. Even within the same cell type, different effectors may also play different roles at different stages of tumor progression. Although $Tiam1^{-/-}$ mice develop fewer skin tumors in response to Ras activation, those that do form show enhanced progression to malignancy (Malliri et al., 2002).

Despite these important developments, RalGDS still lacks validation as a Ras target in human cancers. In contrast, activating mutations in B-Raf occur frequently in many human cancers, particularly in melanoma. These mutations are mutually exclusive with activating mutations in N-Ras, suggesting they act in the same pathway. Likewise, loss of PTEN occurs frequently and is also mutually exclusive with mutations in Ras. Together, these data make a compelling case for a model in which both Raf and PI kinase pathways are activated together in the same tumor: this can be achieved by activating Ras, or by independently activating the two pathways. However, this model does not exclude the possible need to activate RalGDS as well, and potentially other Ras effectors, in tumors with wild-type Ras. The paper from González-García may prompt a more intense analysis of mutations in the RalGDS pathway in such tumors and further analysis of the molecular basis of synergistic interaction between Ras effectors.

Other Ras effectors that have gained credibility through analysis of human tumors include Tiam-1, which appears to be activated by mutation in some renal cell carcinomas, and RASSF, a potentially proapoptotic protein whose expression is suppressed in human tumors. More work is needed to establish the generality and significance of these candidates.

Activated Ras has been implicated in many of the properties of the malignant phenotype (i.e., uncontrolled proliferation, survival, invasion, and metastasis). It is likely that different effector pathways (or combinations of them) will contribute differentially to the various aspects of tumor biology in a cell type-dependant manner.

The availability of knockout mice deficient for specific Ras effectors will greatly help unravel this seemingly endless complexity. Their crossing with mice expressing active Ras under tissue-specific promoters (as well as with each other) will allow the examination of their specific contributions to tumorigenesis in different tissues and during different stages of tumor development. These approaches will help identify effector pathways that Ras may depend on to cause human cancer. Analysis of mutations within these pathways in human tumor DNA will further validadte these candidates. Ultimately, this information could yield a deeper understanding of Ras' role in cancer, and, hopefully, new targets for therapeutic intervention

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