7. BIOLOGY OF RAS IN THYROID CELLS

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INTRODUCTION

Ras is an almost universal component of signaling pathways in vertebrates, invertebrates and yeast where it plays critical roles in development, proliferation, differentiation and survival. In the twenty years since the first identification of mutated Ras genes in human tumors, intensive effort has been devoted to understanding how Ras promotes neoplastic transformation. What has become clear is that Ras promotes transformation in multiple ways. The effects of Ras are diverse due to the significant complexity of Ras-mediated signaling pathways. Mammalian cells express multiple Ras proteins, which localize to discrete membrane microdomains and exhibit differential affinities towards downstream signaling molecules. The existence of a large number of Ras effectors, many of which are members of multi-gene families, together with extensive sites of crosstalk between Ras and other intracellular signaling pathways, further increases the complexity of Ras-mediated signaling.

Mutations in all three cellular Ras genes (H-, K- and N-Ras) have been identified in benign and malignant thyroid tumors. This has generated immense interest in elucidating the cellular consequences of Ras activation in thyroid cells. The recent discovery of B-Raf mutations in thyroid tumors reaffirms the important contribution of Ras-mediated signaling pathways to thyroid cell transformation. Interestingly, the effects of Ras in thyroid cells are unusual in several respects. Unlike primary fibroblasts where expression of activated Ras induces growth arrest, Ras stimulates proliferation in primary human thyrocytes. Recent data suggests that this may be a consequence of

cell type specific effects on cell cycle regulatory proteins. Thyroid cells are one of few cellular models where proliferation is positively regulated by cAMP. As discussed below, crosstalk between cAMP and Ras markedly influences the signaling pathways activated by Ras. Indeed, TSH has been shown to modulate the effects of Ras on differentiation, proliferation and survival. The focus of this chapter is on the effects of Ras activation in thyroid cells, including the role of cellular Ras in TSH driven proliferation and the contribution of sustained Ras activity to thyroid cell transformation.

RAS REGULATION AND SIGNALING

Ras proteins are 21kDa GTP-binding proteins that function as molecular switches, cycling between active GTP- and inactive GDP-bound states. Cellular Ras activity is regulated by the opposing action of guanine nucleotide exchange factors (GEFs) that catalyze GDP dissociation, and GTPase activating proteins (GAPs) that stimulate intrinsic GTPase activity. Multiple RasGEFs and RasGAPs co-exist in most cells, increasing the diversity of signals that regulate Ras activity. Ras proteins are localized to the plasma membrane where they are poised to respond to signals initiated by the activation of cell surface receptors. Cellular Ras activity is maintained at very low levels. In response to signals such as those elicited by growth factors and hormones, Ras becomes activated in a transient manner. For cell surface receptors with tyrosine kinase activity, receptor dimerization induces tyrosine phosphorylation, thereby creating docking sites for signaling molecules such as Grb-2 and Shc, adaptor proteins comprised of SH2 and SH3 domains. Grb-2 is associated with the RasGEF SOS in the cytosol. Recruitment of Grb-2 to the activated receptor localizes SOS to the plasma membrane in close proximity to Ras, facilitating its activation. For G protein-coupled receptors, Ras is activated through second messengers such as diacylglycerol, calcium, and possibly cAMP (Busca et al., 2000; Pak et al., 2002), as well as through heterotrimeric G protein β/γ subunit- and src-mediated pathways.

In its active conformation, Ras binds to a variety of effectors. Effectors are defined as proteins that interact selectively with the GTP-bound form of Ras, and become activated as a consequence of this interaction. Three downstream effector pathways have been characterized in the most detail (Figure 1). They include members of the Raf, PI3K and RalGDS families (reviewed in Reuther et al., 2000; Shields et al.,

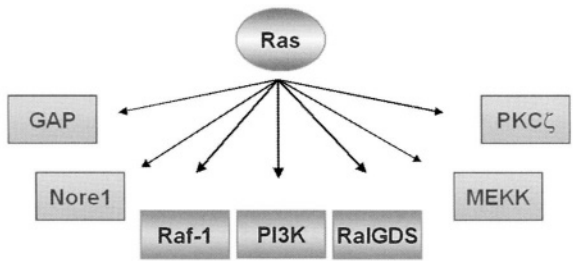

Figure 1. Ras signals through multiple downstream effectors including, but not limited to those illustrated here. In thyroid cells, Ras has been shown to signal through Raf-1, PI3K and RalGDS (shown in bold).