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Minireview: *PRKAR1A*: Normal and Abnormal Functions

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The type 1α regulatory subunit (RIα) of cAMP-dependent protein kinase (PKA) (coded by the *PRKAR1A* gene) is the main component of type I PKA, which regulates most of the serine-threonine kinase activity catalyzed by the PKA holoenzyme in response to cAMP. Carney complex (CNC), or the complex of spotty skin pigmentation, myxomas, and endocrine overactivity, is a multiple endocrine (and not only) neoplasia syndrome that is due to *PRKAR1A*-inactivating mutations. The R1α protein and *PRKAR1A* mRNA have been found to be upregulated in a series of cell lines and human and rodent neoplasms, suggesting this molecule's involvement in tumorigenesis and its potential role in cell cycle regulation, growth, and/or proliferation. Alterations in PKA activity elicit a variety of effects depending on the tissue, developmental stage, degree of differentiation, and cAMP levels. In addition, RIα may have functions independent of PKA. The presence of inactivating germline mutations and the loss of its wild-type allele in some CNC lesions indicate that *PRKAR1A* might function as a tumor suppressor gene in these tissues, but could *PRKAR1A* be a classic tumor suppressor gene? Probably not, and this review explains why. (*Endocrinology* 145: 5452–5458, 2004)

Structure and Functions of PKA and Its Regulatory Subunits

CYCLIC AMP-DEPENDENT protein kinase (PKA), a serine/threonine kinase, is the main mediator of cAMP signaling in mammals (1) (Fig. 1). Phosphorylation mediated by the cAMP/PKA signaling pathway can be elicited by various physiological ligands in cells and is critically involved in the regulation of metabolism, cell proliferation, differentiation, and apoptosis (2–5). The PKA holoenzyme is composed of the genetically distinct catalytic (C) and regulatory (R) subunits. They form the tetrameric holoenzyme R2C2 that dissociates in the presence of cAMP into an R2(cAMP)4 dimer and two free catalytically active C subunits (6). The best known function of the R subunits *in vitro* is inhibition of C subunit kinase activity (7). To date, four major R subunit isoforms (RIα, RIβ, RIIα, and RIIβ) and three isoforms of the C subunit, (Cα, Cβ, and Cγ) have been identified. Two major isozymes, termed PKA types I and II, have been identified based on their patterns of chromatographic elution (5). These isozymes may form from either homo- or heterodimers of the R subunits, yielding holoenzyme complexes of PKA with a number of combinatorial configurations, including RIα2C2, RIβ2C2, RIIα2C2, RIIβ2C2, and RIαRIβC2 (8). In addition, multiple C subunits add to the diversity and complexity of the various holoenzyme complexes (5). Type I PKA contains either regulatory subunit RIα or RIβ in its structure; type II PKA contains either regulatory subunit RIIα or RIIβ (1, 6, 9). Based on *in vitro* studies, the catalytic subunits bind preferentially to regulatory subunits type II; however, type I PKA is more sensitive to cAMP (10), and, thus, PKA-I is the main subtype that mediates cAMP signaling in most human cells.

The four types of regulatory subunits have different expression patterns in mammals. Although RIα has a nearly ubiquitous distribution, RIβ is expressed primarily in brain, testis, and B and T lymphocytes (1, 11). Similarly, RIIα has a wide presence, whereas RIIβ is expressed in brain, adipose, and some endocrine tissues (5). One of the first and most important phenomena described concerning the R subunits is the substantial capacity of the PKA system to self-regulate its assembly, response to cAMP, and disassembly. Overexpression of Cα or Cβ in cell culture results in significant compensation by an increase in RIα subunit protein (12). No compensation by RIIα is observed when Cα and Cβ are overexpressed, suggesting that this phenomenon is specific to RI subunits. The compensatory ability of RIα, but not RIIα, aids in explaining the phenotype displayed by the different knockout mouse models of R subunits (described below).

Within the cellular environment, several cAMP-PKA pathways are operational at any given moment. Activated PKA can phosphorylate different targets in response to different stimuli. Signal specificity is mediated by tissue-specific expression of the R and C subunits, compartmentalization of the tetramer by A kinase anchoring proteins (AKAPs) and other factors (13–15). Compartmentalization of the PKA holoenzymes is an important aspect of PKA response specificity; through it, cAMP messaging is targeted to specific subcellular locations, such as the cytoskeleton, plasma membrane, nucleus, Golgi apparatus, endoplasmic reticulum, and other organelles. Compartmentalization is ensured via interactions of the R subunits with the AKAPs (16, 17). Initially, only PKA-II was observed to be targeted by AKAPs, but recent evidence also demonstrates the association of some AKAPs with PKA-I (18, 19).

Gene knockout (KO) experiments in mice have yielded

Abbreviations: AKAP, A kinase anchoring protein; C, catalytic; CNC, Carney complex; EGFR, epidermal growth factor receptor; FGF, fibroblast growth factor; KO, knockout; LOH, loss of heterozygosity; ODN, oligonucleotide; PKA, cAMP-dependent protein kinase; PKI, PKA-specific inhibitor; R, regulatory; RIα, type 1α regulatory subunit; TSG, tumor suppressor gene.

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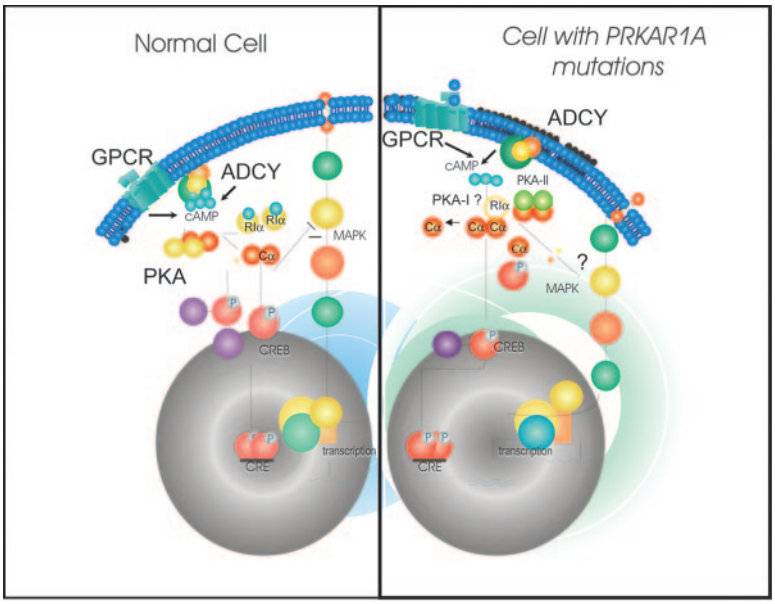

FIG. 1. A diagram of the PKA signaling pathway in normal cells and cells with *PRKAR1A* mutations. After activation of a G protein-coupled receptor (GPCR) and activation of an adenylate cyclase (ADCY) to produce cAMP, the PKA tetramer is activated by binding of cAMP to the regulatory subunits. Catalytic subunits (Cα in most cells) are released after conformational changes in the regulatory subunits; phosphorylation of cytoplasmatic targets ensues. Often this is translated to cross-talking with other intracellular signaling pathways (as the MAPK extracellular signal-regulated kinase 1/2 in the diagram, which in most cells is inhibited by PKA). In the nucleus, PKA catalytic subunits phosphorylate cAMP response element-binding protein, resulting in activation of DNA transcription of cAMP-responsive element-containing genes. In a *PRKAR1A* mutant cell, where there is absent or an ineffective regulatory subunit type 1A, there is either an excess of other regulatory subunits (mostly type II PKA) or defective type I PKA forming (from the few *PRKAR1A* mutant subunits that do not undergo nonsense-mediated mRNA decay; see text), which may also lead to an increased availability of free catalytic subunits. The net effect of all of these changes in CNC cells is an increase in DNA transcription and/or activation of other pathways (such as, perhaps, the MAPK extracellular signal-regulated kinase 1/2 in the diagram), leading to abnormal growth and proliferation.

insight into the specific functions of PKA in relation to its association with various R subunit isoforms. Except for ablation of the RIβ, which does not affect total PKA activity (at least in brain, where it was studied), total and cAMP-stimulated PKA activities are affected to different degrees in the RIα-, RIIα-, and RIIβ-subunit-deficient mice. The RIα-, RIIα-, and RIIβ-subunit-deficient mice exhibit reduced cAMP-stimulated and [PKA-specific inhibitor (PKI)] PKI-inhibited PKA activity, but only the RIα-deficient mice display significantly increased baseline (non-cAMP-stimulated, but PKI-inhibited) PKA activity (20–27). Interestingly, a small increase in this baseline activity is also seen in neonate muscle of RIIα-KO and fat tissue of RIIβ-KO mice. Despite the absence of any major detectable alterations in PKA activity, the RIβ-deficient mice display defective hippocampal long-term depression, depotentiation defects, and reduced injury-induced inflammation and pain (28–30). The RIIβ-deficient mice display a lean phenotype, resistance to diet-induced obesity, increased lipolysis, reduced insulin and very low density lipoprotein cholesterol, diminished motor learning, and neuronal gene expression (20–24). The RIIα-deficient mice are mostly normal (26, 27), whereas the RIα-deficient mice display the most severe phenotype, with gross developmental defects in mesodermal morphogenesis and early embryonic lethality due to incomplete development of the primitive heart tube (25). The later observation and the absence of severe phenotype in the RIIα-, RIIβ-, and RIβ-deficient mice indicate the essential role of the RIα regulatory subunit in maintaining the catalytic subunit under cAMP control during physiological processes. In the latter mouse models, compensation by RIα was also an important factor. It should be mentioned that failure of mesoderm morphogenesis in RIα KO embryos could be rescued by crossing with Cα-subunit-deficient mice; RI$\alpha^{-/-}$C$\alpha^{+/-}$ embryos displayed an intermediate phenotype, with significant rescue of mesoderm-derived structures, whereas RI$\alpha^{-/-}$C$\alpha^{-/-}$ embryos exhibited complete rescue of all of the mesoderm-derived structures and were indistinguishable from early wild-type embryos (31). However, even RI$\alpha^{-/-}$C$\alpha^{-/-}$ embryos died during later development, supporting a role for RIα other than just regulating PKA activity (or, perhaps, a role for the other genes coding for catalytic subunits Cβ and/or Cγ). Interestingly, several mice have been reported with early embryonic lethality caused by defects similar to those induced by RIα deficiency, such as the fibroblast growth factor-4 (FGF-4)/FGF-8, FGF receptor-1, fibronectin, focal adhesion kinase, Src, and Fyn mouse KO models, suggesting molecular pathways that may be interacting with RIα at least during early development (32–40).

The mechanisms by which the RIα-subunit exerts its function outside the PKA holoenzyme are unclear, and they can only be speculated. One possibility is that RIα forms complexes with other proteins in the same way as it does with the catalytic subunits. These complexes could be responsive to cAMP activation or could exert other unknown functions. Recently, yeast two-hybrid experiments identified interaction of RIα with the cytochrome *c* oxidase subunit Vb (41). The complex of RIα with cytochrome *c* oxidase subunit Vb

is responsive to cAMP activation independently of the PKA catalytic subunit, thereby regulating cytochrome *c* oxidase activity and, consequently, energy metabolism (41).

It has also been observed that RIα and RIIα of PKA may be associated with the cytoskeleton in both interphase and mitotic nuclei (42, 43); in particular, the RIα subunit may be associated with the pericentriolar matrix of the centrosome during interphase (44) and the catalytic subunit with microtubules or the mitotic spindle (43). Association with these structures, which are major components of the mitotic apparatus, suggests that RIα may play a very important role in different phases of chromosomal replication, segregation, and/or cell division. Indeed, transfection of mouse hepatoma cells with wild-type or mutant RIα-subunit results in aberrant mitosis with multipolar spindles and mono- or multinucleated giant cells (43).

PRKAR1A and Cancer

Alterations in the expression of RI and RII subunits have been well characterized (at least *in vitro*) during cell proliferation, differentiation, and transformation. Studies in mostly cell lines have suggested that RIα is preferentially expressed in proliferating and transformed cells, whereas increased expression of RIIα is associated with normal nonproliferating and terminally differentiated cells (2). Overexpression of RIα is consistently observed in several cancer tissues (see below) and is associated with poor prognosis in patients with a variety of cancer types. In addition, overexpression of RIIβ, but not RIα, RIIα, or Cα, inhibits cancer cell growth and induces a reverted phenotype in various cancer cell lines (45–47). Thus, uncontrolled proliferation and malignant transformation have been associated with mainly altered RIα expression or changes in the ratio of PKA-I and -II.

Dominant R1α mutations were initially identified in the 1970s and 1980s in Chinese hamster ovary cells (48). Additional mutations were found in S49 mouse lymphoma cells after selection for growth in high concentrations of cAMP analogs that inhibited R1α-cAMP binding (Ka cells), lacked detectable C subunit (kin-8 cells), or had decreased levels of the holoenzyme (Vmax mutants) (48). Enhanced expression of R1α has also been shown in several human cancer tissues and cell lines, including retinoblastoma, renal and breast cancers, the transformed BT5C glioma cell line, malignant osteoblasts, and serous ovarian tumors *vs.* mucinous, endometroid, or clear cell lesions (49–56). Increased R1α expression was shown to be associated with chemical and viral carcinogenesis and oncogene-induced cell transformation, such as *N*-methyl-N^0-nitrosoguanidine-induced gastric tumors in rats, human adenovirus-12-transformed rat 3Y1 cells, murine sarcoma virus (MuSV)-transformed NIH-3T3 clone 13-3B-4 mouse cells, TGF-transformed or v-Ki-*ras*-transformed rat kidney cells, TGF-transformed rat kidney cells, and the human mammary epithelial cell line MCF-10AHE mutated in c-H-*ras* or transformed by the c-*erb*B-2 oncogene (57–63). Interestingly, overexpression of RIα, but not the C subunit, in MCF-10A cells conferred the ability to grow in serum- and growth factor-free conditions (64). In addition, overexpression of RIα in Chinese hamster ovary cells provided growth advantages in monolayer and soft agar conditions, whereas overexpression of the C subunit did not produce such effects (65).

Based on the up-regulation of RIα in several cancers and studies indicating that inhibition of RIα expression through antisense oligonucleotides (ODNs) resulted in the growth arrest of several tumor cell lines, *PRKAR1A* blockade has been considered a possible single-gene targeting approach for the treatment of certain cancers (66). Antisense ODNs designed against the N terminus of RIα subunit specifically inhibit PKA-I expression (not PKA-II) and induce differentiation, cell growth arrest, and/or apoptosis (66). However, it is still unclear how antisense ODNs against RIα exerted these antitumor effects. Antisense targeting of RIα in LS-174T colon and LNCaP prostate cancer cells increased PKA I activity and the cAMP-inducible enzyme phosphodiesterase 4 (67). In contrast, antisense targeting of R1α in HCT-15 multidrug-resistant (MDR) colon carcinoma cells reduced phosphodiesterase 4 activity (67). In both cases, growth arrest was observed.

Inhibition of tyrosine kinase signaling by antisense targeting of R1α has also been suggested in ovarian cancer cells. It has been found that RIα interacts with the ligand-activated epidermal growth factor receptor (EGFR) complex (68). In this study, coimmunoprecipitation using an anti-RIα antibody demonstrated the binding of RIα to the Src homology 3 domains of the Grb2 adaptor protein, which facilitates the localization of the type I PKA to the activated EGFR. Furthermore, treatment of OVCAR-8 cells with an RNA-DNA *PRKR1α* antisense ODN decreased RIα expression, concomitant with reduced expression of EGFR, c-*erb*B-2, and c-erbB-3 and cell growth arrest (69). Other studies have also suggested activation of certain apoptotic pathways by antisense R1α targeting, because inactivation of Bcl-2 by PKA-specific phosphorylation (70) and the association of PKA-I with cytochrome *c* oxidase (41) are well documented. Specifically, antisense R1α induced Bcl-2 hyperphosphorylation and caspase-3 activation, concomitant with hypophosphorylation of Bad, and an increase in Bax, Bak, and Bad proapoptotic proteins in breast and PC3M androgen-independent human prostate cancer cells (70–72). Thus, alterations in serine, threonine, and tyrosine kinase activities as well as activation of apoptosis may contribute in the antitumor effects of R1α-antisense targeting. Nevertheless, recent comprehensive studies with microarray analysis of R1α antisense-targeted tumor cells (73) have indicated that more than 240 genes are significantly altered, raising the possibility of more pathways being involved.

PRKAR1A and the Carney Complex (CNC)

CNC, the complex of spotty skin pigmentation, myxomas, endocrine overactivity, and schwannomas, is a form of multiple endocrine neoplasia (74) (Fig. 2). Patients often have tumors of two or more endocrine glands, including primary pigmented nodular adrenocortical disease, GH- and prolactin-producing pituitary adenoma, thyroid adenoma or carcinoma, testicular neoplasms (primarily large cell calcifying Sertoli cell tumor), and ovarian cysts (75). Nonendocrine tumors that occur frequently are myxomas and ear canal

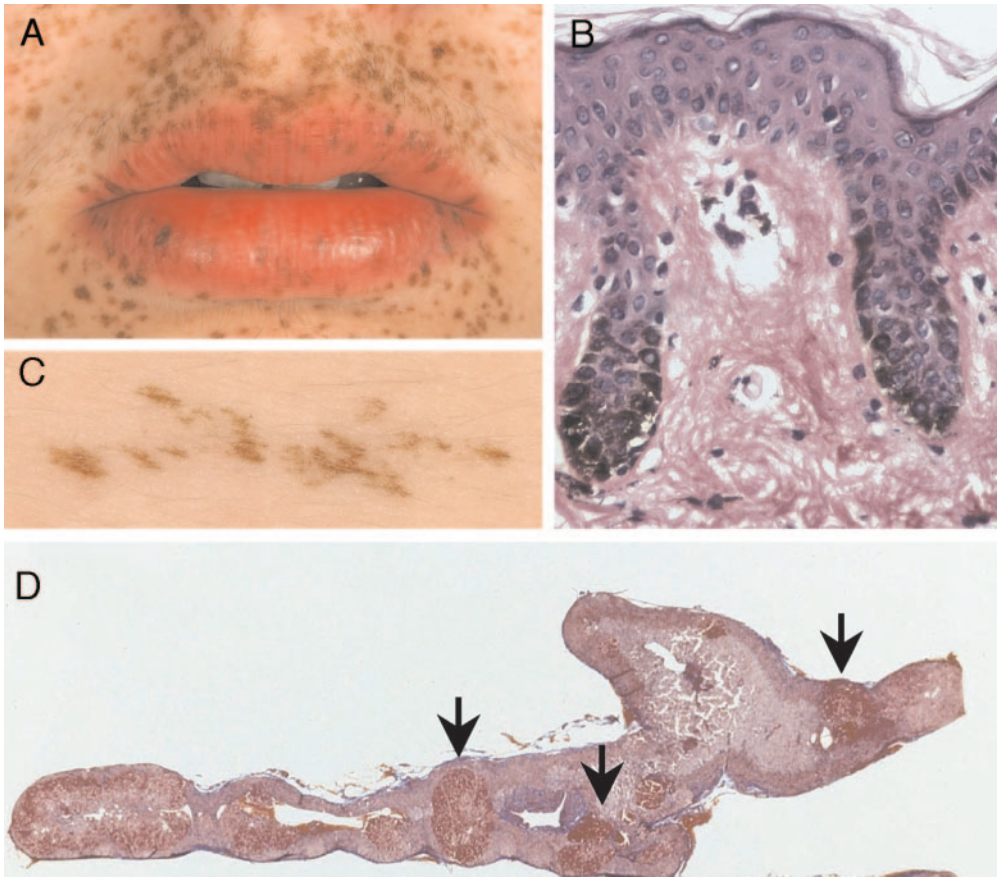

FIG. 2. A, Characteristic perioral and vermilion border pigmentation (lentiginosis) in a patient with a *de novo PRKAR1A*-inactivating mutation. Only about one third of the patients with CNC have such characteristic pigmentation. B, Histology of a lentigo lesion. Unlike regular freckles, in which pigmentation is due to melanin diffusion from a stimulated melanocyte to surrounding cells tissue, in lentiginosis there is both hypertrophy and hyperplasia of the melanocytes with down-growth of the rete pegs and melanin hyperpigmentation of the basal layer. C, Not all lentigo lesions in CNC are like freckles; several patients with CNC have café-au-lait spots (CALS) or other pigmented lesions that mimic freckles or CALS. D, Transverse sections of an adrenal gland demonstrate the characteristic histology of primary pigmented nodular adrenocortical disease, with multiple nodules present (*arrows*), some at the corticomedullary junction, others intruding equally on both layers of the cortex (B and D). [Courtesy of Dr. J. A. Carney, Mayo Clinic, Rochester, MN.]

trichofolliculo-epitheliomas. Additional, but rare, manifestations include psammomatous melanotic schwannoma, breast ductal adenoma, and osteochondromyxoma (75). Most patients belong to families in which the disease is inherited in an autosomal dominant fashion (76).

In most cases of CNC, the endocrine features are reminiscent of McCune-Albright syndrome. For that reason, mutations of the *GNAS* gene or genes related to the cAMP-dependent PKA signaling pathway were long-considered possible candidates underlying the cause of CNC. Linkage analysis and use of loss of heterozygosity (LOH) by microsatellite markers and allelic loss (by fluorescent *in situ* hybridization) allowed us to identify *PRKAR1A* (17q22–24) as the gene mutated in more than half of patients with CNC and/or primary pigmented nodular adrenocortical disease (77, 78). Additionally, genetic linkage analysis has identified other genetic loci harboring a gene(s) for CNC on 2p16 (CNC2 locus) (79, 80). At present, the affected gene on chromosome 2 has not been identified.

In most mutations identified to date in the *PRKAR1A* gene, the sequence change results either directly or indirectly in premature stop codons. The most frequent *PRKAR1A* mutation in CNC is a deletion that results in frameshift c.578delTG in exon 4B of the gene (77, 81); other frequent mutations are present in exons 2 and 6. Analysis of mRNA transcripts in patient lymphocytes treated with cycloheximide showed that mutant mRNAs containing a premature stop codon were unstable due to nonsense-mediated mRNA decay; also, the predicted mutated RIα protein products were absent in these cells (77, 78). Loss of R1α in CNC patients generally leads to increased cAMP-stimulated total (non-PKI-inhibited) kinase activity (81); basal PKA activity did not appear significantly changed in tumors from these patients, but the number of specimens was small, and tissue homogenates were contaminated with normal cells (77).

One of the most prevalent criteria in diagnosing CNC is the increased skin pigmentation seen in these patients (Fig. 2), and it is well documented that the genes required for melanin synthesis are directly regulated by PKA (82). The unregulated PKA activity observed in endocrine and other tissues from CNC patients lacking RIα is consistent with the unregulated PKA activity observed in RIα knockout mouse embryos (31), and this finding supports the conclusion that

this subunit is essential for maintaining appropriate control of PKA activity.

In several cases, tumors from CNC patients display 17q22–24 LOH or allelic loss; the wild-type allele was lost in the lesions where this could be documented (77, 83). In addition, LOH of the *PRKAR1A* locus is frequently detected in some sporadic tumors (84–86). However, in other cases LOH was not identified, and the protein expression was either unaffected or even increased (87) (Stratakis, C. A., unpublished observations). Therefore, haploinsufficiency of the *PRKAR1A* gene may be sufficient for tumor development. According to Knudson's hypothesis, tumor suppressors, unlike oncogenes, generally operate in a recessive manner, requiring loss of both copies for tumorigenesis (88). Thus, the *PRKAR1A* gene does not to seem to follow the definition of a classic tumor suppressor gene (TSG). As we described above, RIα is frequently increased in several cancers. In contrast, CNC, a multiple neoplasia syndrome, results from loss of RIα expression. Thus, *PRKAR1A* could, perhaps, have properties of both an oncogene and a TSG, but satisfies the criteria for neither in human and mouse oncogenesis. The *TP53* gene provides an instructive example of a classic TSG (88) that under certain conditions can act as a dominant oncogene: transfection studies in NIH-3T3 cells showed that *TP53* could function as an oncogene to immortalize and transform cells in conjunction with a mutant *ras* gene (88, 89). If a classic TSG can act as an oncogene under certain conditions, then why not *PRKAR1A,* for which very little is actually known about its role in oncogenesis. More recently, *LKB1*, a serine-threonine kinase that was actually first identified as a TSG (90), was shown to enhance resistance to neoplastic transformation of mouse cells *in vitro* (91). Loss of function mutations in the *LKB1* gene cause Peutz-Jeghers syndrome (75), a disease with which CNC shares some manifestations. Although *LKB1* mediates its TSG activity through a pathway that is distinct from that of PKA/*PRKAR1A* (92), *LKB1* is phosphorylated by PKA (93).

Summary

Based on the evidence presented in this review, it is clear that the biology of cAMP/PKA signaling is complex and warrants further exploration. Loss of one or both alleles of the RIα of PKA in humans produces a multiple neoplasia syndrome; in mice (and most likely in humans), loss of both alleles produces an embryonic lethal defect. Alterations in PKA activity elicit a variety of effects depending on the tissue, developmental stage, degree of differentiation, cAMP levels, as well as the duration of PKA activation. For example, although generally cAMP is known to be antimitogenic, it is mitogenic in many neuroendocrine cell types, and the phenotype in CNC confirms this. The biology of PKA signaling is further complicated by recent evidence indicating that RIα may have functions independent of PKA. Even the known interaction of RIα with the PKA catalytic subunit may result in properties that blur the traditional distinctions between oncogenes and TSGs, as it is true for other serine-threonine kinases functioning as TSGs (*i.e. LKB1*). Having said that, more recent data from our laboratory indicate that *Prkar1a* down-regulation in mice leads to tumors like one would expect from any TSG (94–96).

Note Added in Proof

Refs. 97 and 98 report more complete phenotyping of a mouse with germline down-regulation of Prkar1a expression.

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