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The role of Raf kinase inhibitor protein (RKIP) in health and disease

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Abstract

Raf kinase inhibitor protein (RKIP) is a member of the phosphatidylethanolamine-binding protein (PEBP) family. RKIP plays a pivotal modulatory role in several protein kinase signaling cascades. RKIP binds inhibits Raf-1-mediated phosphorylation of MEK through binding to Raf-1. Protein kinase C (PKC) phosphorylates RKIP, resulting in release of Raf-1 and activation of MEK and ERK. The phosphorylated RKIP binds to and inhibits G-protein-coupled receptor kinase, resulting in sustained G-protein signaling. The regulatory role that RKIP has in cell signaling is reflected in its role in physiology and pathophysiology. RKIP is involved in neural development, cardiac function and spermatogenesis and appears to have serine protease activity. In addition to its roles in physiology, dysregulated RKIP expression has the potential to contribute to pathophysiological processes including Alzheimer's disease and diabetic nephropathy. RKIP has been shown to fit the criteria of being a metastasis suppressor gene, including having decreased expression in prostate cancer metastases and restoring RKIP expression in a prostate cancer cell line diminishes metastasis in a murine model. Clearly, RKIP has multiple molecular and cellular functions. In this review, RKIP's molecular roles in intracellular signaling, its physiological functions and its role in disease are described.

Keywords: Metastasis; RKIP; MEK-1; Signal transduction; Cancer; Prostate

1. Introduction

Raf kinase inhibitor protein (RKIP), a member of the phosphatidylethanolamine-binding protein (PEBP) family, is a conserved, small, cytosolic protein originally purified from bovine brain [1]. RKIP (also known as PEBP-1 and PBP) has wide tissue expression in a variety of different mammalian species such as monkey [2], rat [3], chicken [4] and human [5,6]. The PEBP family of proteins is highly conserved and does not share significant homology with any other protein family [7]. Human RKIP mRNA is 1434 bp long and is transcribed from a gene comprised of four exons spread across approximately 10 kb [5] of chromosome 12q24.23 with a *PEBP* homologue on chromosome 2p [8,9]. The human RKIP mRNA shares a 95% similarity to bovine mRNA and an 85.5% similarity to rat mRNA [6]. The human RKIP mRNA encodes a 187 amino acid protein that shares a 186 amino acid overlap with the bovine 21 kDa RKIP and a 187 amino acid overlap with rat 23 kDa RKIP [5].

RKIP has been assigned multiple functions and is associated with an increasing number of diseases through its involvement with signal transduction pathways. In this review, we describe RKIP's molecular role in signaling, its physiological functions and its role in disease.

2. RKIP in signaling

RKIP has been shown to be involved with several cell signaling cascades. A yeast two-hybrid assay screen of a human T-cell library was used to identify proteins that bound to Raf-1 kinase binding domains [10]. The protein identified was designated RKIP whose sequence was analogous to the human and monkey 23 kDa protein PEBP [10]. Using anti-RKIP antibody, anti-sense RKIP and sense RKIP expression vectors, Yeung et al. discovered that

Abbreviations: GRK, G protein receptor-coupled kinase; GPCR, G protein coupled receptor; HCNP, hippocampal cholinergic neurostimulating peptide; MAPK, mitogen activated protein kinase; NFκB, nuclear factor kappa B; PEBP, phosphatidylethanolamine-binding protein; PKC, protein kinase C; RKIP, Raf kinase inhibitor protein

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RKIP could bind Raf-1, MEK-1 and weakly bind to ERK-2, interfering with MEK phosphorylation and activation by Raf-1. However, RKIP was not a substrate for Raf-1 or MEK. RKIP did not bind to Ras, nor possess kinase activity. It appears that RKIP acts to set the threshold for Raf-1 activation and subsequent activation of the MEK/ERK pathway. Raf-1 dissociates from its complex with MEK in the presence of RKIP. As a result, downstream mitogen activated protein kinase (MAPK) signaling is interrupted and diminished. As stated earlier, RKIP can bind to Raf-1 or MEK, yet not at the same time, and binding to either one is enough to cause downstream inhibition [11]. In addition, it was postulated that RKIP may be involved in growth, transformation, and differentiation [10], which are often deregulated in many forms of cancer.

Evidence for a role for RKIP in cell growth comes from its interaction with protein kinase C (PKC), which phosphorylates proteins that regulate growth, differentiation and transcription. PKC phosphorylates RKIP on serine 153 and alleviates RKIP's inhibition of Raf-1 [12]. PKC is normally recruited to the plasma membrane and activated by diacylglycerol, whose location near the plasma membrane may place it in close proximity to RKIP, which also binds to phospholipids present in cell membranes [13].

Expression of RKIP altered NFκB signaling induced by tumor necrosis factor alpha (TNF-alpha), independent of its regulatory effects upon the MAPK cascade [14]. This regulation occurred upstream of NFκB as a result of inhibition of IKKβ. As a result, IKK can no longer phosphorylate or activate IκB, allowing for NFκB to remain sequestered with IκB. Interacting in a similar fashion with Raf-1 and MEK, RKIP was found to inactivate several kinases of the NFκB family [14]. RKIP appears to have multifunctional roles concerning regulation of different cell signaling cascades.

G protein signaling has been shown to be facilitated by RKIP [15]. G-protein-coupled receptor kinase 2 (GRK-2) is a critical negative feedback inhibitor of G-protein-coupled receptors (GPCR). GRK-2 phosphorylates activated GPCRs, which uncouples them from the active G protein:GPCR complex resulting in initiating GPCR internalization and recycling and inactivating G protein signaling. The importance of GRK-2 activity in controlling G protein signaling implies that GRK-2 activity needs to be tightly regulated. It was demonstrated that RKIP is a physiological inhibitor of GRK-2 [16]. Specifically, after stimulation of GPCR, RKIP dissociates from Raf-1 and associates with GRK-2 and blocks its activity. This switch is triggered by the protein kinase C (PKC)-dependent phosphorylation of the RKIP on serine 153 [12,16].

In summary, RKIP impacts several signaling pathways (summarized in Fig. 1) and intriguingly demonstrates a new paradigm in signal transduction. Namely, a receptor that activates PKC may promote activation of several different signaling pathways through both inducing release of RKIP from Raf, promoting MEK/ERK signaling and inducing association of RKIP to GRK-2, which promotes G protein signaling.

3. RKIP functions

RKIP's location in many different organs enables it to play a role in a variety of processes (Fig. 2). RKIP, is the

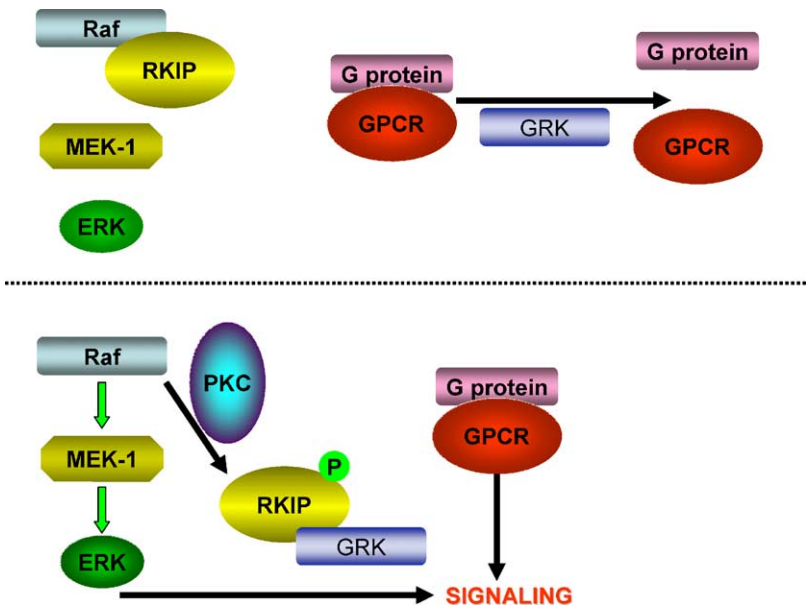

Fig. 1. RKIP modulates Raf and G protein signaling. Upper panel: RKIP binds to Raf, inhibiting Raf-mediated activation of MEK and ERK. Meanwhile, G protein receptor-coupled kinase (GRK) phosphorylates G protein coupled receptor (GPCR), inducing its release from G proteins and inhibition of G protein signaling. Lower panel: protein kinase C (PKC) phosphorylates RKIP inducing its release from Raf and its association with GRK. The dissociated Raf activates MEK, which in turn activates ERK. Additionally, binding of RKIP to GRK, dissociates GRK from the G protein:GPCR complex, which results in stabilization of the G protein:GPCR complex and prolonged G protein signaling.

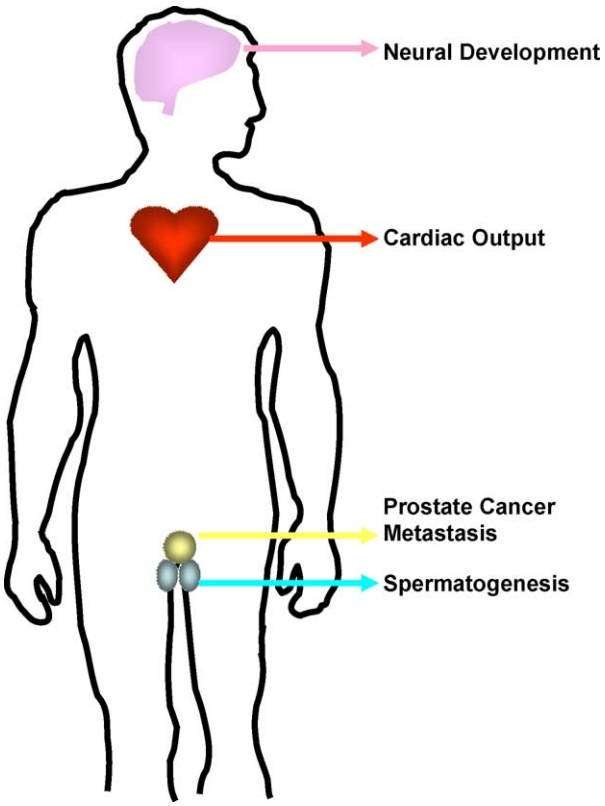

Fig. 2. Functions of RKIP. RKIP has been localized to many tissues; however, the functions of RKIP in most of those tissues are unknown. The figure indicates tissues in which functions of RKIP have been postulated based on experimental data.

precursor of the hippocampal cholinergic neurostimulating peptide (HCNP), which is the natural N-terminal fragment previously described to be released by hippocampal neurons [17,18]. HCNP may play an important role in the septal cholinergic development of the hippocampus, which is important for memory and learning [19–22]. Additionally, examination of human neonatal brain and spinal cord revealed high RKIP expression in the cytoplasm of oligodendrocytes in the white matter and Schwann cells in the nerve roots, but only low expression within the neurones and neuropil [23]. The findings are consistent with a role for RKIP in the organization of phospholipids in the myelin sheath.

In addition to a role in neural development, RKIP and its product HCNP have other physiological functions. For example, in an isolated and perfused frog heart preparation, HCNP acts on the cardiac mechanical performance exerting a negative inotropism and counteracting the adrenergic stimulation of isoproterenol [24]. These data, in combination with the observation that RKIP and HCNP were secreted with catecholamines into the circulation, suggests that RKIP and HCNP might be considered as endocrine factors involved in cardiac physiology [24].

RKIP exerts inhibitory activity against several serine proteases including thrombin, neuropsin, and chymotrypsin, whereas trypsin, tissue type plasminogen activator, and elastase are not affected [25]. RKIP does not share significant homology with other serine protease inhibitors, suggesting that it is the prototype of a novel class of serine protease inhibitors. RKIP is found on the surface of cells, but does not have a recognized secretory signal, thus it may only work locally. However, recombinant overexpression of RKIP in rat fibroblast cells results in the ability to detect RKIP in the cell medium [25]. The mechanism through which RKIP is released from cells is not known. One possibility is that it may be secreted through non-classical signal secretory mechanisms that could be due to interactions with granule membrane lipid and lipid rafts.

RKIP has been identified in the testis and epididymis, where it has been implicated in spermatogenesis [2]. In the adult rat testis, RKIP was localized to elongating spermatids, residual bodies, and interstitial Leydig cells. In the adult rat epididymis, RKIP was localized to epithelial cells of the caput, corpus, and cauda regions and to the cytoplasmic droplets of spermatozoa in the lumen of the epididymidis. However, in prepubertal testes, RKIP was localized only to Leydig cells from day 1 postpartum and was not detected in any other cell type until the differentiation of spermatids. These data suggest that RKIP is involved in the organization of sperm membranes during spermatogenesis and the presence of RKIP in Leydig cells suggests diverse roles for this protein as a lipid carrier or binding protein [26].

In summary, RKIP is a multifunctional protein whose exact function still remains uncertain. Its roles in reproduction, cardiology and neurology present interesting and exciting avenues for future research.

4. RKIP in non-neoplastic disease

The function of RKIP and its cleavage product, HCNP, are actively being researched in Alzheimer's disease and dementia [19,27–29]. HCNP accumulates and is strongly expressed in Hirano bodies [21,27,28]. These hippocampal, eosinophillic rod-like structures occur mainly in the CA1 region of the hippocampus and are pathologic findings of Alzheimer's disease in elderly patients [27,30,31]. Patients with these inclusions may have a disturbance of the septo-hippocampal cholinergic system, thus effecting learning and memory. In addition, high levels of HCNP have been found in the cerebrospinal fluid of some early onset Alzheimer's patients with clinical signs [29]. It is still unclear what the exact mechanism of RKIP or HCNP is within the nervous system and further work needs to be performed to delineate its precise role is the pathophysiology of Alzheimer's disease.

Through its role in PKC signaling, RKIP may mediate diabetic nephropathy. Specifically, PKC activation has been shown to play a role in the pathogenesis of diabetic nephropathy [32,33]. Thus, molecules downstream of

activated PKC, such as RKIP, could be important mediators of PKC-dependent pathogenesis. Use of peptide mass fingerprinting identified an unknown protein that was up-regulated in mouse diabetic kidneys [34]. Bioinformatic analysis indicated that the protein was RKIP. These data suggest that RKIP has a potential functional role in PKC-dependent pathogenic pathways of diabetic nephropathy [34].

5. RKIP in neoplastic disease

Aberrant RKIP expression also plays a critical role in cancer. Recently, our lab has identified a novel anti-metastasis function for RKIP in prostate cancer [8]. We compared levels of RKIP expression in non-metastatic prostate cancer cell lines and metastatic prostate cancer cell lines. The metastatic prostate cancer cells had much less RKIP expression than non-metastatic prostate cancer cells. Immunochemistry of tissues from patients indicated moderate to high RKIP protein expression in normal prostate and primary prostate cancers; however, its expression was downregulated or undetectable in prostate cancer metastases. Restoration of RKIP expression in metastatic prostate cancer cell lines was associated with decreased in vitro invasiveness of the cells. Furthermore, RKIP restoration in metastatic prostate cancer cells reduced spontaneous lung metastasis, but not primary tumor growth rate, in mice injected with tumor cells orthotopically. These observations define RKIP as a metastasis suppressor gene which, by definition, suppress metastasis without affecting tumorigenicity [35].

The mechanisms through which RKIP suppresses metastasis are not known. However, increased RKIP expression was associated with decreased vascular invasion and decreased ability to form new blood vessels in the primary tumors in the rodent model [8] suggesting that RKIP may act at the early angioinvasive stages of metastasis. Taken together, these data demonstrate that RKIP is a clinically relevant prostate cancer metastasis suppressor gene that regulates the Raf/MEK/ERK pathway. RKIP could be a promising molecular target for compounds designed for cancer treatment.

6. Conclusions

Although the existence of RKIP, initially identified as PEBP, has been recognized for many years, only recently has it role in cell signaling been truly appreciated. This has led to a recent flurry of activity to understand its roles in physiology and pathophysiology. Clearly, due to its role in modulating PKC signaling through regulation of Raf and G-protein signaling, RKIP has the potential to modulate many processes. This is reflected in the myriad of functions it performs in different tissues. At this early stage of discovery regarding RKIP's role in many signaling pathways, it is too early to confidently reconcile the contribution of RKIP to different biological processes with its role in signaling. Furthermore, additional studies are needed to determine the precise relationship between RKIP and its role in several diseases, including Alzheimer's disease, diabetes and cancer. The more that is defined about RKIP and its role in health and disease may help identify RKIP or proteins downstream of RKIP as important targets for therapeutic interventions.

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