



Review Article

Regulation on tumor metastasis by Raf kinase inhibitory protein: New insight with reactive oxygen species signaling

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ABSTRACT

Targeted therapy aiming at the metastatic signal pathway, such as that triggered by receptor tyrosine kinase (RTK), for the prevention of tumor progression is promising. However, RTK-based targeted therapy frequently suffered from drug resistance due to the co-expression of multiple growth factor receptors that may raise compensatory secondary signaling and acquired mutations after treatment. One alternative strategy is to manipulate the common negative regulators of the RTK signaling. Among them, Raf kinase inhibitory protein (RKIP) is highlighted and focused on this review. RKIP can associate with Raf-1, thus suppressing the downstream mitogen-activated protein kinase (MAPK) cascade. RKIP also negatively regulates other metastatic signal molecules including NF- κ B, STAT3, and NOTCH1. In general, RKIP achieves this task via associating and blocking the activity of the critical molecules on upstream of the aforementioned pathways. One novel RKIP-related signaling involves reactive oxygen species (ROS). In our recent report, we found that PKC δ -mediated ROS generation may interfere with the association of RKIP with heat shock protein 60 (HSP60)/MAPK complex via oxidation of HSP60 triggered by the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate. The departure of RKIP may impact the downstream MAPK in two aspects. One is to trigger the Mt \rightarrow cytosol translocation of HSP60 coupled with MAPKs. The other is to change the conformation of HSP60, favoring more efficient activation of the associated MAPK by upstream kinases in cytosol. It is worthy of investigating whether various RTKs capable of generating ROS can drive metastatic signaling via affecting RKIP in the same manner.

KEYWORDS: Heat shock protein 60, Hepatocellular carcinoma, Metastasis, Raf kinase inhibitory protein, Reactive oxygen species

INTRODUCTION

The poor prognosis of tumor is due to the high recurrence rate caused by metastasis after surgical removal. Metastasis is a complicated pathological process beginning with epithelial-mesenchymal transition (EMT) of the primary tumor cells which then migrate and invade into surrounding tissue followed by entering into (intravasate) and moving out (extravasate) blood circulation and finally proliferating in the secondary loci. The tumor microenvironment contains a lot of growth factors and cytokine such as hepatocyte growth factor (HGF) [1] and transforming growth factor β (TGF β) [2] collectively called metastatic factors, capable of triggering tumor progression via a lot of molecular pathways [3-5]. Moreover, deregulation of the receptors of these metastatic factors was closely associated with tumor progression. Among them, receptor tyrosine kinase (RTK) including c-Met [6-8], EGFR [7,9] and platelet-derived

growth factor receptor-alpha [10,11] were frequently found to be overexpressed or mutated that activate various signaling cascades such as mitogen-activated protein kinase (MAPK) [4,12-15], NF- κ B [16], AKT [17,18], STAT3 [19,20], NOTCH1 [21], and G protein-coupled receptor kinase 2 [22] leading to tumor progression. In the past decades, targeted therapy aiming at RTK and its downstream pathway for the prevention of tumor progression has been intensively studied. One unresolved issue for RTK signaling-based targeted therapy is drug resistance [7,23-26] due to the co-expression of multiple growth factors that may raise compensatory


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secondary signaling after treatment with specific tyrosine kinase inhibitors (TKIs) [27]. For example, EGFR and HER3 overexpression might be responsible for acquired resistance to a specific inhibitor of HER2, trastuzumab [28]. In addition, c-Met amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling [29]. In addition, resistance to TKIs was frequently observed due to acquired mutation of RTKs after long-term treatment. For example, a secondary EGFR^{T790M} mutation was responsible for clinically acquired resistance to the first- and second-generation EGFR-TKIs drugs such as gefitinib, erlotinib, and afatinib [30]. In addition, a secondary mutation in the activation loop (Y1230) of MET, the receptor of HGF, can contribute to acquired resistance to MET inhibitors PHA-665752 and PF-2341066 [31]. Therefore, an alternative cancer-targeted therapy that effectively blocks signaling from multiple RTKs without resistance needs to be developed. One promising strategy is to manipulate the common negative regulators of the RTK signaling. Especially, tumor metastasis suppressors, which directly interact with various critical signaling molecules downstream of RTKs, can be employed as more efficient antagonists of metastatic signaling. Among them, Raf kinase inhibitory protein (RKIP) is highlighted [32-34] and will be focused on this review.

THE NEGATIVE REGULATORY ROLE RAF KINASE INHIBITORY PROTEIN IN PREVENTING TUMOR METASTASIS

RKIP was initially identified to be a cytosolic protein isolated from the bovine brain and called phosphatidylethanolamine-binding protein 1 (PEBP1) ascribed to its phospholipid-binding potential [35]. However, in 2000, PEBP1 was found to suppress the Raf1-MAPK pathway [36-38] and was renamed as RKIP. This further triggered numerous studies extending RKIP's negatively regulatory function to other signaling cascades downstream of various cell surface receptors including RTKs (see below section). Meanwhile, RKIP was found to be a critical player regulating a lot of pathophysiological systems including tumor progression. In the past decades, RKIP was emerging to be a negative regulator in metastasis of a lot of tumors such as lung cancer (for review, lung cancer [32]); hepatocellular carcinoma (HCC) [39], gastric cancer [40,41], colon cancer [42], and breast cancer [43]. Reduced expression of RKIP was found to be associated with malignancy and poor prognosis in several tumor types (for review [44]) such as breast cancer [34], prostate cancer [45], colorectal cancer [46], HCC [47], melanoma [48], gastric cancer [49], pancreatic ductal adenocarcinoma [50], thyroid carcinomas [51], esophageal cancer [52], and acute myeloid leukemia [53]. Furthermore, downregulation of RKIP was responsible for sorafenib resistance via reactivation of the Raf/MEK/ERK pathway in HCC cell lines [54]. Moreover, downregulation of RKIP in the advanced stages of gastric cancer facilitated the development of gastric cancer stem cells with increased expression of CD44 and peroxiredoxin 2, two of the cancer stem cell markers [55]. On the other hand, RKIP overexpression can reverse tumor chemo/immune/radi-resistance and support anticancer host immunosurveillance [56]. Furthermore,

ectopic RKIP expression or upregulation of RKIP by chemo/immune-modulatory agents increased tumor chemo- and radiosensitivity by suppressing PI3K activation [54,57].

THE MECHANISM FOR RAF KINASE INHIBITORY PROTEIN TO SUPPRESS TUMOR PROGRESSION: REGULATION ON METASTATIC SIGNALING

As mentioned above, RKIP exerts its suppressive effect on tumor metastasis via its impact on critical signal molecules. In addition to the Raf-MAPK cascade, RKIP negatively regulates a lot of other signal molecules involved in tumor progression including NF- κ B [58,59], STAT3 [60], NOTCH1 [61], and G protein-coupled receptor kinase 2 (GRK2) [62,63]. On the other hand, RKIP can sustain the expression of GSK3, a suppressor of multiple oncogenic pathways including Wnt [64]. The inhibitory effect of RKIP on the aforementioned metastatic pathways can impact the expression and/or activation of a lot of downstream transcription and posttranscription machineries. For example, RKIP may indirectly regulate Snail [65,66] and Yin Yang 1 [67,68], a well-known metastatic transcriptional factor, via NF- κ B inhibition. Moreover, RKIP may inhibit local breast cancer invasion by antagonizing the transcriptional activation of MMP-13, mediated by the ERK2 signaling pathway [69].

The underlying mechanisms for RKIP to suppress cancer signaling are diverse and complicated. In general, RKIP achieves this task via blockade of the activity of the critical molecules on upstream of the aforementioned metastatic signaling cascades. As its name suggested, RKIP was initially found to compete with MEK for association with Raf-1, thus interrupting MEK phosphorylation and suppressing downstream MAPK. Further studies demonstrated that RKIP inhibits the activity of NF- κ B via interaction with I κ B kinase (IKK) complex, IKK α and IKK β , or with upstream IKK activators, including TGF β -activated kinase 1 (TAK1) and NF- κ B-inducing kinase (NIK) [58]. In addition, RKIP was found to associate with melanoma differentiation antigen-9/syntenin, which disturbs the assembly of stable c-Src/focal adhesion kinase (FAK) signaling complexes, required for the activation of NF- κ B and melanoma progression. RKIP can also block the activation of STAT3 by suppressing its interaction with upstream kinases including cellular Src (c-Src), interleukin 6 (IL-6), Janus kinase 1/2 (JAK1/2), and Raf [60]. In addition, RKIP directly interacts with the full length of NOTCH1, preventing its proteolytic cleavage and NICD release and decreasing mesenchymal markers such as N-cadherin and Snail in H1299 cells [61].

INVOLVEMENT OF REACTIVE OXYGEN SPECIES IN RAF KINASE INHIBITORY PROTEIN REGULATED SIGNALING

One potential mechanism for RKIP to regulate downstream signaling involves the reactive oxygen species (ROS). Initially, ROS was well known to be a defending molecule against pathogenic microorganisms. Later, it was found to be essential for mediating major signal pathways to trigger a lot of

pathophysiological processes including tumor progression (for review: [70-72]). Conventionally, ROS was known to enhance signal transduction via oxidative activation of a signal kinase or inactivation of negative regulatory molecules (for reviews, [73,74]). For example, oxidative activation of c-Src may lead to anoikis resistance by activating the PI3K/PKB α and ERK to trigger pro-survival pathways [75]. On the other hand, oxidative inactivation of negative signaling regulators such as protein tyrosine phosphatases (PTPs) and phosphatase and tensin homolog (PTEN) can indirectly elevate PI3K-AKT and MAPK signaling [74,76]. In addition, oxidation of a scavenger enzyme thioredoxin may disrupt its interaction with apoptosis signaling kinase 1 which is then activated, serving as the upstream kinases in the MAPK cascade [77]. Moreover, ROS generation can be induced by a lot of growth factors and cytokines including HGF [78,79], EGF [80,81], PDGF [82,83], TGF β [84-86], and integrin engagement [87-89] for activation of similar downstream signalings including MAPK, PI3K-AKT, and NF- κ B to trigger EMT, migration, invasion, and tumor progression (for review, [90]). It is worthy of noting that the signal pathways activated by ROS happen to be the same as those suppressed by RKIP described above. Interestingly, several reports described the negative relationship of RKIP with ROS status in several contexts. For example, in acute liver injury, reduced RKIP expression significantly enhanced the levels of ROS and the pro-inflammatory factors such as tumor necrosis factor- α as well as IL-6 [91]. On the other hand, RKIP together with the epithelial markers E-cadherin and ZO-1 can be downregulated by ROS leading to injury on proximal tubular epithelial cells [92]. However, the underlying mechanism for the negative correlation of RKIP with ROS is still obscure. One potential molecule involved in the negative regulation of ROS generation by RKIP is mitochondrial Mn-dependent superoxide dismutase (MnSOD also called SOD2) which is responsible for the conversion of O $_2^-$ to H $_2$ O $_2$ in the mitochondria. MnSOD and the mitochondria H $_2$ O $_2$ produced by it play critical roles in triggering cancer progression within the tumor microenvironment. For example, IL-6, an essential growth factor for multiple myeloma cells, induces myeloma therapy resistance via NF- κ B-dependent MnSOD expression and mtROS production [93]. Furthermore, ROS-p38MAPK/Akt signaling mediated the upregulation of MnSOD expression induced by heat shock [94]. Interestingly, MnSOD was found to negatively correlate with RKIP in renal cell carcinoma [95]. Since it was implicated that RKIP negatively regulates ROS generations as described above [91], RKIP may downregulate MnSOD via suppressing the ROS-MAPK signaling. On the other hand, previous studies also suggested that ROS can downregulate RKIP gene expression for triggering tumor progression. For example, RKIP can be decreased by a lot of transcriptional factors such as Snail and SP1 [96], well known to be induced by ROS signaling triggered by a lot of metastatic factors [71,87,89]. Taken together, the negative relationship between RKIP and ROS signal transduction is promising.

POTENTIAL MECHANISMS FOR RAF KINASE INHIBITORY PROTEIN TO RELEASE RAF KINASE INHIBITORY PROTEIN FROM ONCOGENIC SIGNALING

Recently, we found that ROS may disturb the association of RKIP with an important ROS signal target, heat shock protein 60 (HSP60), which is one of the chaperones in mitochondria (Mt), which is mediated by PKC δ in HCCs (HepG2 and HCC340) and stimulated with the tumor promoter 12-O-tetradecanoyl-phorbol-13-acetate (TPA) [97]. In the resting state, RKIP was closely associated with HSP60-MAPK complex in both Mt and cytosol. Treatment of TPA can release RKIP upon oxidation of HSP60, leading to enhanced activation of MAPK in HCCs. The departure of RKIP from oxidized HSP60 may impact the downstream MAPK in two aspects. One is to trigger the Mt \rightarrow cytosol translocation of HSP60 coupled with MAPKs, which may be easier to be activated by upstream kinases in the cytosol. The other is to change the conformation of HSP60 favoring more efficient activation of the associated MAPK [97]. Based on this finding, it is worthy of investigating whether the aforementioned metastatic factors capable of generating ROS, including HGF, EGF, PDGF, and TGF β , can drive metastatic signaling via reversing the suppressive effect of RKIP in the same manner. Among them, we have found that HGF triggered-ROS signaling can oxidize HSP60 for activating ERK (MAPK) required for HCC progression [78]. Therefore, it is tempting to investigate whether HGF and the other metastatic factors may trigger ROS-dependent MAPK activation via oxidation of HSP60 and release of RKIP from HSP60/MAPK complex as that observed in HCC stimulated by TPA [96] [Figure 1].

POTENTIAL RAF KINASE INHIBITORY PROTEIN TARGET SIGNAL MOLECULES INVOLVED IN REGULATION OF RAF KINASE INHIBITORY PROTEIN

Since a lot of ROS-mediated signal pathways including PI3K-AKT, NF- κ B, STATs, and Notch can also be negatively regulated by RKIP as described above, it is very probable that the ROS-generating metastatic factors may trigger the dissociation of RKIP from the redox-sensitive targets for activation of the downstream signaling, just like the dissociation of RKIP from HSP60 for activating MAPK pathway. For example, TGF- β was known to trigger oxidative activation of Src to activate FAK and downstream AKT and MAPK signaling [74], whereas RKIP can interact with c-Src to block the activation of STAT3 [60]. In addition, ROS can activate NF- κ B signaling and induce EMT-related morphological changes via promoting IKK-mediated degradation of I κ B and induce the nuclear translocation of NF- κ B [74], whereas RKIP was known to inhibit the NF- κ B activity via interaction with IKK, TAK, and NIK complex as described above[58]. Thus, it is tempting to investigate whether ROS signaling induced by the relevant metastatic factors can trigger the dissociation of RKIP from critical molecules such as c-Src, IKK, and Notch to activate STAT3,

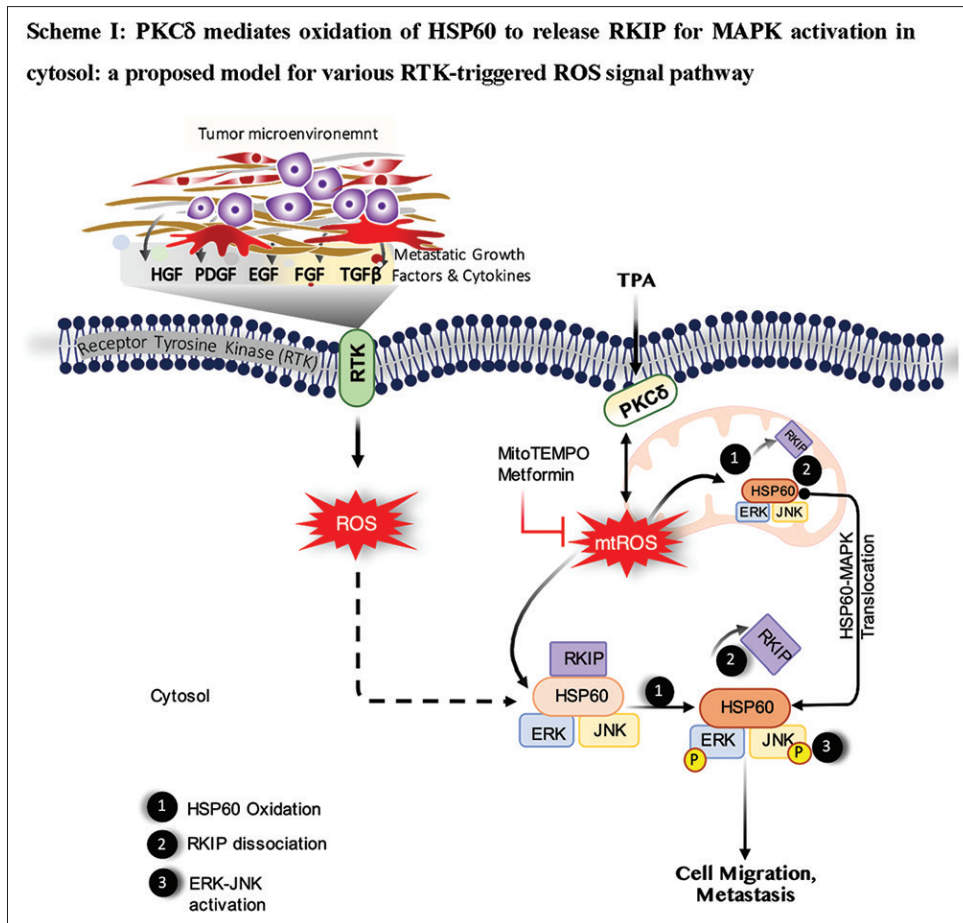


Figure 1: Upon stimulation of hepatocellular carcinoma by tetradecanoyl-phorbol-13-acetate, mitochondria reactive oxygen species is generated which can be blocked by mitochondria reactive oxygen species scavenger MitoTEMPO and Metformin. The mitochondria reactive oxygen species thus generated may oxidize heat shock protein 60 resulting in Raf kinase inhibitory protein dissociation from heat shock protein 60/mitogen-activated protein kinase (ERK and JNK) complex, triggering Mt → cytosol translocation of heat shock protein 60/mitogen-activated protein kinase. On the other hand, some mitochondria reactive oxygen species that diffuse into cytosol may also oxidize cytosolic heat shock protein 60, releasing Raf kinase inhibitory protein. Both the pathways contribute to the robust activation of mitogen-activated protein kinases in the cytosol. It can be proposed that the same molecular event may occur in the signal pathway driven by other metastatic factors capable of generating reactive oxygen species *via* receptor tyrosine kinase in the tumor environment (indicated on the upper left panel). Solid lines: established pathway. Dashed line: proposed pathway

NF- κ B, and Notch signaling, respectively, leading to tumor progression [Figure 2].

CONCLUSION AND PERSPECTIVE

RKIP was well established to be a negative regulator of tumor metastasis via its impact on critical signal cascade downstream of oncogenic receptors such as RTKs by binding to the signal module on upstream of RTKs. Since we found that RKIP can be released upon oxidation of HSP60 resulted from TPA-triggered PKC activation and ROS generation [Figure 1], it is worthy of investigating whether various factors capable of generating ROS can drive various oncogenic signaling via affecting RKIP in the same manner [Figure 2].

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Conflicts of interest

There are no conflicts of interest.

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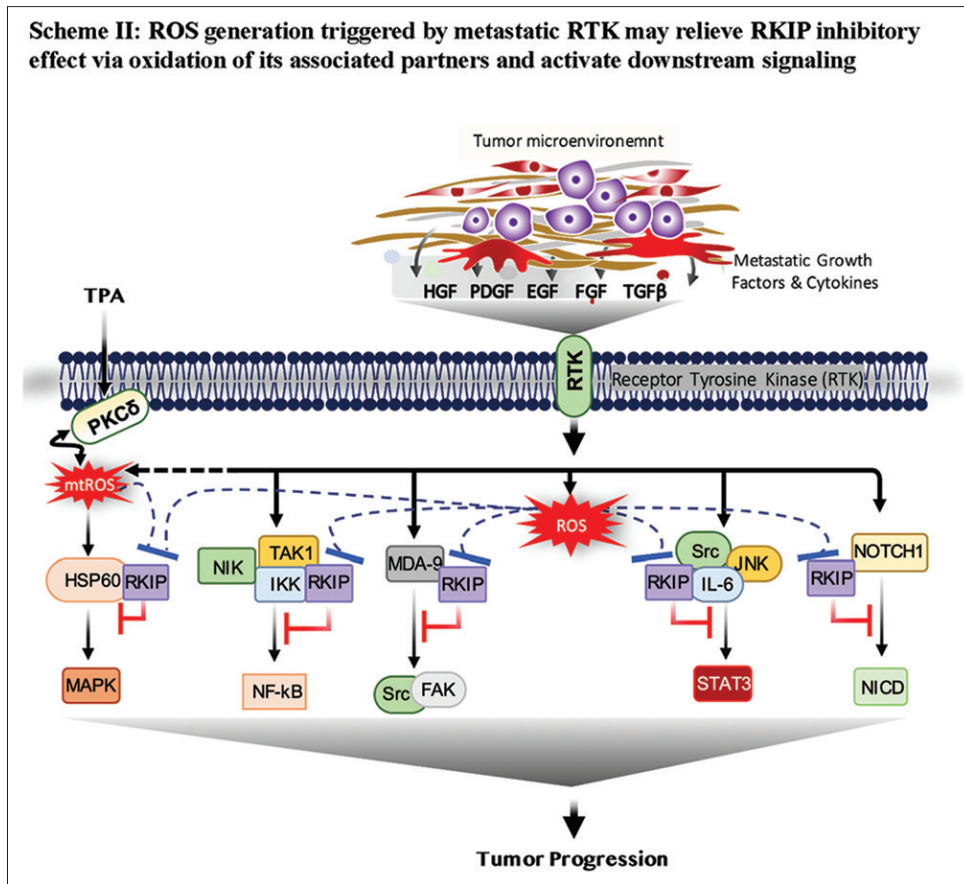


Figure 2: A lot of metastatic factors and cytokines secreted in the tumor microenvironment can trigger various metastatic signal pathways most of them can be suppressed by Raf kinase inhibitory protein via associating with upstream signal molecules as indicated. According to what has been observed in the tetradecanoyl-phorbol-13-acetate-triggered pathway that activates mitogen-activated protein kinase through mitochondria reactive oxygen species-mediated heat shock protein 60 oxidation, Raf kinase inhibitory protein may be released upon oxidation of critical upstream signal molecules resulting in reactivation of downstream signaling for tumor progression. Solid lines: established pathway. Dashed line: a proposed pathway

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