Tzu Chi Medical Journal 2021; 33(4): 332-338

Review Article

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

*Chi-Tan Hu[a,b,c], Jaya Prakash Mandal[d], Wen-Sheng Wu[d,e,f]**

[a]Division of Gastroenterology, Department of Medicine, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, [b]Research Centre for Hepatology, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, [c]School of Medicine, Tzu Chi University, Hualien, Taiwan, [d]Institute of Medical Sciences, Tzu Chi University, Hualien, Taiwan, [e]Division of General Surgery, Department of Surgery, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, Hualien, Taiwan, [f]Department of Laboratory Medicine and Biotechnology, College of Medicine, Tzu Chi University, Hualien, Taiwan

Submission : 17-Dec-2020
Revision : 19-Jan-2021
Acceptance : 18-Feb-2021
Web Publication : 04-May-2021

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-κB, 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 PKCδ-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→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 begining 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 (TGFβ) [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-κB [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

Access this article online
Quick Response Code:
Website: www.tcmjmed.com
DOI: 10.4103/tcmj.tcmj_296_20

*Address for correspondence:
Prof. Wen-Sheng Wu,
Division of General Surgery, Department of Surgery, Hualien Tzu Chi Hospital, Buddhist Tzu Chi Medical Foundation, 707, Section 3, Chung-Yang Road, Hualien, Taiwan.
E-mail: wuws@gms.tcu.edu.tw

How to cite this article: Hu CT, Mandal JP, Wu WS. Regulation on tumor metastasis by Raf kinase inhibitory protein: New insight with reactive oxygen species signaling. Tzu Chi Med J 2021; 33(4): 332-8.

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-κB [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-κB 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-κB via interaction with IkappaB kinase (IKK) complex, IKKα and IKKβ, or with upstream IKK activators, including TGFβ-activated kinase 1 (TAK1) and NF-κB-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-κB 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/PKBα 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], TGFβ [84-86], and integrin engagement [87-89] for activation of similar downstream signalings including MAPK, PI3K-AKT, and NF-κB 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_2O_2 in the mitochondria. MnSOD and the mitochondria H_2O_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-κB-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 PKCδ 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 → 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 TGFβ, 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-κB, 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-κB signaling and induce EMT-related morphological changes via promoting IKK-mediated degradation of IκB and induce the nuclear translocation of NF-κB [74], whereas RKIP was known to inhibit the NF-κB 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,

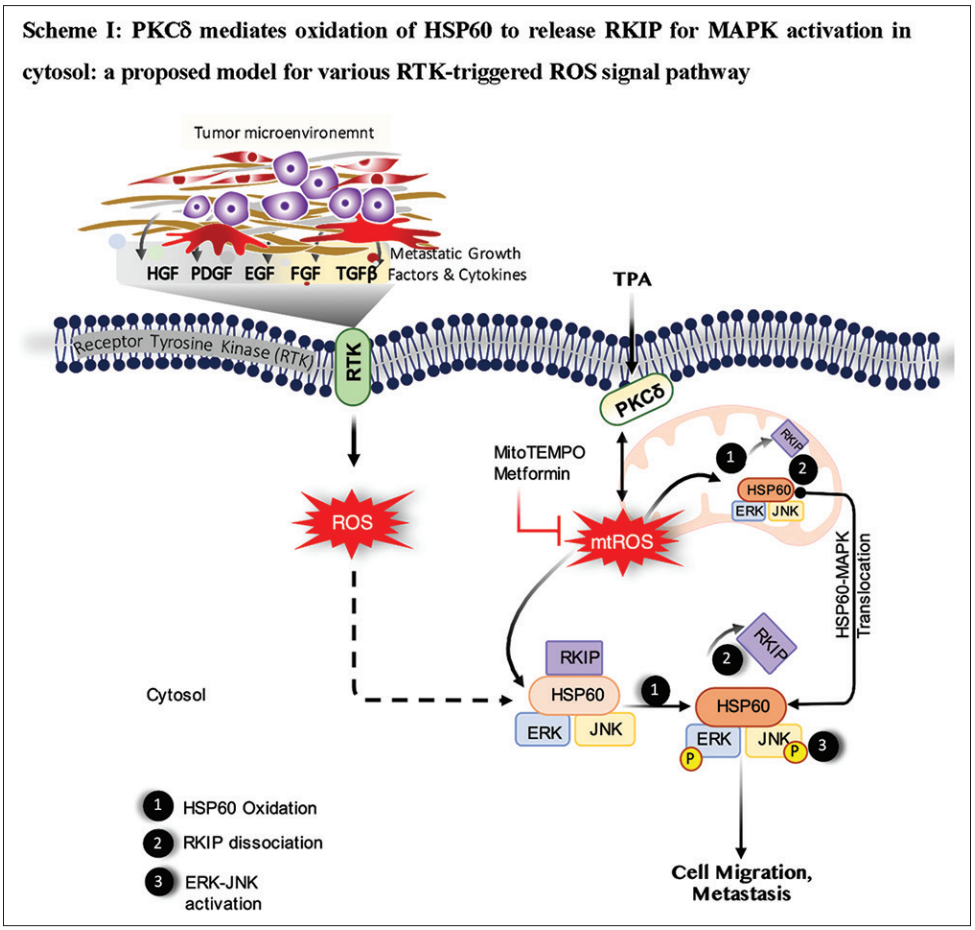

Figure 1: Upon stimulation of hepatocellular carcinoma by tetradecanoyl-phorbol-13-acetate, mitochondria reactive oxygen species is generated which can be blocked by mitochondria reactive oxygen species scavenger MitoTEMPO and Metformin. The mitochondria reactive oxygen species thus generated may oxidize 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 kinase s 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-κB, 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].

Acknowledgments

We thank Dr. Ren-In You at Tzu Chi University in Hualien, Taiwan, for discussing and suggesting the writing of this review paper.

Financial support and sponsorship

This study was financially supported by the project of Tzu Chi Medical Mission Project (TCMMP108-03-01) and TCMMP108-03-02) granted by the Tzu Chi Medical Foundation and by the Ministry of Science and Technology (MOST 108-2320-B-320-003).

Conflicts of interest

There are no conflicts of interest.

References

1. Goyal L, Muzumdar MD, Zhu AX. Targeting the HGF/c-MET pathway in hepatocellular carcinoma. Clin Cancer Res 2013;19:2310-8.
2. Mazzocca A, Antonaci S, Giannelli G. The TGF-β signaling pathway as a pharmacological target in a hepatocellular carcinoma. Curr Pharm Des 2012;18:4148-54.
3. Ye QH, Zhu WW, Zhang JB, Qin Y, Lu M, Lin GL, et al. GOLM1 modulates EGFR/RTK cell-surface recycling to drive hepatocellular carcinoma metastasis. Cancer Cell 2016;30:444-58.
4. You RI, Wu WS, Cheng CC, Wu JR, Pan SM, Chen CW, et al.

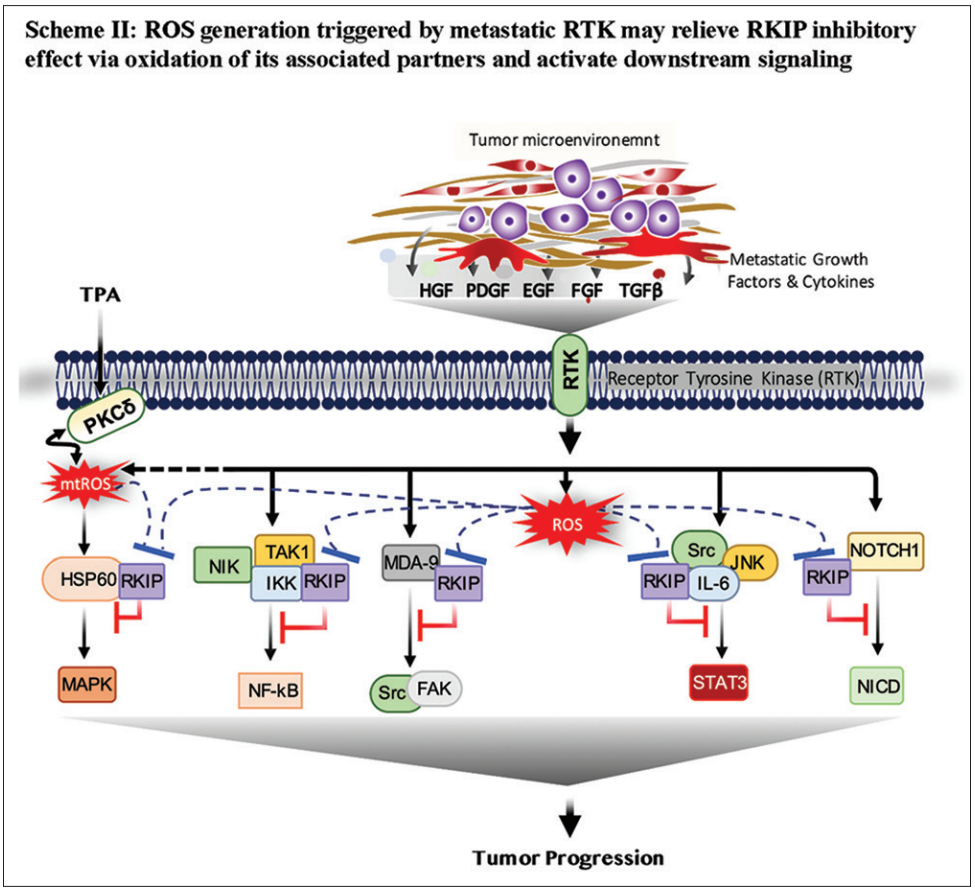

Figure 2: A lot of metastatic factors and cytokines secreted in the tumor microenvironment can trigger various metastatic signal pathways most of them can be suppressed by Raf kinase inhibitory protein via associating with upstream signal molecules as indicated. According to what has been observed in 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

Involvement of N-glycan in multiple receptor tyrosine kinases targeted by ling-zhi-8 for suppressing HCC413 tumor progression. Cancers (Basel) 2018;11:9.

5. Yoo BK, Gredler R, Chen D, Santhekadur PK, Fisher PB, Sarkar D. c-Met activation through a novel pathway involving osteopontin mediates oncogenesis by the transcription factor LSF. J Hepatol 2011;55:1317-24.
6. Giordano S, Columbano A. Met as a therapeutic target in HCC: Facts and hopes. J Hepatol 2014;60:442-52.
7. Llovet JM, Bruix J. Molecular targeted therapies in hepatocellular carcinoma. Hepatology 2008;48:1312-27.
8. Whittaker S, Marais R, Zhu AX. The role of signaling pathways in the development and treatment of hepatocellular carcinoma. Oncogene 2010;29:4989-5005.
9. Berasain C, Perugorria MJ, Latasa MU, Castillo J, Goñi S, Santamaría M, et al. The epidermal growth factor receptor: A link between inflammation and liver cancer. Exp Biol Med (Maywood) 2009;234:713-25.
10. Wei T, Zhang LN, Lv Y, Ma XY, Zhi L, Liu C, et al. Overexpression of platelet-derived growth factor receptor alpha promotes tumor progression and indicates poor prognosis in hepatocellular carcinoma. Oncotarget 2014;5:10307-17.
11. Carvalho I, Milanezi F, Martins A, Reis RM, Schmitt F. Overexpression of platelet-derived growth factor receptor alpha in breast cancer is associated with tumour progression. Breast Cancer Res 2005;7:R788-95.
12. Chen J, Ji T, Wu D, Jiang S, Zhao J, Lin H, et al. Human mesenchymal stem cells promote tumor growth via MAPK pathway and metastasis by epithelial mesenchymal transition and integrin α5 in hepatocellular carcinoma. Cell Death Dis 2019;10:425.
13. Chen L, Guo P, He Y, Chen Z, Chen L, Luo Y, et al. HCC-derived exosomes elicit HCC progression and recurrence by epithelial-mesenchymal transition through MAPK/ERK signalling pathway. Cell Death Dis 2018;9:513.
14. Imperial R, Toor OM, Hussain A, Subramanian J, Masood A. Comprehensive pancancer genomic analysis reveals (RTK)-RAS-RAF-MEK as a key dysregulated pathway in cancer: Its clinical implications. Semin Cancer Biol 2019;54:14-28.
15. Sundaram MV. RTK/Ras/MAPK signaling. WormBook 2006;11:1-19. doi: 10.1895/wormbook.1.80.1.
16. Cheng Y, Che X, Zhang S, Guo T, He X, Liu Y, et al. Positive cross-talk between CXC chemokine receptor 4 (CXCR4) and epidermal growth factor receptor (EGFR) promotes gastric cancer metastasis via the nuclear factor kappa B (NF-kB)-dependent pathway. Med Sci Monit 2020;26:e925019.
17. Liu Z, Zhao T, Li Z, Sun K, Fu Y, Cheng T, et al. Discovery of [1,2,3] triazolo[4,5-d] pyrimidine derivatives as highly potent, selective, and cellularly active USP28 inhibitors. Acta Pharm Sin B 2020;10:1476-91.
18. Yue CH, Chen CH, Lee WT, Su TF, Pan YR, Chen YP, et al. Cetyltrimethylammonium bromide disrupts the mesenchymal characteristics of HA22T/VGH cells via inactivation of c-Met/PI3K/Akt/mTOR pathway. Anticancer Res 2020;40:4513-22.

19. Bian C, Liu Z, Li D, Zhen L. PI3K/AKT inhibition induces compensatory activation of the MET/STAT3 pathway in non-small cell lung cancer. Oncol Lett 2018;15:9655-62.
20. Vultur A, Villanueva J, Krepler C, Rajan G, Chen Q, Xiao M, et al. MEK inhibition affects STAT3 signaling and invasion in human melanoma cell lines. Oncogene 2014;33:1850-61.
21. Zheng Y, Wang Z, Xiong X, Zhong Y, Zhang W, Dong Y, et al. Membrane-tethered Notch1 exhibits oncogenic property via activation of EGFR-PI3K-AKT pathway in oral squamous cell carcinoma. J Cell Physiol 2019;234:5940-52.
22. Chen Y, Long H, Wu Z, Jiang X, Ma L. EGF transregulates opioid receptors through EGFR-mediated GRK2 phosphorylation and activation. Mol Biol Cell 2008;19:2973-83.
23. Zhu AX, Stuart K, Blaszkowsky LS, Muzikansky A, Reitberg DP, Clark JW, et al. Phase 2 study of cetuximab in patients with advanced hepatocellular carcinoma. Cancer 2007;110:581-9.
24. Thomas MB, Chadha R, Glover K, Wang X, Morris J, Brown T, et al. Phase 2 study of erlotinib in patients with unresectable hepatocellular carcinoma. Cancer 2007;110:1059-67.
25. Philip PA, Mahoney MR, Allmer C, Thomas J, Pitot HC, Kim G, et al. Phase II study of Erlotinib (OSI-774) in patients with advanced hepatocellular cancer. J Clin Oncol 2005;23:6657-63.
26. Ramanathan RK, Belani CP, Singh DA, Tanaka M, Lenz HJ, Yen Y, et al. A phase II study of lapatinib in patients with advanced biliary tree and hepatocellular cancer. Cancer Chemother Pharmacol 2009;64:777-83.
27. Stommel JM, Kimmelman AC, Ying H, Nabioullin R, Ponugoti AH, Wiedemeyer R, et al. Coactivation of receptor tyrosine kinases affects the response of tumor cells to targeted therapies. Science 2007;318:287-90.
28. Narayan M, Wilken JA, Harris LN, Baron AT, Kimbler KD, Maihle NJ. Trastuzumab-induced HER reprogramming in "resistant" breast carcinoma cells. Cancer Res 2009;69:2191-4.
29. Engelman JA, Zejnullahu K, Mitsudomi T, Song Y, Hyland C, Park JO, et al. MET amplification leads to gefitinib resistance in lung cancer by activating ERBB3 signaling. Science 2007;316:1039-43.
30. Lu X, Yu L, Zhang Z, Ren X, Smaill JB, Ding K. Targeting EGFR L858R/T790M and EGFR L858R/T790M/C797S resistance mutations in NSCLC: Current developments in medicinal chemistry. Med Res Rev 2018;38:1550-81.
31. Jie Qi, Michele A, McTigue MA, Andrew R, Eugene L, James G, et al. Multiple mutations and bypass mechanisms can contribute to development of acquired resistance to MET inhibitors. Cancer Res 2011;71:1081-91.
32. Raquel-Cunha A, Cardoso-Carneiro D, Reis RM, Martinho O. Current Status of Raf Kinase Inhibitor Protein (RKIP) in Lung Cancer: Behind RTK Signaling. Cells 2019;8:442.
33. Giovannetti E, Labots M, Dekker H, Galvani E, Lind JS, Sciarrillo R, et al. Molecular mechanisms and modulation of key pathways underlying the synergistic interaction of sorafenib with erlotinib in non-small-cell-lung cancer (NSCLC) cells. Curr Pharm Des 2013;19:927-39.
34. Hao C, Wei S, Tong Z, Li S, Shi Y, Wang X, et al. The effects of RKIP gene expression on the biological characteristics of human triple-negative breast cancer cells *in vitro*. Tumour Biol 2012;33:1159-67.
35. Bernier I, Jollès P. Purification and characterization of a basic 23 kDa cytosolic protein from bovine brain. Biochim Biophys Acta 1984;790:174-81.
36. Yeung K, Seitz T, Li S, Janosch P, McFerran B, Kaiser C, et al. Suppression of Raf-1 kinase activity and MAP kinase signalling by RKIP. Nature 1999;401:173-7.
37. Yeung K, Janosch P, McFerran B, Rose DW, Mischak H, Sedivy JM, et al. Mechanism of suppression of the Raf/MEK/extracellular signal-regulated kinase pathway by the Raf kinase inhibitor protein. Mol Cell Biol 2000;20:3079-85.
38. Yesilkanal AE, Rosner MR. Raf kinase inhibitory protein (RKIP) as a metastasis suppressor: Regulation of signaling networks in cancer. Crit Rev Oncog 2014;19:447-54.
39. Walker EJ, Rosenberg SA, Wands JR, Kim M. Role of Raf kinase inhibitor protein in hepatocellular carcinoma. For Immunopathol Dis Therap 2011;2:195-204.
40. Nisimova L, Wen S, Cross-Knorr S, Rogers AB, Moss SF, Chatterjee D. Role of Raf kinase inhibitor protein in *Helicobacter pylori*-mediated signaling in gastric cancer. Crit Rev Oncog 2014;19:469-81.
41. Jia B, Liu H, Kong Q, Li B. RKIP expression associated with gastric cancer cell invasion and metastasis. Tumour Biol 2012;33:919-25.
42. Cross-Knorr S, Lu S, Perez K, Guevara S, Brilliant K, Pisano C, et al. RKIP phosphorylation and STAT3 activation is inhibited by oxaliplatin and camptothecin and are associated with poor prognosis in Stage II colon cancer patients. BMC Cancer 2013;13:463.
43. Al-Mulla F, Marafie M, Zea Tan T, Paul Thiery J. Raf kinase inhibitory protein role in the molecular subtyping of breast cancer. J Cell Biochem 2014;115:488-97.
44. Escara-Wilke J, Yeung K, Keller ET. Raf kinase inhibitor protein (RKIP) in cancer. Cancer Metastasis Rev 2012;31:615-20.
45. Fu Z, Smith PC, Zhang L, Rubin MA, Dunn RL, Yao Z, et al. Effects of raf kinase inhibitor protein expression on suppression of prostate cancer metastasis. J Natl Cancer Inst 2003;95:878-89.
46. Al-Mulla F, Hagan S, Behbehani AI, Bitar MS, George SS, Going JJ, et al. Raf kinase inhibitor protein expression in a survival analysis of colorectal cancer patients. J Clin Oncol 2006;24:5672-9.
47. Xu YF, Yi Y, Qiu SJ, Gao Q, Li YW, Dai CX, et al. PEBP1 downregulation is associated to poor prognosis in HCC related to hepatitis B infection. J Hepatol 2010;53:872-9.
48. Schuierer MM, Bataille F, Hagan S, Kolch W, Bosserhoff AK. Reduction in Raf kinase inhibitor protein expression is associated with increased Ras-extracellular signal-regulated kinase signaling in melanoma cell lines. Cancer Res 2004;64:5186-92.
49. Wang J, Yang YH, Wang AQ, Yao B, Xie G, Feng G, et al. Immunohistochemical detection of the Raf kinase inhibitor protein in nonneoplastic gastric tissue and gastric cancer tissue. Med Oncol 2010;27:219-23.
50. Kim HS, Kim GY, Lim SJ, Kim YW. Loss of Raf-1 kinase inhibitory protein in pancreatic ductal adenocarcinoma. Pathology 2010;42:655-60.
51. Kim HS, Kim GY, Lim SJ, Kim YW. Raf-1 kinase inhibitory protein expression in thyroid carcinomas. Endocr Pathol 2010;21:253-7.
52. Birner P, Jesch B, Schultheis A, Schoppmann SF. RAF-kinase inhibitor protein (RKIP) downregulation in esophageal cancer and its metastases. Clin Exp Metastasis 2012;29:551-9.
53. Zebisch A, Wölfler A, Fried I, Wolf O, Lind K, Bodner C, et al. Frequent loss of RAF kinase inhibitor protein expression in acute myeloid leukemia. Leukemia 2012;26:1842-9.
54. Kim JS, Choi GH, Jung Y, Kim KM, Jang SJ, Yu ES, et al. Downregulation of Raf-1 kinase inhibitory protein as a sorafenib resistance mechanism in hepatocellular carcinoma cell lines. J Cancer Res Clin Oncol 2018;144:1487-501.
55. Yang SF, Ma R, Pan LL, Cao J, Sheng N. RKIP and peroxiredoxin 2 expression predicts the proliferative potential of gastric cancer stem cells. Oncol Lett 2018;15:3173-7.
56. Bonavida B, Jazirehi A, Vega MI, Huerta-Yepez S, Baritaki S. Roles Each of Snail, Yin Yang 1 and RKIP in the Regulation of Tumor Cells Chemo-immuno-resistance to Apoptosis. Immunopathol Dis Therap 2013;4:10.1615.
57. Yuan L, Yi HM, Yi H, Qu JQ, Zhu JF, Li LN, et al. Reduced RKIP enhances nasopharyngeal carcinoma radioresistance by increasing ERK and AKT activity. Oncotarget 2016;7:11463-77.
58. Yeung KC, Rose DW, Dhillon AS, Yaros D, Gustafsson M, Chatterjee D, et al. Raf kinase inhibitor protein interacts with NF-kappaB-inducing kinase and TAK1 and inhibits NF-kappaB activation. Mol Cell Biol 2001;21:7207-17.
59. Tang H, Park S, Sun SC, Trumbly R, Ren G, Tsung E, et al. RKIP inhibits

NF-kappaB in cancer cells by regulating upstream signaling components of the IkappaB kinase complex. FEBS Lett 2010;584:662-8.
60. Yousuf S, Duan M, Moen EL, Cross-Knorr S, Brilliant K, Bonavida B, et al. Raf kinase inhibitor protein (RKIP) blocks signal transducer and activator of transcription 3 (STAT3) activation in breast and prostate cancer. PLoS One 2014;9:e92478.
61. Noh HS, Hah YS, Ha JH, Kang MY, Zada S, Rha SY, et al. Regulation of the epithelial to mesenchymal transition and metastasis by Raf kinase inhibitory protein-dependent Notch1 activity. Oncotarget 2016;7:4632-46.
62. Lorenz K, Schmid E, Deiss K. RKIP: A governor of intracellular signaling. Crit Rev Oncog 2014;19:489-96.
63. Fu X, Koller S, Abd Alla J, Quitterer U. Inhibition of G-protein-coupled receptor kinase 2 (GRK2) triggers the growth-promoting mitogen-activated protein kinase (MAPK) pathway. J Biol Chem 2013;288:7738-55.
64. Al-Mulla F, Bitar MS, Al-Maghrebi M, Behbehani AI, Al-Ali W, Rath O, et al. Raf kinase inhibitor protein RKIP enhances signaling by glycogen synthase kinase-3β. Cancer Res 2011;71:1334-43.
65. Baritaki S, Huerta-Yepez S, Sahakyan A, Karagiannides I, Bakirtzi K, Jazirehi A, et al. Mechanisms of nitric oxide-mediated inhibition of EMT in cancer: Inhibition of the metastasis-inducer Snail and induction of the metastasis-suppressor RKIP. Cell Cycle 2010;9:4931-40.
66. Pires BR, Mencalha AL, Ferreira GM, de Souza WF, Morgado-Díaz JA, Maia AM, et al. NF-kappaB is involved in the regulation of EMT genes in breast cancer cells. PLoS One 2017;12:e0169622.
67. Wottrich S, Kaufhold S, Chrysos E, Zoras O, Baritaki S, Bonavida B. Inverse correlation between the metastasis suppressor RKIP and the metastasis inducer YY1: Contrasting roles in the regulation of chemo/immuno-resistance in cancer. Drug Resist Updat 2017;30:28-38.
68. Bonavida B. RKIP-mediated chemo-immunosensitization of resistant cancer cells via disruption of the NF-κB/Snail/YY1/RKIP resistance-driver loop. Crit Rev Oncog 2014;19:431-45.
69. Datar I, Feng J, Qiu X, Lewandowski J, Yeung M, Ren G, et al. RKIP Inhibits Local Breast Cancer Invasion by Antagonizing the Transcriptional Activation of MMP13. PLoS One 2015;10:e0134494.
70. Riemann A, Schneider B, Ihling A, Nowak M, Sauvant C, Thews O, et al. Acidic environment leads to ROS-induced MAPK signaling in cancer cells. PLoS One 2011;6:e22445.
71. Wu WS, Wu JR, Hu CT. Signal cross talks for sustained MAPK activation and cell migration: The potential role of reactive oxygen species. Cancer Metastasis Rev 2008;27:303-14.
72. Wu JR, Hu CT, You RI, Pan SM, Cheng CC, Lee MC, et al. Hydrogen peroxide inducible clone-5 mediates reactive oxygen species signaling for hepatocellular carcinoma progression. Oncotarget 2015;6:32526-44.
73. Holmström KM, Finkel T. Cellular mechanisms and physiological consequences of redox-dependent signalling. Nat Rev Mol Cell Biol 2014;15:411-21.
74. Jiang J, Wang K, Chen Y, Chen H, Nice EC, Huang C. Redox regulation in tumor cell epithelial-mesenchymal transition: Molecular basis and therapeutic strategy. Signal Transduct Target Ther 2017;2:17036.
75. Zhu P, Tan MJ, Huang RL, Tan CK, Chong HC, Pal M, et al. Angiopoietin-like 4 protein elevates the prosurvival intracellular O2(-):H2O2 ratio and confers anoikis resistance to tumors. Cancer Cell 2011;19:401-15.
76. Kwon J, Lee SR, Yang KS, Ahn Y, Kim YJ, Stadtman ER, et al. Reversible oxidation and inactivation of the tumor suppressor PTEN in cells stimulated with peptide growth factors. Proc Natl Acad Sci U S A 2004;101:16419-24.
77. Nadeau PJ, Charette SJ, Toledano MB, Landry J. Disulfide bond-mediated multimerization of Ask1 and its reduction by thioredoxin-1 regulate H (2) O (2)-induced c-Jun NH (2)-terminal kinase activation and apoptosis. Mol Biol Cell 2007;18:3903-13.
78. Lin CY, Hu CT, Cheng CC, Lee MC, Pan SM, Lin TY, et al. Oxidation of heat shock protein 60 and protein disulfide isomerase activates ERK and migration of human hepatocellular carcinoma HepG2. Oncotarget 2016;7:11067-82.
79. Lee KH, Kim JR. Reactive oxygen species regulate the generation of urokinase plasminogen activator in human hepatoma cells via MAPK pathways after treatment with hepatocyte growth factor. Exp Mol Med 2009;41:180-8.
80. Flinder LI, Timofeeva OA, Rosseland CM, Wierød L, Huitfeldt HS, Skarpen E. EGF-induced ERK-activation downstream of FAK requires rac1-NADPH oxidase. J Cell Physiol 2011;226:2267-78.
81. Cho KH, Choi MJ, Jeong KJ, Kim JJ, Hwang MH, Shin SC, et al. A ROS/STAT3/HIF-1α signaling cascade mediates EGF-induced TWIST1 expression and prostate cancer cell invasion. Prostate 2014;74:528-36.
82. Frijhoff J, Dagnell M, Augsten M, Beltrami E, Giorgio M, Östman A. The mitochondrial reactive oxygen species regulator p66Shc controls PDGF-induced signaling and migration through protein tyrosine phosphatase oxidation. Free Radic Biol Med 2014;68:268-77.
83. Damiano S, Fusco R, Morano A, De Mizio M, Paternò R, De Rosa A, et al. Reactive oxygen species regulate the levels of dual oxidase (Duo×1-2) in human neuroblastoma cells. PLoS One 2012;7:e34405.
84. Krstic J, Trivanovic D, Mojsilovic S, Santibanez JF. Transforming growth factor-beta and oxidative stress interplay: Implications in tumorigenesis and cancer progression. Oxid Med Cell Longev 2015;2015:654594.
85. Cruz-Bermúdez A, Laza-Briviesca R, Vicente-Blanco RJ, García-Grande A, Coronado MJ, Laine-Menéndez S, et al. Cancer-associated fibroblasts modify lung cancer metabolism involving ROS and TGF-β signaling. Free Radic Biol Med 2019;130:163-73.
86. Hiraga R, Kato M, Miyagawa S, Kamata T. No×4-derived ROS signaling contributes to TGF-β-induced epithelial-mesenchymal transition in pancreatic cancer cells. Anticancer Res 2013;33:4431-8.
87. Hu CT, Wu JR, Cheng CC, Wang S, Wang HT, Lee MC, et al. Reactive oxygen species-mediated PKC and integrin signaling promotes tumor progression of human hepatoma HepG2. Clin Exp Metastasis 2011;28:851-63.
88. Svineng G, Ravuri C, Rikardsen O, Huseby NE, Winberg JO. The role of reactive oxygen species in integrin and matrix metalloproteinase expression and function. Connect Tissue Res 2008;49:197-202.
89. Wu WS. The signaling mechanism of ROS in tumor progression. Cancer Metastasis Rev 2006;25:695-705.
90. Paoli P, Giannoni E, Chiarugi P. Anoikis molecular pathways and its role in cancer progression. Biochim Biophys Acta 2013;1833:3481-98.
91. Lin X, Wei J, Nie J, Bai F, Zhu X, Zhuo L, et al. Inhibition of RKIP aggravates thioacetamide-induced acute liver failure in mice. Exp Ther Med 2018;16:2992-8.
92. Zhou X, Zang X, Guan Y, Tolbert T, Zhao TC, Bayliss G, et al. Targeting enhancer of zeste homolog 2 protects against acute kidney injury. Cell Death Dis 2018;9:1067.
93. Brown CO, Salem K, Wagner BA, Bera S, Singh N, Tiwari A, et al. Interleukin-6 counteracts therapy-induced cellular oxidative stress in multiple myeloma by up-regulating manganese superoxide dismutase. Biochem J 2012;444:515-27.
94. Banerjee Mustafi S, Chakraborty PK, Dey RS, Raha S. Heat stress upregulates chaperone heat shock protein 70 and antioxidant manganese superoxide dismutase through reactive oxygen species (ROS), p38MAPK, and Akt. Cell Stress Chaperones 2009;14:579-89.
95. Noriyuki H, Marimu S, Yoshihiko T, Tadashi K. Approach to spot overlapping problem in 2D-PAGErevealed clinical and functional significance of RKIP and MnSOD in renal cell carcinoma. EuPA Open Proteomics 2014;4:129-39.
96. Zaravinos A, Bonavida B, Chatzaki E, Baritaki S. RKIP: A key regulator in tumor metastasis initiation and resistance to apoptosis: Therapeutic targeting and impact. Cancers (Basel) 2018;10:287.
97. Mandal JP, Shiue CN, Chen YC, Lee MC, Yang HH, Chang HH, et al. PKCδ mediates mitochondrial ROS generation and oxidation of HSP60 to relieve RKIP inhibition on MAPK pathway for HCC progression. Free Radic Biol Med 2021;163:69-87.