RAF-MEK-MAPK Pathway Targeted by Tumor Suppression and Anticancer Therapeutic Agents

Zhang X*, Zhou J, Li T, Zheng B*, Huang Z**

1Department of Pathophysiology, School of Basic Medical Science, and Chinese American Collaborative Cancer Institute, Provincial Key Laboratory of Medical Molecular Diagnostics, People’s Republic of China
2Department of Microbiology, School of Laboratory Medicine, Guangdong Medical University, Dongguan, Guangdong, People’s Republic of China
3Department of Pathophysiology, School of Basic Medical Science, and Chinese American Collaborative Cancer Institute, Provincial Key Laboratory of Medical Molecular Diagnostics, People’s Republic of China

Abstract

RAS-RAF-MEK-MAPK pathway comprises a group of kinases which regulates the activities of effector proteins in growth, proliferation and apoptosis. The extracellular signals from growth factors, cytokines and other stimuli transmitted by surface receptors and upstream signaling molecules are integrated by this cascade of kinases whose activity is regulated by the interaction of oncoproteins and tumor suppressors. The anomaly of the signaling pathway would lead to occurrence of malignancies. The RAS-RAF-MEK-MAPK pathway is therefore targeted by anticancer therapeutic agents. The present paper discussed the interaction of individual component with tumor suppressors and the impact of their inhibitor on the efficacy of anticancer therapy, and improvement on small molecule inhibitor of RAS-RAF-MEK-MAPK pathway with modified targeting has been proposed.

Keywords: Oncogene; RAS; RAF-MEK-MAPK pathway; Tumor suppression; Tyrosine Kinase Inhibitor (TKI); Anti-cancer therapy

Introduction

Kinas, notably those regulated the action of RAF-MEK-MAPK and AKT-mTOR-P13K pathways have attracted increasing attention to identify anticancer therapeutic targets, in view of their activity in integration of signals of growth, proliferation, angiogenesis and apoptosis. Oncoprotein RAS activates the RAF/MEK/ERK (Extracellular Signal-Regulated Kinases) pathways, involving proteins of MAPK family as end effector and pathways of PI3K (Phosphatidylinositol 3-Kinase)/Akt/NF-κappaB (Nuclear Factor-Kappa B) pathway, p120-GAP/p190-B/Rac/NF-κappa-B, and Raf/MEKK1/IKK (I-Kappa-B Kinase)/I-Kappa-B/NIKappa-B pathway activate transcription factor NF kappa-B through signaling molecules PI3K and Akt [1]. RAS proteins are encoded by members of oncogene RAS family with H-, Ki-and N-RAS, whose mutational activation has been seen more than 50% of human cancer cases [2], leading to cancerous cell growth. Inhibition of the kinases has demonstrated efficacy in therapy against cancers, and is also targeted by tumor suppressor genes (TSGs) when exerting their anti-oncogenic activities, through downregulation of cell cycle entry and angiogenesis, and potentiation of apoptosis. Mitogen-activated protein kinase (MAPK) is a serine/threonine kinase, which activates transcription factors and other cytoplasmic factors leading to mitogenesis [3]. The modulation of RAF-MEK-MAPK pathway in the context of oncogenes-TSGs interaction, and of intervention of anticancer drugs is to be discussed in the present paper.

The Implications of RAF-MEK-MAPK Pathway in Promotion of Cell Growth and Proliferation

The family of RAS gene comprises of a group of oncogenes that are frequently mutated in human tumors like pancreas, lung, and colorectal cancers and neuroblastoma. The prominent members of the family include N-Ras (neuroblastoma cell line), H-Ras (Harvey murine sarcoma virus), and the alternatively spliced K-Ras (Kirsten murine sarcoma virus). Among these, K-Ras is most frequently constitutively activated in human cancers [2]. The genes of this family code for RAS proteins, which reversely binds guanine nucleotides of GDP and GTP. The metabolic forms of guanine phosphate correspond with functional statuses of RAS, a small molecule G protein. RAS is activated when recruiting adapter proteins such as Grb2 that in turn engages guanine nucleotide exchange factors (GEFs) like SOS to the cell membrane, GDP bound to RAS is replaced by GTP transferred by factors SOS [4,5].

RAS is normally activated in response to the binding of extracellular signals, such as growth factors, RTKs (Receptor Tyrosine Kinases), TCR (T-Cell Receptors) and PMA (Phorbol-12 Myristate-13 Acetate). The GTP associated Ras triggers the activation of a sequential three-kinase phosphorylation cascade through RAF, MEK, and ERK. RAF-MEK-ERK is essential for the regulation of cellular proliferation and survival [6]; the pathway integrates a wide range of signals into major cellular programs such as proliferation, differentiation, or apoptosis. And half of all human malignancies display aberrations in the RAS-RAF-MEK-ERK pathway.

RAF is a downstream effector kinase of RAS, and is found in three isoforms: A, B and C-RAF (also called RAF-1 or C-RAF-1). Many studies showed the role of RAF kinase as a potential cellular oncogene for cancer therapy [7]. RAF-1 is a 74 kDa mitochondrial protein, ubiquitously expressed in adult tissues, with highest expression in muscle, cerebellum, and fetal brain. It was the first RAF isoform identified. The agents targeting the RAF family as a whole or C-RAF extensively examined in many pre-clinical studies and more recently some of them are in clinical trials [8-11]. B-RAF is a 94 kDa mitochondrial protein identified as second RAF isoform which acts as mutational target in various human cancers.

*Corresponding author: Dr. Xiangqing Zhang, M.D., Ph.D.; Zhiwei He, M.D., Ph.D.; Zunnan Huang, Ph.D., Department of Pathophysiology, Guangdong Medical University, 1 Xincheng Avenue, Songshan Lake Scientific and Industrial Park, Dongguan, Guangdong 523808, People’s Republic of China, Tel: 00867692886405, Fax: 008676922896100; E-mail: zhangx_2006@126.com

Received April 18, 2017; Accepted May 09, 2017; Published May 12, 2017

Citation: Zhang X, Zhou J, Li T, Zheng B, He Z, et al. (2017) RAF-MEK-MAPK Pathway Targeted by Tumor Suppression and Anticancer Therapeutic Agents. J Mol Genet Med 11: 264 doi:10.4172/1747-0862.1000264

Copyright: © 2017 Zhang X, et al. This is an open-access article distributed under the terms of the Creative Commons Attribution License, which permits unrestricted use, distribution, and reproduction in any medium, provided the original author and source are credited.
It is the strongest RAF kinase in terms of induction of MEK activity. A-RAF isoform is the weakest activator of MEK, and can only activate MEK1 but not MEK2. At present, no mutations in A-RAF have been found in human cancers [13,14].

MEK activates MAPK, the members of the family include extracellular signal regulated kinase (ERK) or MAPK, p38 MAPK, and JNK [15]. While different MAPK family members involve the same pathway with similar components with similar activities, the downstream effects notably regulators of cell cycle are not completely overlapped between different molecules.

In the field of anticancer drug, success in screening drugs targeting to the upstream factor RAS have been limited [16]. RAF and MEK, however, are important intermediates in the MAPK pathway [17]. researchers invested efforts to screen inhibitors of RAF and MEK as agents in anticancer therapy.

The Regulation of RAF-MEK-MAPK Involving Interactions between Oncogenes and Tumor Suppressor Genes

The pathway is activated by oncoproteins, and targeted by tumor suppressors while exerting their anticancer potential, and in some context, the activity involves interactions between TSGs and oncogenes. TSGs are implicated in the genesis of malignancies in case of inactivation, through loss of heterozygosity (LOH) mutation, or epigenetic inactivation, in a manner of loss-of-function. As a result, the transformed cells are no longer harnessed in growth and proliferation by the regulation of cell cycle progression [18,19]. In fact, cell cycle progression, and hence cell proliferation is regulated by cyclin dependent kinases (CDKs) in complexed with cyclins transcriptionally activated mostly by RAS-RAF-MEK-MAPK pathway [20]. Tumor suppressors have been shown to downregulate the signaling axis of MAPK-cyclin, for example, JNK-cyclin D1, and some through interactions with RAS proteins [21-23].

FHIT is tumor suppressor mapped on 3p14, a frequently lost chromosomal region in human cancers; its inactivation is an early event in development of the cancer [24-26]. Data obtained from lung cancer lines have not just indicate the importance of FHIT in carcinogenesis but also its potential to serve as an early biomarker for lung cancer [27]. A new role of FHIT in down-regulating the Ras/Rho GTPase-associated oncogenic signaling pathway has been suggested [28].

We have reported that BLU, a TSG mapped on the same chromosomal region as RASSF1, i.e. 3p21 which is frequently lost in nasopharyngeal carcinoma (NPC) and a variety of human tumors, mainly of epithelial origin, suppressed the signaling of JNK pathway, and reduced the level of cyclin D1 to arrest cell cycle at G1 phase when it is re-expressed in negative NPC cells [29]. Structurally, BLU protein contains a zinc finger MYND domain on its amino-terminus. The molecular mechanisms underlying its downregulation of JNK-cyclin D1 axis remain to be elucidated.

Transcription factor AP1 is formed by, heteromerization of c-FOS and c-JUN, whose phosphorylation is catalyzed by JNK and another protein c-FOS. AP1 binds to the genomic DNA sequences upstream to the coding portion of a number of genes coding for cell cycle regulator, notably CCND1 coding for cyclin D1. Known as product of proto-oncogene, cyclin D1 promotes proliferation in malignancy through interaction with oncogenic molecules [30]. It has been reported that over-expressed cyclin D1 facilitates the infection of nasopharyngeal epithelial cells by a lymphotropic human herpesvirus, Epstein-Barr virus (EBV), a ubiquitous human virus that is tightly associated with the occurrence of NPC and Burkitt lymphoma (BL) [31]. It has been proposed that MAPK pathway and cyclin D1 forms a signaling axis to regulate cell proliferation, and amplification of the chromosomal region that harbors CCND1 is a frequent abnormality at cytogenetic level during the occurrence of human tumors [32,33].

We reported that re-expression of BLU downregulated JNK signaling through reducing phosphorylation on JNK and inhibiting formation of AP1. It is reasoned that the effect was due to the inhibition of upstream kinase(s) by reducing their levels via epigenetic mechanism. In fact, we have shown that BLU inhibited the expression of IKK alpha, reduced the level of NFkappaB and hence NFkappaB dependent anti-apoptotic factors, so as to promote death receptor induced apoptosis [34]. It is speculated that BLU binds HDACs or SIRT, to repress transcription of genes coding for kinases in the pathway of RAF-MEK1-2/MAPK to downregulate the pathway and exert tumor suppression.

Previous study has shown that lymphoid-specific helicase (LSH), a SNF2-SWI chromatin remodeler, plays an essential role in cancer progression via regulation of fumarate hydratase (FHI) [35]. Mechanistically, together with histone methyltransferase G9a, LSH is critical for the normal development of mammals and is involved in the establishment and maintenance of DNA methylation [36]. Since apart from depositing of H3K9me2 [37], G9a and its partner modifier GLP also interact with DNA methyltransferases (DNMTs) and protect proper DNA methylation at certain loci [38], LSH might also directly interact with DNMTs and affect the patterns of DNA methylation in the cells. Therefore, in human NPC cells, investigation of chromatin loading of LSH and the patterns of DNA methylation at the BLU locus might shed light on the mechanisms that required for the regulation of BLU and the progression of these malignant carcinomas.

The tumor suppression of a family of proteins RASSF is exerted by downregulation of RAS-RAF-MEK1-2/ERK pathway. RASSF1A, the founding member of the RASSF family and RASSF5/NORE inhibits tumor growth and proliferation by targeting to signaling molecules of the MAPK family [23,35]. Phosphorylated ERK (pERK) is a key downstream component of the Ras/Raf/MEK/ERK signaling pathway. After phosphorylation, it translocates to the nucleus, and regulates various transcription factors such as Ets family transcription factors (Elk-1) [39].

The Implications of RAF-MEK-MAPK on Malignant Transformation of Cells

The aberrant activation of the RAF/MEK/MAPK signaling pathway is correlated with the occurrence of hepatocellular carcinoma (HCC) and a variety of human cancers. The activity of the RAF/MAPK signaling pathway was significantly higher, and the activity of ERK1/2 and MEK1 were upregulated threefold to fourfold in neoplastic liver specimens when compared to normal liver tissue adjacent to the HCC lesions [40,41]. Furthermore, it has been reported that the over-expression of RAF-1 could be regarded as an indicator of HCC prognosis [42]. These data suggest that the RAF/MEK/MAPK pathway may serve as an attractive target in the therapy of HCC.

The Applications of Components of RAF-MEK-MAPK Pathway in Anticancer Therapy

Epidermal growth factor receptor (EGFR) signaling is triggered by the binding of its ligand, resulting in the dimerization of EGFR molecules or heterodimerization with other closely related receptors, such as HER2/neu. EGFR is overexpressed in 40-80% of non–small
Table 1: Small molecules generated for targeting the components of the RAS-RAF-MEK-MAPK signaling pathway.

| Targeting component | Small molecule inhibitor | References |
|---------------------|--------------------------|------------|
| EGFR Tyrosine Kinase | Genistein; AG 1478; gefitinib, erlotinib; afatinib, icotinib | [62,63] |
| Mutant BRAF         | PLX4032; dabrafenib, sorafenib | [64,65] |
| MEK1/2              | PD 98059; Pimasertib U0126, Selumetinib (AZD6244, ARRY-142886) cobalt(l) complex 2 | [55,56,66-69] |
| P38 MAPK            | SB202190, SB203580 | [70,71] |
| JNK                 | SP600125 | [72] |
| ERK                 | PD98059 | [73] |

In conclusion, small molecule inhibitors to RAS-RAF-MEK-MAPK pathway have been validated as effective in therapy against a variety of cancers, and have gained wide application (Table 1). To circumvent the problems like acquired resistance, agents with multiple targeting are to be developed, and modality with combination of agents should be considered based on cancer related gene profile of the individual patients.

**Acknowledgments**

Our work is supported by Medical Science Research Fund, Guangdong Provincial Commission of Health and Family Plan (2014A287).

**References**

1. Wellbrock C, Karasarides M, Marais R (2004) The Raf proteins take centre stage. Nat Rev Mol Cell Biol 5: 875-885.
2. Matallanas D, Crespo P (2010) New druggable targets in the Ras pathway? Curr Opin Mol Ther 12: 674–683.
Zhang X, Zhou J, Li T, Zheng B, He Z, et al. (2017) RAF-MEK-MAPK Pathway Targeted by Tumor Suppression and Anticancer Therapeutic Agents. J Mol Genet Med 11: 264 doi:10.4172/1747-0862.1000264

3. Gay B, Suarez S, Caravatti P, Furet P, Meyer T, et al. (1999) Selective GRB2 SH2 inhibitors as anti-Ras therapy. Int J Cancer 83: 235-241.

4. Agathanggelou A, Cooper WN, Latif F (2005) Role of the Ras-association domain family 1 tumor suppressor gene in human cancers. Cancer Res 65: 3497–3508.

5. Rowinsky ER, Windle JJ, Von Hoff DD (1999) Ras protein farnesyltransferase: A strategic target for anticancer therapeutic development. J Clin Oncol 17: 3633-3652.

6. Arozarena I, Calvo F, Crespo P (2011) Ras, an actor on many stages: Posttranslational modifications, localization, and site-specific events. Genes Cancer 2: 182–194.

7. Hagemann C, Rapp UR (1999) Isotype-specific functions of Raf kinases. Exp Cell Res 253: 34-46.

8. Schreck R, Rapp UR (2011) Raf kinases: oncogenesis and drug discovery. Int J Cancer 119: 2261-2271.

9. Sridhar SS, Hedley D, Siu LL (2005) Raf kinase as a target for anticancer therapeutics. Mol Cancer Ther 4: 677-685.

10. Strumberg D, Seeber S (2005) Raf kinase inhibitors in oncology. Onkologie 28: 101-107.

11. Thompson N, Lyons J (2005) Recent progress in targeting the Raf/MEK/ERK pathway with inhibitors in cancer drug discovery. Curr Opin Pharmacol 5: 350-356.

12. Garnett MJ, Marais R (2004) Guilty as charged: B-RAF is a human oncogene. Cancer Cell 6: 313-319.

13. Nantel A, Huber M, Thomas DY (1999) Localization of endogenous Grb10 to the mitochondria and its interaction with the mitochondrial-associated Raf-1 pool. J Biol Chem 274: 35719-35724.

14. Yuryev A, Ono M, Goff SA, Macaluso F, Wennogle LP (2000) Isoform specific localization of A-RAF in mitochondria. Mol Cell Biol 20: 4870-4878.

15. Schaeffer HJ, Weber MJ (1999) Mitogen-activated protein kinases: Specific messages from ubiquitous messengers. Mol Cell Biol 19: 2435–2444.

16. Wong KK (2009) Recent developments in anti-cancer agents targeting the Ras/Raf/MEK/ERK pathway. Recent Pat Anticancer Drug Discov 4: 28–35.

17. Sebolt-Leopold JS, Herrera R (2004) Targeting the mitogen-activated protein kinase cascade to treat cancer. Nat Rev Cancer 4: 937–947.

18. Herman JG, Baylin SB (2000) Gene silencing in cancer in association with promoter hypermethylation. N Engl J Med 349: 2042-2054.

19. Baylin SB, Chen WY (2005) Aberrant gene silencing in tumor progression: implications for control of cancer. Cold Spring Harb Symp Quant Biol 70: 427-433.

20. Albanese C, Johnson J, Watanabe G, Eklund N, Vu D (1995) Transforming p21ras mutants and e-Fos-2 activate the c-jun D1 promoter through distinguishable regions. J Biol Chem 270: 23389-23397.

21. Whang YM, Kim YH, Kim JS, Yoo YD (2005) RASSF1A suppresses the c-Jun-NH2-kinase pathway and inhibits cell cycle progression. Cancer Res 65: 3682–3690.

22. Yoo YA, Na AR, Lee MS, Yoon S, Kim JS (2006) RASSF1A suppresses histidine triad-mediated tumor suppression of lung cancer by targeting multiple components of the Ras/Rho GTPase molecular switch. Cancer Res 67: 10379-10388.

23. Zhang X, Liu H, Li B, Huang P, Shao J, et al. (2012) Tumor suppressor BLU inhibits proliferation of nasopharyngeal carcinoma cells by regulation of cell cycle, c-Jun N-terminal kinase and the cyclin D1 promoter. BMC Cancer 12: 267.

24. Yu Z, Wang C, Wang M, Li Z, Casimiro MC, et al. (2008) A cyclin D1/microRNA 17/20 regulatory feedback loop in control of breast cancer cell proliferation. J Cell Biol 182: 509-517.

25. Tsang CM, Yip YL, Lo KW, Deng W, To KF, et al. (2012) Cyclin D1 overexpression supports stable EBV infection in nasopharyngeal epithelial cells. Proc Natl Acad Sci USA 109: E3473-E3482.

26. Liu Y, Hock JM, Sullivan C, Fang G, Cox AJ, et al. (2010) Activation of the growth and tumour suppressor NORE1A is a required for protein that suppresses growth of CDC6 and cyclin D1 in low-dose arsenite-induced cell proliferation. J Cell Biochem 111: 1546-1555.

27. Qin L, Yang YB, Yang YX, Gong YZ, Li XL (2014) Inhibition of smooth muscle cell proliferation by ezetimibe via the cyclin D1-MAPK pathway. J Pharmacol Sci 125: 283-291.

28. Zhou J, Huang Z, Wang Z, Liu S, Grandien A (2016) Tumor suppressor BLU promotes TRAIL-induced apoptosis by downregulating NF-κB signaling in nasopharyngeal carcinoma. Oncotarget 14125.

29. He X, Yan B, Liu S, Jia J, Lai W, et al. (2016) Chromatin Remodeling Factor LSH Drives Cancer Progression by Suppressing the Activity of Fumarate Hydratase. Cancer Res 76: 5743-5755.

30. Myant K, Termanis A, Sundram AY, Boe T, Li C (2011) LSH and G9a/GLP complex are required for developmentally programmed DNA methylation. Genome Res 21: 83-94.

31. Shinkai Y, Tachibana M (2011) H3K9 methylation transferase G9a and the related molecule GLP. Genes Dev 25: 781-788.

32. Zhang T, Termanis A, Özkan B, Bao XX, Culley J (2016) G9a/GLP Complex Maintains Imprinted DNA Methylation in Embryonic Stem Cells. Cell Rep 15: 77-85.

33. Moshnikova A, Frye J, Shay JW, Minna JD, Khokhlatchev AV (2006) The growth- and tumour suppressor NORE1A is a required for protein that suppresses growth by inhibition of the ERK pathway. J Biol Chem 281: 8143-8152.

34. Roberts PJ, De CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 26: 3291.

35. McKillop IH, Schmidt CM, Cahill PA, Sitzmann JV (1997) Altered expression of the Ras/Raf/MEK/ERK pathway in adenocarcinoma of the breast: implications for control of cancer. J Thorac Oncol 3: 179-185.

36. Myant K, Termanis A, Sundram AY, Boe T, Li C (2011) LSH and G9a/GLP complex are required for developmentally programmed DNA methylation. Genome Res 21: 83-94.

37. Shinkai Y, Tachibana M (2011) H3K9 methylation transferase G9a and the related molecule GLP. Genes Dev 25: 781-788.

38. Zhang T, Termanis A, Özkan B, Bao XX, Culley J (2016) G9a/GLP Complex Maintains Imprinted DNA Methylation in Embryonic Stem Cells. Cell Rep 15: 77-85.

39. Moshnikova A, Frye J, Shay JW, Minna JD, Khokhlatchev AV (2006) The growth- and tumour suppressor NORE1A is a required for protein that suppresses growth by inhibition of the ERK pathway. J Biol Chem 281: 8143-8152.

40. Roberts PJ, De CJ (2007) Targeting the Raf-MEK-ERK mitogen-activated protein kinase cascade for the treatment of cancer. Oncogene 26: 3291.

41. McKillop IH, Schmidt CM, Cahill PA, Sitzmann JV (1997) Altered expression of the Ras/Raf/MEK/ERK pathway in adenocarcinoma of the breast: implications for control of cancer. J Thorac Oncol 3: 179-185.

42. Myant K, Termanis A, Sundram AY, Boe T, Li C (2011) LSH and G9a/GLP complex are required for developmentally programmed DNA methylation. Genome Res 21: 83-94.

43. Shinkai Y, Tachibana M (2011) H3K9 methylation transferase G9a and the related molecule GLP. Genes Dev 25: 781-788.
A novel epidermal growth factor receptor inhibitor promotes apoptosis in non-small cell lung cancer cells resistant to erlotinib. Cancer Res 67: 6253–6262.

50. Engelman JA, Zejnullahu K, Gale CM, Lifshitz E, Gonzales AJ, et al. (2007) PF00299804, an irreversible pan-ERBB inhibitor, is effective in lung cancer models with EGFR and ERBB2 mutations that are resistant to gefitinib. Cancer Res 67: 11924–11932.

51. Li D, Shimamura T, Ji H, Chen L, Hariingsma HJ, et al. (2007) Bronchial and peripheral murine lung carcinomas induced by T790M-L858R mutant EGFR respond to HKi-272 and rapamycin combination therapy. Cancer Cell 12: 81–93.

52. Mc Cubrey JA, Steelman LS, Chappell WL, Abrams SL, Wong EWT, et al. (2007) Role of the Raf/MEK/ERK pathway in cell growth, malignant transformation and drug resistance. Biochim Biophys Acta 1773: 1263-1284.

53. Ripple MO, Kim N, Springett RJ (2013) Acute mitochondrial inhibition by mitogen-activated protein kinase/extracellular signal-regulated kinase kinase (MEK) 1/2 inhibitors regulates proliferation. J Biol Chem 288: 2933-2940.

54. Gandhi J, Zhang J, Xie Y, Sch J, Shigematsu H, et al. (2009) Alterations in genes of the EGFR signaling pathway and their relationship to EGFR tyrosine kinase inhibitor sensitivity in lung cancer cell lines. PLoS One 4(2): e4576.

55. Takada Y, Ichikawa1 H, Patara A, Swisher S, Aggarwal BB (2007) Genetic deletion of PKR abrogates TNF-induced activation of ikappaBa kinase, JNK, Akt and cell proliferation but potentiates p44/p42 MAPK and p38 MAPK activation. Oncogene 26: 1201–1212.

56. Singer G, Oldt R, Cohen Y, Wang BG, Sidransky D, et al. (2003) Mutations in BRAF and KRAS characterize the development of low-grade ovarian serous carcinoma. J Natl Cancer Inst 95: 484–486.

57. Adji AA, Cohen RB, Franklin W, Morris C, Wilson D, et al. (2008) Phase I pharmaco-kinetic and pharmacodynamic study of the oral, small-molecule mitogen-activated protein kinase kinase 1/2 inhibitor AZD6244 (ARRY-142886) in patients with advanced cancers. J Clin Oncol 26: 2139–2146.

58. Meng J, Peng H, Dai B, Guo W, Wang L, et al. (2009) High level of AKT activity is associated with resistance to MEK inhibitor AZD6244 (ARRY-142886). Cancer Biol Ther 8: 2071–2078.

59. Engelman JA, Chen L, Tan X, Crosby K, Guimaraes AR, et al. (2009) Effective use of PI3K and MEK inhibitors to treat mutant Kras G12D and PIK3CA H1047R murine lung cancers. Nat Med 15: 1351–1356.

60. Pratilas CA, Hanrahan AJ, Hallivoe E, Persaud Y, Soh J, et al. (2008) Genetic predictors of MEK dependence in non-small cell lung cancer. Cancer Res 68: 9375–9383.

61. Wee S, Jagani Z, Xiang KX, Loo A, Dorsch M, et al. (2009) PI3K pathway activation mediates resistance to MEK inhibitors in KRAS mutant cancers. Cancer Res 69: 4286–4293.

62. Yoon YK, Kim HP, Han SW, Oh DY, Im SA, et al. (2010) KRAS Mutant Lung Cancer Cells Are Differentially Responsive to MEK Inhibitor Due to AKT or STAT3 Activation: Implication for Combinatorial Approach. Molec Carcinog 49: 353–362.

63. Li D, Ambrogio L, Shimamura T, Kubo S, Takahashi M, et al. (2008) BIBW2992, an irreversible EGFR/HER2 inhibitor highly effective in preclinical lung cancer models. Oncogene 27: 4702–4711.

64. Shi Y, Zhang L, Liu X, Zhou C, Zhang L, et al. (2013) Icotinib versus gefitinib in previously treated advanced non-small-cell lung cancer (ICOGEN): a randomised, double-blind phase 3 non-inferiority trial. Lancet Oncol 14: 953–961.

65. Hauschild A, Grob JJ, Demidov LV, Jouary T, Gutzmer R, et al. (2012) Dabrafenib in BRAF-mutated metastatic melanoma: a multicentre, open-label, phase 3 randomised controlled trial. Lancet 380: 358–365.

66. Long GV, Tretzer U, Davies MA, Kefford RF, Ascierto PA, et al. (2012) Dabrafenib in patients with V600E or V600K BRAF-mutant melanoma metastatic to the brain (BREAK-3): A multicentre, open-label, phase 3 trial. Lancet Oncol 13: 1087–1095.

67. Mohr S, Mocorniss TS, Latapina EG (1998) Macrophages resistant to endogenously generated nitric oxide-mediated apoptosis are hypersensitive to exogenously added nitric oxide donors: Dichotomous apoptotic response independent of caspase 3 and reversal by the mitogen-activated protein kinase (MEK) inhibitor PD98059. Proc Natl Acad Sci USA 95: 5045–5050.

68. Morgillo F, Cascone T, D’Auto E, Martinelli E, Troiani T, et al. (2011) Antitumour efficacy of MEK inhibitors in human lung cancer cells and their derivatives with acquired resistance to different tyrosine kinase inhibitors. Br J Cancer 105: 382–392.

69. Favata MF, Horiiuchi KY, Manes EJ, Daulerio AJ, Stradley DA, et al. (1998) Identification of a novel inhibitor of mitogen-activated protein kinase protein kinase. J Biol Chem 273: 18623-18632.

70. Li H, Zhou T, Liu H, Xu F, Niu Y, et al. (2017) Discovery of a cobalt complex with high MEK1 binding affinity. Bioorg Med Chem Lett S0960-894X (17) 30255-X.

71. Sicard P, Clark JE, Jacquet S, Mohammadi S, Arthur JS, et al. (2010) The activation of p38 alpha, and not p38 beta, mitogen-activated protein kinase is required for ischemic preconditioning. J Mol Cell Cardiol 48: 1324–1328.

72. Nemoto S, Xiang J, Huang S, Lin A (1998) Induction of apoptosis by SB202190 through inhibition of p38beta mitogen-activated protein kinase. J Biol Chem 273: 16415–16420.

73. Shin M, Yan C, Boyd D (2002) An inhibitor of c-jun amino-terminal kinase (SP600125) represses c-Jun activation, DNA-binding and PMA-inducible 92-kDa type IV collagenase expression. Biochim Biophys Acta. 1589: 311–316.

74. Kumar B, Sinclair J, Khandrika L, Koul S, Wilson S, et al. (2009) Differential effects of MAPKs signaling on the growth of invasive bladder cancer cells. Int J Oncol 34: 1557–1564.

75. Dobbelstein M, Moll U (2014) Targeting tumour-supportive cellular machineries in anticancer drug development. Nat Rev Drug Discov 13: 179–196.

76. Flaherty KT, Infante JR, Daud A, Gonzalez R, Kefford RF, et al. (2012) Combined BRAF and MEK inhibition in melanoma with V600E mutations. N Engl J Med 367: 1694–1703.

77. Wang Z, Luo S, Wan Z, Chen C, Zhang X, et al. (2016) Glabridin arrests cell cycle and inhibits proliferation of hepatocellular carcinoma by suppressing braf/MEK signaling pathway. Tumor Biol 37: 5837–5846.