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The MAPK pathway across different malignancies: *A new perspective*

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Abstract

The MAPK/ERK pathway is activated by upstream genomic events and/or activation of multiple signaling events where information coalesces at this important nodal pathway point. This pathway is tightly regulated under normal conditions by phosphatases and bidirectional communication with other pathways, such as the AKT/m-TOR pathway. Recent evidence indicates that the MAPK/ERK signaling node can function as a tumor suppressor as well as the more common pro-oncogenic signal. The effect that predominates depends on the intensity of the signal and the context or tissue in which the signal is aberrantly activated. Genomic profiling of tumors has revealed common mutations in MAPK/ERK pathway components, such as *BRAF*. Currently approved for the treatment of melanoma, inhibitors of B-RAF kinase (BRAFi) are being studied alone and in combination with inhibitors of the MAPK and other pathways to optimize treatment of many tumor types. Therapies targeted toward MAPK/ERK components have variable response rates when used in different solid tumors, such as colorectal cancer and ovarian cancer. Understanding the differential nature of activation of the MAPK/ERK pathway in each tumor type is critical in developing single and combination regimens, as different tumors have unique mechanisms of primary and secondary signaling and subsequent sensitivity to drugs.

Keywords

MAPK; BRAF; ERK; MEK; signaling; melanoma; ovarian cancer; colorectal cancer

Introduction

Great advances have been made toward understanding the genomic characterization of solid and hematological malignancies. Collaborative projects, such as The Cancer Genome Atlas (TCGA)[1], have revealed the molecular complexity of tumors, and also the challenges inherent in data interpretation and clinical application. Differentiation of targetable driver mutations from genetic and genomic noise and passenger mutations is one of the most important goals of genome and epigenome analysis.[2, 3] Driver mutations lead to

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dysregulation of signaling pathways, increasing malignant behavior[4]. Many of these gain-of-function driver mutations have been shown to be druggable, leading to the development of small molecules and antibodies that target specific events such as the ligand-binding site of the receptor, or the ATP binding site in the kinase domain of specific kinase proteins.

The mitogen-activated protein kinase (MAPK) cascade is a critical pathway for human cancer cell survival, dissemination, and resistance to drug therapy.[5] The MAPK/ERK (extracellular signal regulated kinases) pathway is a convergent signaling node receiving input from numerous stimuli, including internal metabolic stress and DNA damage pathways, and altered protein concentrations, as well as via signaling from external growth factors, cell-matrix interactions, and communication from other cells.[6] Mutated genes responsible for regulation of cell fate, genome integrity, and survival can lead to increased protein amplification and alter the tumor microenvironment, thus over-activating the pathway.[7] These mutations can occur upstream in membrane receptor genes, such as epithelial growth factor receptor (*EGFR*)[8], in signal transducers (*RAS*)[9], regulatory partners (*Sprouty*)[10], and in downstream kinases belonging to MAPK/ERK pathway itself (*BRAF*; Figure 1).[11,12] Several mutations involving the MAPK/ERK pathway have been identified in human cancers and are ripe for targeting. Current and future drug development efforts will need to alter and regulate tumor signaling in this complex network of co-dependent pathways.

The MAPK pathway and its regulation

There are four independent MAPK pathways composed of four signaling families: the MAPK/ERK family or classical pathway, and Big MAP kinase-1 (BMK-1), c-Jun N-terminal kinase (JNK), and p38 signaling families.[13] These families share a basic organization composed of two serine/threonine kinases and one double specificity threonine/tyrosine kinase.[14] Generically, these kinases are designated from upstream to downstream, closer to the nucleus, as MAPK kinase-kinase (MAPKKK), MAPK kinase (MAPKK) and MAPK (Figure 1).[5] The canonical MAPK/ERK pathway is composed of three types of MAPKKK: A-RAF, B-RAF and RAF-1 or C-RAF kinases. *BRAF* is the gene most commonly mutated at this level in human cancer. One level below are the MAPKKs, which are composed of MEK1 and MEK2. Finally, further downstream are ERK1 and ERK2, which are the final effectors of the MAPK pathway.[15]

ERK phosphorylation results in the activation of multiple substrates that are responsible for stimulation of cell proliferation. Spatial localization of ERK determines target substrates and later effects within the cell.[6] When located in the cytoplasm, ERK phosphorylates cytoskeletal proteins that affect cell movement and trafficking,[16] metabolism, cell adhesion, and nodal regulation of other pathways.[17] Cytoplasmic substrates include ribosomal S6 kinases (RSK) that regulate glycogen synthase kinase 3 (GSK3) involved in metabolism, and L1 adhesion molecule, a protein of neural origin, that participates in cell adhesion.[18, 19] Minutes after MAPK/ERK activation, ERK detaches from cytoplasmic anchoring proteins, and translocates to the nucleus to exert its transcriptional regulation.[20] Active ERK in the nucleus causes phosphorylation and activation of various transcription factors, such as carbamoyl phosphate synthetase II (CPS II) linking with synthesis of DNA or p90RSK and

promoting cell cycle progression. These two events are integral in MEK/ERK stimulation of cell proliferation.[21, 22] In immune cells, activated ERK is also a component of the innate response in different steps of the inflammatory cascade, increasing the expression of tumor necrosis factor alpha (TNF-α) and inducible nitric oxide synthase (iNOS).[23]

In addition to spatial activation, the final effect of MAPK/ERK pathway is modulated by timing, duration, and intensity of its signal. Winters et al examined the MAPK/ERK cascade in different times points in colorectal cancer cell lines under the combination of carboxyamidotriazole, a intracellular calcium regulator, plus the selective cyclooxygenase 2 inhibitor, celecoxib. Suppression of ERK activation occurred in the first hour of treatment, in contrast with the sustained ERK phosphorylation after 9 days of treatment.[24] Indeed cells interpret and respond differently to small changes in the levels of MAPK/ERK activation. As described by Murphy et al, c-FOS, an early gene product of MAPK/ERK activation, works as a sensor of the duration of ERK stimulation. When the MAPK/ERK signal is transient, c-FOS is unstable and degraded in the nucleus, but if the signal is sustained c-FOS is phosphorylated, and specific domains are exposed promoting more ERK activation. [25] The pro-carcinogenic or pro-apoptotic signaling of this pathway is dependent upon the timing and duration of MAPK/ERK activation.

Specific proteins, such as kinase suppressor Ras-1 (KSR1), work as the main scaffold for proteins related to MAPK/ERK pathway activation. Cytoplasmic proteins, Sprouty and Spred, directly inhibit the pathway[26] by removing activating phosphate groups from ERK, therefore decreasing its ability to phosphorylate its substrates.[12] Thus, there are regulatory events both in the cytoplasm and the nucleus, along with spatial and temporal regulation that fine tune the output of the MAPK/ERK pathway.

Overactivated and oncogenic drivers of the MAPK pathway as therapeutic targets

Cellular proliferation is driven by an intricate network of regulated, interdependent signals. The complexity of the MAPK pathway is not random; it allows for the periodic environmental adaptation necessary for activation and regulation of the coordinated events critical for cell survival.[27] MAPK/ERK pathway activation and subsequent interactions are highly regulated processes that are deregulated in cancer cells. Stimulation of growth factor receptors in the cell membrane leads to activation of two different but interconnected pivotal pathways: the phosphoinositide 3-kinase (PI3K) signal causing activation of AKT and its downstream substrates, and the MAPK/ERK pathway (Figure 1). Both drive cell proliferation, survival, and dissemination. The PI3K/AKT pathway also promotes anabolism; whereas, the MAPK/ERK pathway is more active in proliferation and invasion.[5] Upregulation of MAPK/ERK signaling occurs as a result of overexpression or aberrant activation of receptor tyrosine kinases (RTKs) or their immediate downstream targets, PI3K, SRC, and RAS.

Normal MAPK/ERK function is also responsible for tumor suppression through induction of senescence and ubiquitinization and degradation of proteins necessary for cell cycle activity and survival.[28] Senescence involves the inhibition of cell proliferation through terminal cell

cycle arrest.[29] Abnormal activation of MAPK/ERK by RAS causes degradation of proteins required for both migration and progression through the cell cycle, as shown in a model of normal fibroblasts and validated in prostate benign tumors. In these tumors, high levels of phospho-ERK were found coexpressed with markers of senescent $p16^{INK4a}$ and PML, a marker of protein degradation.[28] In addition, a screening study using a panel of silencing RNAs (shRNAs) against *MEK1* increased lymphomagenesis in MYC-expressing lymphoid cells, demonstrating that *MEK1* has tumor suppressor properties, and that the function of MEK1 kinase is context dependent. [30]

Genetic mutations can dysregulate kinase activity and hyperactivate the MAPK pathway during induction and progression of tumorigenesis. Many oncogenic driver mutations have been identified in genes upstream of MAPK/ERK, varying across cancer types, as shown in Table 1. These may include exon 21 mutations in *EGFR* or del19*EGFR*, mutations in *KRAS*, and the classic V600E*BRAF* mutation. These mutated genes lead to downstream overactivation of the MAPK/ERK pathway. In general, mutations affecting MAPK/ERK pathway are singular, independent events. Infrequently, two mutations can be found in the RAS/RAF/MEK/ERK pathway within the same tumor, demonstrating tumoral molecular heterogeneity.[31] The sensitivity of mutation detection depends upon the dominant population of cells represented in the tumor sample that is tested and thus may not be illustrative of the tumor as a whole.[32]

Discovery of specific oncogene mutations that activate the MAPK pathway has spurred development of targeted therapies that apply to multiple tumor types. Studies in tumor cells with mutant V600E*BRAF* have demonstrated that RAF kinase inhibitors prevent ERK signaling.[33] The selective MEK inhibitor, PD0325901, decreased cyclin D1 protein expression, and thereby decreased cell proliferation in *BRAF* mutant melanoma xenograft models.[34] High plasma concentrations of the RAF inhibitor, vemurafenib, are associated with strong ERK pathway inhibition. Patients with advanced stage, V600E*BRAF*-mutated metastatic melanoma receiving vemurafenib treatment who achieved >80% inhibition of cytoplasmic ERK phosphorylation, shown in paired pre- and on-treatment biopsy samples, demonstrated clinical evidence of partial remission.[35] Recently, immunomodulatory effects of BRAF inhibition were examined and shown to explain part of the efficacy of vemurafenib in melanoma. BRAF and MEK inhibition increased the expression of melanoma antigens in melanoma cell lines. This could increase T cell recognition of the tumor leading to a successful immunotherapeutic approach. [36]

The next proteins downstream, MEK1 and MEK2, have now been successfully targeted. Selumetinib has shown some activity in metastatic biliary cancers in a study of 28 patients yielding a response rate (RR) of 12% and progression free survival (PFS) of 3.7 months. Only two patients were found to have *RAS* mutations and neither responded to therapy.[37] Selumetinib was also studied in 24 patients with metastatic papillary or poorly differentiated thyroid cancer refractory to radioiodine treatment. It increased the iodine-124 uptake in twelve patients with responses to radioiodine in 5 of 8. Mutations were detected in seven of the eight patient treated; responses were reported in four patients with *NRAS* and one with a *BRAF* mutation.[38] Docetaxel with or without selumetinib was examined in a randomized phase II trial of 87 patients with metastatic non-small cell lung cancer (NSCLC) with *KRAS*

mutations. The RR was 37% in the experimental arm vs 0 ($p < 0.0001$), with PFS 5.3 versus 2.1 months ($p=0.014$).[39] There are currently many ongoing phase II clinical trials exploring the use of agents targeting BRAF (Table 3) and MEK kinases (Table 4). Many of these trials apply mutational analyses for study eligibility to enrich for patients who may be most likely to benefit. Data suggest that this pathway behavior is not consistent in all settings, making targeting the addictive oncogenic pathway a challenge across different tumor types.

The MAPK/ERK pathway is a double-edged sword. Generally, therapeutic inhibition of elements within this pathway has yielded some benefit. However, small molecule inhibitor therapy aimed at specific protein targets within the MAPK/ERK pathway has resulted in development of secondary malignancies. RAF inhibitors, as a class, may cause abnormal skin cell proliferation leading to keratoacanthomas or squamous cell cancers in approximately 10 to 20% of the patients.[40-42] The development of these lesions is due to paradoxical activation of the normal MAPK/ERK pathway in the genomically normal skin keratinocytes.[42] The combination of BRAF and MEK inhibitors in metastatic melanoma resulted in improved treatment safety by counterbalancing the activation of the normal MAPK/ERK pathway yielding a marked reduction in the frequency of the paradoxical oncogenic skin changes.[43, 44] The combination of dabrafenib (BRAFi) with trametinib (MEKi) caused keratoacanthomas in 7% and rare squamous cell skin cancers compared with a 19% frequency with dabrafenib alone.[43] This safer combination is now under evaluation in numerous other cancers.

MAPK/ERK pathway susceptibility varies by tumor type

Different morphomolecular human tumors have demonstrated unexpected differential responses to signal interruption and may develop unique mechanisms of primary and secondary resistance (Table 2).[45-47] Colorectal and low grade serous ovarian cancers harbor the same mutations as seen melanoma, *KRAS* and *BRAF*, with very different responses to inhibition of the MAPK/ERK pathway (Figure 2). The relative importance of MAPK/ERK in cancer cells is thus dependent on the cell and/or tissue of origin, magnitude of addictive dependence to the pathway, and mechanisms of escape or alternative signaling.

Melanoma

The success of RAF kinase inhibition was a turning point in the treatment of melanoma. But, as with other agents, resistance to treatment occurred and was mapped to the MAPK/ERK pathway. Primary resistance to vemurafenib in V600E*BRAF* melanomas can occur through increased cellular proliferation in response to loss of function of tumor suppressors or dysregulation of mechanisms that prevent apoptosis. PTEN deficiency is a major mechanism through which the prosurvival AKT signaling pathway becomes constitutively activated. This was observed in melanoma cell lines treated with vemurafenib. This PTEN deficiency was accompanied by loss of induction of the pro-apoptotic BIM/BCL2L11 protein, and resulted in primary resistance in these cell lines.[48] Selective cytoplasmic redistribution of the transcription factor FOXO3a led to decreased transcription of pro-apoptotic proteins.[49, 50] These findings, coupled with the fact that not every mutation-positive tumor will respond to B-RAF inhibitor therapy despite activating mutations in *BRAF*, underscores the need for future research into mechanisms of primary resistance.

Tumors with oncogenic driver mutations in the MAPK/ERK pathway have been observed to progress despite initial response to targeted intervention, secondary resistance. Multiple secondary mechanisms of resistance have been identified in melanoma, including new activating mutations in *MAPK/ERK* pathway genes [51] and *NRAS* [52], increased dimers of splice variants of wild type *BRAF* [33, 53, 54], amplification of wild type *BRAF* and *MEK* [55], and increased *CRAF*.[56] New studies have demonstrated that C-RAF activates the MAPK/ERK pathway through acquisition of secondary mutations that increase its half-life, avoiding degradation, and allowing hetero-dimerization with B-RAF.[57] Many of these mechanisms result in paradoxical hyperactivation of ERK.[13] Intratumoral heterogeneity allows multiple mechanisms to be found within a single patient's tumor, such as unique mutations in *NRAS* and an alternative splice variant of *BRAF*. [58] These findings support the concept that tumors demonstrate clonal evolution and plasticity over time, adapting to microenvironment and pharmacologic exposures.[59]

Colorectal Cancer

Targeted kinase inhibitors (KIs) that have been beneficial in melanoma have not yielded similar activity in patients with colorectal cancer (CRC). RAF signaling is downstream of RAS in the MAPK/ERK pathway, such that the presence of *BRAF* and *KRAS* mutations in CRC should lead to sensitivity to RAF-targeted agents and to circumvent inhibition of upstream signals, such as those emanating from receptor kinases. Consistent with that latter expectation, *BRAF* and *KRAS* mutations were demonstrated to be negative predictors of benefit to cetuximab and panitumumab, EGFR inhibitor therapy, in phase III clinical trials.[60, 61] They did not predispose to susceptibility to inhibitor therapy as observed in melanoma. It is now routine clinical practice to test for *KRAS* mutation prior to initiation of EGFR inhibitors. Thus, rather than functioning as therapeutic targets in CRC, these genomic events in the MAPK/ERK pathway are validated negative predictive biomarkers for EGFR inhibitor intervention.

CRC resistance to B-RAF inhibition has been attributed to differential activation of EGFR in the cell membrane, reinforcing the differential relevance of EGFR expression across tumor types. Treatment of *BRAF*-mutant CRC cell lines with vemurafenib resulted in a strong increase in ^{1068}Y-EGFR phosphorylation and receptor activation, through inhibition of CDC25C phosphatase that regulates ^{1068}Y-EGFR phosphorylation. Blockade of MAPK/ERK by BRAF or MEK inhibitors prevented CDC25C activation resulting in increased ^{1068}Y-EGFR and subsequent activation of other downstream pathways, such AKT. The combination of suppression of EGFR in combination with vemurafenib markedly inhibited proliferation in CRC cells and may be a mechanism to increase clinical activity. [62]

Cross-communication between the MAPK/ERK pathway and parallel pathways, such as the PI3K-AKT and Wnt-Ca^{++} pathways, is critical to abnormal proliferation and therapy resistance. These parallel pathways are activated when the MAPK/ERK pathway is attenuated, and drive cellular proliferation. Inhibition of PI3K or AKT, or use of hypomethylating agents that secondarily block AKT signaling, can overcome this mechanism of resistance in vitro.[63] Understanding of mechanisms of induction of parallel signaling is needed to guide development of combination therapies. Recently, Spreafico et al

demonstrated a potential role of noncanonical Wnt/Ca^{++} signaling pathway in overcoming resistance of CRC to MEK inhibitors using cyclosporine (Wnt/Ca^{++} modulator) in a model of patient-derived tumor xenografts.[64] These models demonstrated that drug combinations blocking both a targeted pathway and its associated counter-regulatory signal can effectively abrogate resistance of CRC to BRAF or MEK inhibitors.

Ovarian Cancer

Ovarian cancers can be classified into distinct types, some of which are characterized by genetic mutations that may involve the MAPK/ERK pathway. Type II ovarian cancers include high-grade serous tumors, with defects in DNA repair via loss of normal p53 regulation in almost all tumors. [65,66] Type I ovarian tumors include low grade serous and endometrioid, clear cell, mucinous and borderline tumors (BOT); low grade serous cancers have mutations in *KRAS* (27-36%) and *BRAF* (33-50%), and in *PIK3CA*, whereas nearly all mucinous tumors may have *KRAS* mutations. [67-69] One study reported that 57% of BOT or low-grade serous tumors had V600*BRAF* or *KRAS* codon 12 mutations. Provocatively, patients with *BRAF* mutation had no recurrence after a median follow up of 3.6 years.[70] Ho et al showed *KRAS* or *BRAF* mutations in 86% of cystadenomas adjacent to borderline tumors, and BOT had mutations in 88% of cases.[71] There is a loss of frequency of *BRAF* and *KRAS* mutations in the transition from nonmalignant to malignant disease, from cystadenoma or BOT to invasive low grade serous cancer. The mechanism of this selective process by which there is loss of an otherwise recognized oncogenic mutation during the process of acquisition of an invasive phenotype is unknown. This is the only example identified where there is such loss of a perceived gain-of-function mutation.

The identification of these mutations led to the logical hypothesis that such ovarian neoplasms were a new frontier for experimentation with targeted BRAF and MEK inhibitor therapy. Gynecologic Oncology Group study 239, a phase II trial of the MEK inhibitor, selumetinib, in 52 previously treated patients with low grade serous ovarian tumors yielded a 15% overall response rate and a median progression free survival of 11 months. This was compared to a historical PFS of 7 months. Mutational analysis was performed in tumor from 34 patients; *KRAS* and *BRAF* mutations were found in 41% and 6%, respectively, although mutation did not correlate with response or longer PFS.[72] These examples argue against a preponderant role of the MAPK/ERK pathway as a targeting oncogenic driver in these tumors, despite the presence of mutations. Studies of sorafenib in predominantly high serous histology recurrent ovarian cancer patients did not demonstrate biochemical activity of reduced ERK activation pre and on-treatment, perhaps consistent with the lack of genomic events in the MAPK/ERK pathway in those tumors.[73]

Interactions between the MAPK/ERK pathway and estrogen receptor-α (ERα) have also been identified in preclinical studies. MEK inhibition caused an increase in ERα expression independent of AKT signaling in ovarian cell lines positive for ERα. The addition of the ER inhibitor fulvestrant caused synergistic suppression of tumor growth in vitro and in an in vivo model.[74] This may be a direction for clinical study using modulation of the MAPK/ERK pathway to secondarily regulate a parallel pathway. This reinforces how ovarian cancer is a challenging environment in which to study the tumor specific effects of

MAPK/ERK pathway activation. Its broad range of cellular diversity and complexity of pathways activation lends itself to combination therapy, necessitating greater understanding of the interaction of the pathways.

Conclusion

The downstream MAPK/ERK signaling node, predominantly activated by upstream SRC/RAS/RAF signaling, is also regulated by modulation through parallel pathways. This creates a complexity within and between tumors that impedes the ability to translate therapeutic findings across tumors. Tissue and subtype specificity in signaling adds a level of complexity to application of novel targeted agents, even against an otherwise dominant pathway. The MAPK/ERK pathway stimulates cellular proliferation and invasion; however, its activation also can increase cellular apoptosis or antagonize pro-oncogenic input from other signals. The MAPK/ERK pathway demonstrates both oncogene and tumor suppressor effects depending on the tissue-specific tumor microenvironment. While cancers share common mutations, different cell types have developed unique responses to the mutations. These mutations may behave as oncogenic drivers, passenger mutations, or regulatory events. The role of the MAPK/ERK pathway in the tumor microenvironment has long been recognized. This pathway is critical in the process of physiologic and malignant invasion, angiogenesis, and most recently, a clear role for MAPK/ERK has been demonstrated in the tumor-immune system interactions. Hence, MAPK/ERK activation is a multi-faceted target under varied regulatory bodies. Regulatory mechanisms may lead to activation of alternative pathways, and paradoxical hyperactivation of the normal MAPK/ERK pathway. One unintended and unexpected consequence of KRAS/BRAF inhibitor drug therapy is increased activity of the normal MAPK/ERK pathway, which can lead to the development of secondary malignancies. Some novel combination therapies have demonstrated increased treatment efficacy by addressing both a specific target and its counter-regulatory effect in the complex milieu of cellular signaling. In shaping future approaches toward personalized medicine, the challenge is clear: we must strike a delicate balance between exploiting shared genetic targets and acknowledging unique features of human cancers.

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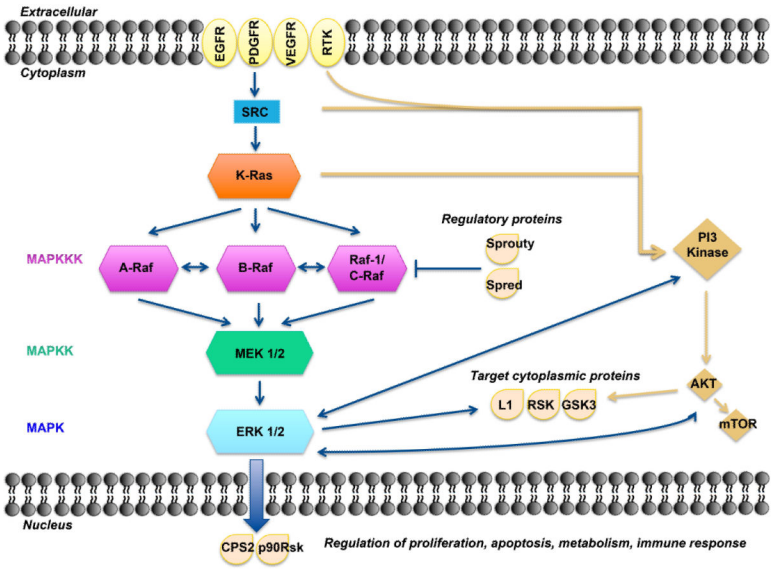

Figure 1.

A model of the MAPK/ERK pathway. After membrane receptor activation, adaptor proteins recruit RAS proteins to activate steps concluding with ERK activation. Successive steps of phosphorylation amplify the signal, Raf→MEK→ERK, until ERK activates its cytoplasmic and/or nuclear targets. Regulatory phosphatases, Sprouty and Spred, modulate the intensity of the signal. The PI3K-AKT pathway interacts with the MAPK/ERK node under normal conditions and in the cancer cell. Target cytoplasmic proteins include RSK, ribosomal S6 kinases; GSK3, glycogen synthase kinase 3; L1, adhesion molecule L1. Additional proteins in nucleus include CPS2, p90Rsk.

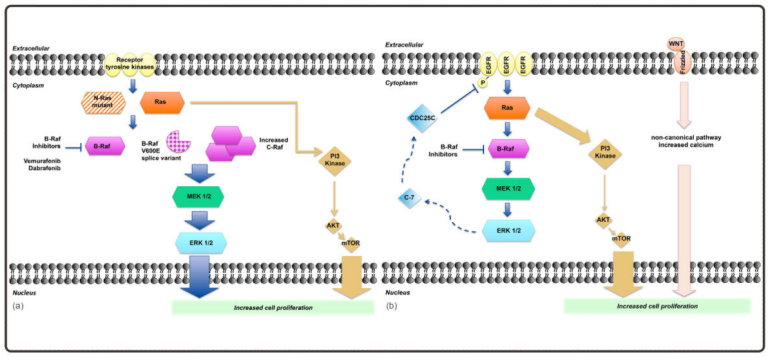

Figure 2.

Mechanisms of resistance to BRAF inhibitors. A) In melanoma mechanisms of secondary resistance to BRAF inhibitors include BRAF splice variants expression, CRAF activation (all of which activate MAPK/ERK) or signaling trough alternatives pathways AKT/m-TOR etc. (see table 2) B) In Colorectal cancer, primary resistance to BRAF inhibitors is caused by direct activation of EGFR and AKT/m-TOR pathway.

Table 1

Frequency of mutations in the activators and components of MAPK/ERK pathway across different tumors.

Tumor	RAS	BRAF	MEK	ERK
Melanoma	15-29%[75, 76]	50-60%[77]	3-8%[78, 79]	N/R
NSCLC	12-30%[80]	4%[81]	N/R	N/R
Colorectal	34.1%[82]	5-20%[77, 83]	<3%[78]	N/R
Ovarian HGSOC LGSOC	0-12%[84] 27-36%[68, 69]	N/R 33-50%[68, 69]	N/R	N/R
Thyroid	9*-27%[85]	45-69%[86, 87]	N/R	N/R
Hairy Cell	N/R	79-100%[88, 89]	N/R	N/R

Table 2

Mechanisms of primary and secondary resistance to TKIs in different tumor types.

Tumor	Mechanism of resistance	
	Primary	**Secondary**
Lung	MET amplification[90] BIM polymorphism[91, 92]	T790M mutation[93] EGFR amplification[94] Her2 amplification[95] PIK3CA mutations[94] MET amplification[96]
Melanoma	NF1 loss[97, 98] PTEN loss[48]	BRAF amplification[55] NRAS amplification Increase in CRAF[52] Splice variant BRAF[54] Increase activation AKT[99] NF1 loss[97]
Colorectal	EGFR activation[62, 100] PI3K/AKT activation[63] Wnt/Ca++ activation[64]	N/A
Ovarian	PI3K activation[101] Activation of ERα[74]	N/A

Table 3

Phase II Ongoing Clinical trials for BRAF inhibitors in patients with solid tumors

	Tumor type	
	Melanoma	**Other Tumor Types**
BRAF inhibitors		
Vemurafenib	• NCT01495988 • NCT01813214 • NCT01611675 • NCT01942993 • NCT01638676 • NCT01781026 • NCT01586195 • NCT00949702 • NCT01378975 • NCT01248936	• NCT01709292 - thyroid-locally advanced • NCT01286753 - papillary thyroid • NCT01524978 - any BRAFV600 mutant tumor • NCT01771458 - any tumor
Dabrafenib	• NCT01682213 • NCT01721603 • NCT01266967 • NCT01153763	• NCT01340846 - any BRAFV600 mutant tumor • NCT01723202 - thyroid • NCT01336634 - NSCLC with BRAF mutation

Table 4

Phase II trials of MEK inhibitors across tumor types

	Tumor type	
	Melanoma	**Other Tumor Types**
Selumetinib	• NCT01143402 • NCT00866177	• NCT00888134 - any tumor type • NCT00553332 - biliary • NCT01160718 - breast • NCT00780676 - breast • NCT00514761 - colorectal • NCT01011933 - endometrial • NCT01089101- glioma • NCT01752569- Kaposi's sarcoma • NCT00604721 - liver • NCT00372788 - NSCLC • NCT01306045 - NSCLC, thymic • NCT00372944 - pancreatic • NCT00551070 - ovarian, peritoneum • NCT00559949 - papillary thyroid • NCT01843062- thyroid
Trametinib	• NCT01978236 • NCT01037127 • NCT01328106 BRAF inhibitor + trametinib: • LCCC 1128: NCT01726738 • NCT01072175 • NCT01972347 • NCT01928940 • NCT01619774 • NCT01978236	• NCT01827384 - any tumor type • NCT01943864 - biliary • NCT01553851 - oral squamous BRAF inhibitor + trametinib: • NCT01723202- thyroid • NCT01750918- colorectal
Pimasertib	• NCT01693068	• NCT01936363- ovarian
Refametinib		• NCT01915589 -hepatocellular • NCT01915602- hepatocellular