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Review Article

MAPK SIGNALLING PATHWAY: ROLE IN CANCER PATHOGENESIS

MAUSAM J. NAIK

Maliba Pharmacy College, Uka Tarsadia University, Surat 394350, Gujarat, India
Email: mjnaik96@gmail.com

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ABSTRACT

Cancer is one of the prime causes of death presently. In normal cells, the firmly regulated pathway relays extracellular signals from the cell membrane to nucleus through a cascade of phosphorylation events. The Mitogen-Activated Protein Kinase (MAPK) cascades are among the most thoroughly studied signal transduction systems and have been proven to participate in a diverse array of cellular programs consisting of cell differentiation, cell movement, cell division and cell death. Constitutive activation of the MAPK cascade is associated with the carcinogenesis and melanoma development because of activating mutations within the B-RAF and RAS genes or other genetic or epigenetic modifications in their components or upstream activation of cell-surface receptors (e. g., EGFR and Flt-3) and chimeric chromosomal translocations (e. g. BCR-ABL) leading to elevated signaling activity eliciting cellular proliferation, invasion, metastasis, migration, survival and angiogenesis. Even in the absence of apparent genetic mutations, MAPK pathway has been stated to be activated in over 50% of Acute Myelogenous Leukemia (AML) and acute lymphocytic leukemia. In this brief review, we are about to outline the current advances in understanding the regulation of Mitogen-activated protein kinase signaling system and how can we generate specificity.

Keywords: MAPK pathway, B-RAF mutations, Cancers, MAPK dysregulation, Genetic mutations

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INTRODUCTION

Cells respond to diverse extracellular signals by transmitting intracellular signals to coordinate appropriate responses. Proliferation, survival, differentiation, adhesion and motility of malignant cells are regulated by different intracellular signalling pathways. Signalling pathways are a group of molecule in the cell working in a cascade to control one or more cell functions such as cell division or death. These are mainly classified into subtypes: (a) Intracrine signalling (b) Autocrine signalling (c) Juxtacrine signalling (d) Paracrine signalling (e) Endocrine signalling. Abnormal activation of these signalling pathways leads to disturbed/ deranged cellular proliferation. Some of the possible cellular/ molecular mechanisms involved in cancer are (a) Degradation of interstitial collagens in extracellular matrix which is an integral component of tumor invasion and metastasis (b) Promotion of cell division by protooncogenes or by cell cycle suppression of tumor suppressor genes (c) Mutations in the p53 genes (d) Mutations in BRCA-1 antibodies. Many pathways are involved in pathogenesis of cancer namely JAK-STAT signalling pathway, Mitogen-Activated Protein Kinase (MAPK) pathway, Phosphatidylinositol-3-kinases (PI3K)/Protein Kinase B (AKT) signalling pathway, Notch pathway, HEDgehog pathway, mTOR pathway yet MAPK pathway is of keen intrest due to its drug-resistant nature in cancer pathogenesis. This review will highlight several studies that are caried out from the year 1996 to 2018 in order to have a better understanding of MAPK pathway and its role in cancer pathogenesis.

Expression of constitutively active components of the ERK pathway in addition to activation of PI3K/Akt/mTOR signaling either through mutation of pathway components or through activation of upstream signaling molecules cause deregulation of proliferation, resistance to apoptosis, transformation of cells and changes in metabolic characteristic of transformed cells which ultimately leads to carcinogenesis [1]. When the balance between cell division and growth on one hand, and programmed cell death (i.e. apoptosis) on the other is disturbed, it leads to carcinogenesis/oncogenesis. Growth factors, cytokines, and serum provide both mitogenic and anti-apoptotic signals to cells and thus play an important role in maintaining the homeostatic balance between cell proliferation and cell death. Due to this exquisite balance, proteins and signaling pathways regulating cell growth, differentiation and development undergo oncogenic changes regularly than other molecule groups [2]. The PI3K and MAPK pathways interact in multiple ways by co-regulating their functions leading molecular alterations, mutation or amplification of cell surface receptors which as a consequence leads to deregulated signaling and uncontrolled cell growth and survival causing oncogenic transformation and progression [3, 4].

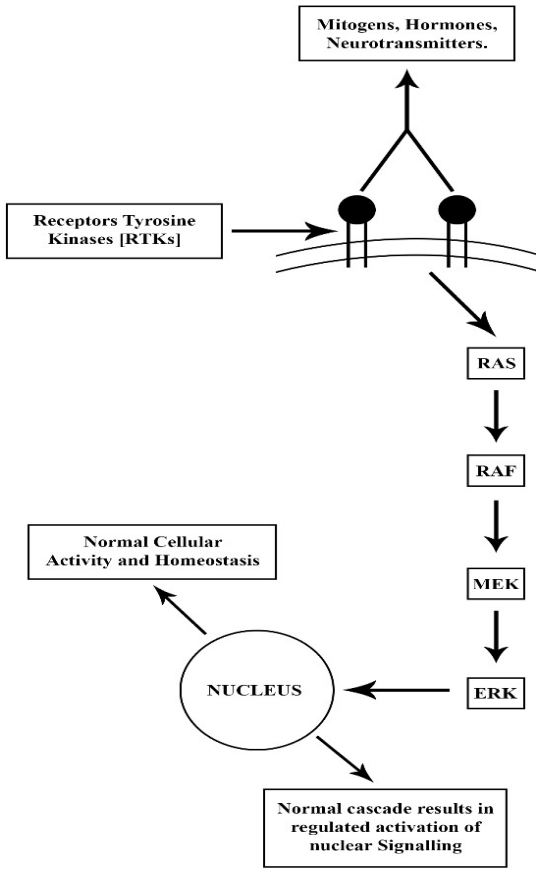

Fig. 1: The mitogen-activated protein kinase (MAPK) signalling cascade

MAPK signalling pathway

MAPKs are Proline-directed kinases which phosphorylate sites containing Serine/Threonine-Proline (S/T-P) motif that recognize the Proline at+1 position in the substrate [5, 6]. The classical MAPK pathway consists of RAS, RAF, MEK and ERK which consecutively proceed the proliferative signals generated at the cell surface receptors and through cytoplasmic signaling into the nucleus [7].

In normal cells the signaling cascade is stimulated by the binding of mitogens, hormones, or neurotransmitters to Receptor Tyrosine Kinases (RTKs) which upon dimerization instigate actuation of kinase activity in the cytoplasmic domain, triggering the activation of oncogenic RAS to increase cellular RAS-GTP levels [8, 9]. Those RTKs that connect for RAS or different parts of the RAS superfamily include: Epidermal Growth Factor Receptor (EGFR), c-KIT (CD117), Platelet-Derived Growth Factor Receptor (PDGFR), Vascular Endothelial Growth Factor Receptor (VEGF), fibroblast growth factor receptor and Fms-Related Tyrosine Kinase-3 (FLT-3) [10]. The activation of RAS leads to auto phosphorylation of C-terminal tyrosine residues that bind to Src Homology 2 (SH2) alternately Phosphotyrosine Binding (PTB) domains, for example, the adaptor protein Growth Factor Receptor-Bound Protein 2 (GRB2) [11, 12]. Mechanistically, the phosphorylated SH2 domain of the GRB2 acquires Son of Sevenless (SOS) into close vicinity with inactive membrane restricted GDP-bound RAS as a result of prenylation and converts it into an active GTP-bound RAS [13, 14]. GTP-bound RAS in turn now binds with Raf-1 and B-Raf and target either one or both to the membrane and increase the kinase activity [15]. A-Raf activates MEK-1, B-Raf activate both MEK-1 and MEK-2 yet activate MEK-1 superiorly than MEK-2 and C-Raf activate both MEK-1 and MEK [16-18]. Once activated, RAF isoforms are able to phosphorylate the MAPK kinases, MEK1 and MEK2 and dual-specificity kinases. For example MKK4, a dual specificity kinase and member of MAPK which has the ability to directly phosphorylate Serine/Threonine along with Tyrosine residues leading to activation of two downstream pathways, C-Jun Terminal kinase (JNK) as well as P38 [19]. These kinases are perceived by MEK and phosphorylate tyrosine at Tyr-185 and then proceed to phosphorylation of threonine at Thr-183 residues in the Thr-X-Tyr activation loop of the MAPKs also known as ERK1 and ERK2 [20-22]. ERK1 and ERK2 direct: (a) increased proliferation, due to tumor suppressor inactivation and down regulation of cyclin-dependent kinases (b) increased survival through modulation of MITF and protection against FAS-induced apoptosis (c) invasion and metastasis due to extracellular matrix remodeling and angiogenesis [23-26]. ERK1 and ERK2 additionally are also associated with phosphorylating cytosolic signaling proteins including p90 Ribosomal S6 Kinase (RSK) and MAPK-interacting Serine/Threonine kinase and transcription factors, for example erythroblastosis virus E26, Elk-1, cAMP Response Element Binding Protein (CREB), c-Fos and c-Jun [27-33]. The Raf/MEK/ERK pathway can also modulate the activity of many proteins involved in apoptosis including: Bcl-2, Bad, Bim, Mcl-1, caspase 9 and Survivin where Bcl-2 and multi-drug-resistance gene expression are responsible for aberrant activation of MAPK pathway [34-37]. While activation of the MAPK pathway seems essential in the biology of melanoma, mechanisms other than RAS or BRAF mutation may also contribute to the constitutive MAPK signaling in invasive melanoma. These include: (1) increased coupling of RAS to cell surface RTKs (such as c-KIT) resulting in upregulation of RTKs expression (2) overexpression and accumulation of wild-type RAS protein (3) constitutive expression of growth factors such as hepatocyte growth factor or fibroblast growth factor (4) upregulated growth factor receptors such as c-MET (receptor for hepatocyte growth factor) leads to aberrant signaling or (5) negative regulators of ERK expression has been decreased [38-42]. Moreover, increase expression of VEGF-R receptors has been observed in AML which could result in activation of this pathway. Constitutive activation of the Raf/MEK/ERK pathway has been implicated in invasion, metastases, angiogenesis and radioresistance. Reactive oxygen species either through growth factor receptor activation including EGFR and PDGF or through reactive oxygen intermediate-induced receptor activation activate RAS and initiate MAPK signaling cascade. ROS will induce the activation of the ERK1/2 signaling pathway in Ras negative cells [43, 44].

Genetic modification prompting activation of MAPK pathway

Dysregulation of the MAPK pathway often takes place in malignancies wherein receptor tyrosine kinase (RTKs), generally, the Epidermal Growth Factor Receptor(EGFR), are constitutively active as result of somatic mutation, gene amplification, increased autocrine or paracrine signalling. In addition to the mutations within the components of the pathway inclusive of the RAS, BRAF and MEK genes may also result in the constitutive activation of the signalling cascade [45, 46].

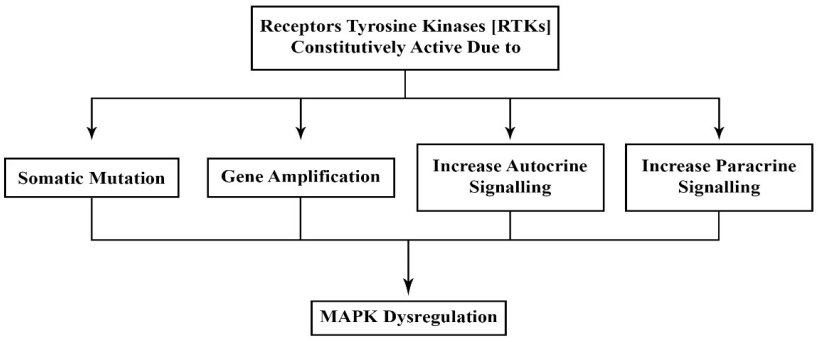

Fig. 2: Contributors for MAPK dysregulation

A recent study showed that Mitogen-Activated Protein Kinase 14 (MAPK 14), also called p38α plays a key role in inflamation cytokine induction such as Tumor Necrosis Factor(TNF α) [47]. A study confirmed that MAPK is directly involved in resistance development of breast cancer cells against Gefitinib drug (EFGR inhibitor). The principle mechanism via which MAPK prevent induction of apoptosis is p90rsk-1 mediated phosphorylation of proapoptic BAD in serine 112. Phosphorylation of this site inhibits apoptosis through sequestering BAD in cytosol and preventing its interaction with Bcl-XL [48]. In RAS genes maximum somatic mutations are detected typically in codons 12, 13, 59 and 61 leading to single-amino-acid substitutions [49]. All mutations compromise the hydrolysis activity through intrinsic and GTPase-activating-protein-stimulated of GTP. Activating point mutations in RAS genes arise in about 30% of human cancers. A current study propose that 10–50% of individuals diagnosed with myelodysplastic syndrome or AML have RAS mutations which are often point mutation that modify RAS activity and additionally perturb the Raf/MEK/ERK kinase cascade [50-52].

Mutations in KRAS account for approximately 85% of overall RAS mutations and is most often mutated RAS isoform in human cancer followed by NRAS (about 15%) including the brain, uveal and mucosal primaries and are absent in cancer of soft parts and HRAS (less than 1%) [53, 54]. Somatic mutations of KRAS were found at a high percentage in pancreatic cancer (69%), in 16% of lung cancers

and in approximately 35% of colon cancer, while they may be rarely found in breast cancer (approximately 3%). Mutations in NRAS were found in cancer (19%) and with lower frequency in colon cancer (2%) and breast cancer (2%). A study showed that the oncogenic BRAF antagonize COT expression largely through altered protein stability and the wild type BRAF produce COT which induce Thr 202/Tyr 204 phosphorylation of ERK1 *in vitro* indicating that COT expression might also potentiate ERK activation in a MEK-independent manner [55]. Mutations rates for BRAF gene is 50%-70% many of which are clustered inside the P-loop (exon 11) and within the activation segment (exon 15) of the kinase domain [56]. These mutations destabilize the inactive conformation of the protein, disrupting the interaction between the P-loop and the activation segment which typically locks the kinase in the inactive conformation ensuing in constitutive activation of the MAPK pathway [57, 58]. The substitution of a valine residue at position 600 for glutamic acid (V600E) accounts for about (80%-90%) of the BRAF mutations observed in human cancers [59-63]. Activating BRAF mutations have additionally been documented in a variety of human cancers inclusive of papillary thyroid carcinoma, colorectal cancer, cholangiocarcinoma, and esophageal carcinoma (Barrett's), gastric cancer, squamous cell carcinoma of the head and neck, lung cancer, ovarian tumors, in addition to AML and non-Hodgkin's lymphoma [64-75]. Whilst MAPK activation may be essential for melanoma initiation, facts suggest that additional molecular events are also required probably exerted via V600E BRAF modulation of different pathways such as Hypoxia-Inducible Factor 1-a (HIF 1-a) [76]. Other mutations bring about BRAF proteins with impaired kinase activity in comparison with wild type BRAF. The impaired-activity BRAF mutants aren't capable of activating MEK directly however can stimulate CRAF that in turn activates MEK while the activated BRAF mutants signal to MEK directly. BRAF kinase mutations arise in about 8% of human carcinomas most frequently in melanoma (41%), thyroid (45%), colorectal (10%-14%) and mismatch repair-deficient tumors (31%) [77]. A low frequency (1-3%) of BRAF mutations has been observed in a number of different tumor types along with breast cancer. A recent study suggested that BRAF has 3 AKT phosphorylation sites: (a) Thr439 (b) Ser428 and (c) Ser364 (conserved in RAF1). *In vitro* alanine substitution at Thr439 results in BRAF activation via loss of AKT-induced inhibition with gradually increased BRAF activity as the additional sites are mutated [78].

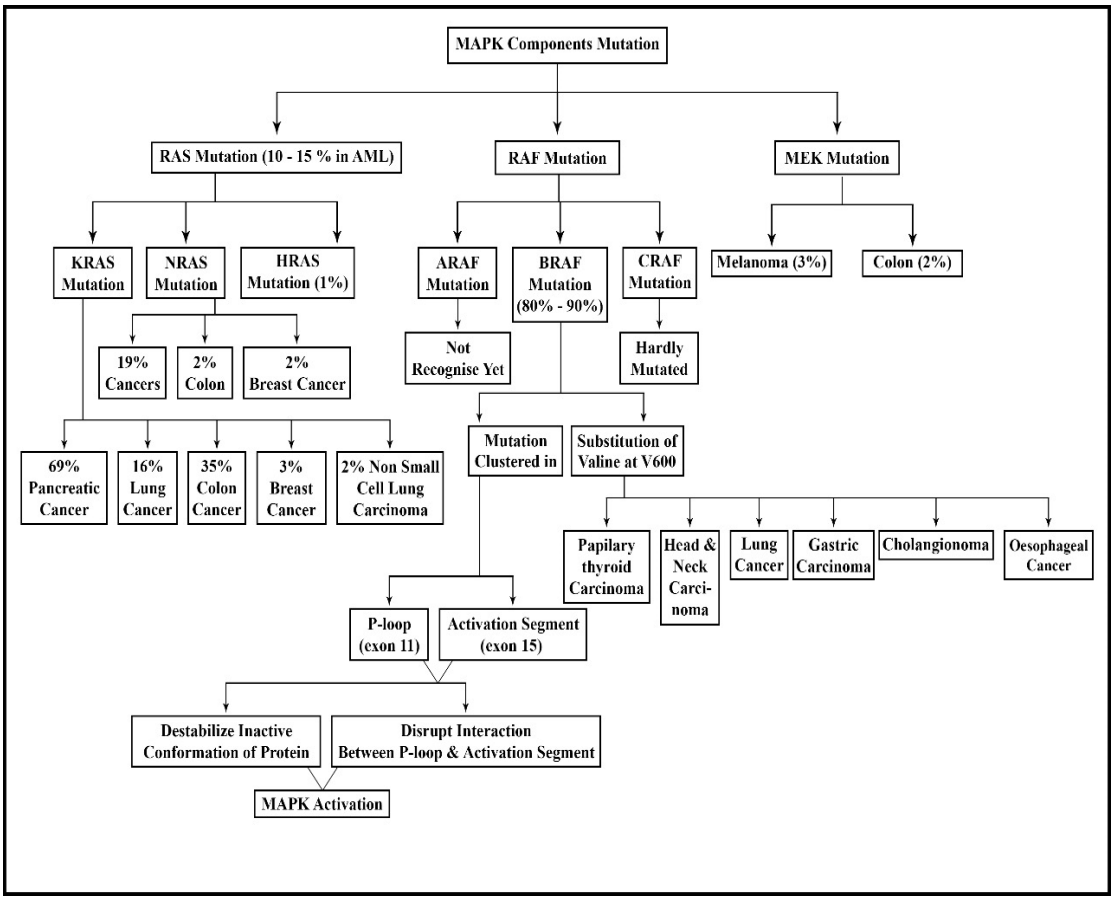

Fig. 3: Mutations of MAPK cascade components

Mutations of ARAF have not been recognized while CRAF is hardly ever mutated. A study confirmed that RAF inhibitors efficiently blocks MAPK signaling led to reduced growth in tumors which arise because of V600E BRAF mutation however results in activation of CRAF by inducing dimerization, membrane localization and interaction with Ras-GTP which ultimately activate MAPK pathway and results in enhanced growth [79]. MEK mutations have been rarely detected in human cancers inclusive of melanomas (3%) and colon (2%) carcinomas. Those mutations lead to a gain of function of kinase activity ensuing inactivation not only of MEK but also of ERK [80]. Numerous different researches have demonstrated that EGFR and KRAS mutations are mutually exclusive in Non-Small-Cell Lung Carcinoma (NSCLC) [81, 82]. In melanomas lacking B-RAF or RAS mutations the signaling cascade is induced via different autocrine mechanisms which includes C-MET overexpression, a receptor for hepatocyte growth factor or via down-regulation of MAPK pathway inhibitory proteins consisting of RAF-1 inhibitory protein or SPRY-2 [83]. Mutations in upstream receptors including Flt-3 (20–30%), kit (7–17% of AMLs), Fms (12% of MDS) and Granulocyte Colony-Stimulating Factor Receptor (G-CSF-R) have been documented in AML and could cause the activation of the Ras/Raf/MEK/ERK pathway [84]. In a study it has been proven that MAPK activity regulates

proliferation through regulation of cyclin D1 expression and increase expression of cyclin D1 provide BRAF inhibitor resistance that is more advantageous through elevated CDK4 expression, frequently deregulated in cancer via couple of mechanisms [85].

CONCLUSION

The MAPK pathway plays a crucial role in controlling cellular proliferation, survival and invasion. Constitutive activation of MAPK is common event in human cancer and is often the result of molecular alteration of key components of signaling cascade. The challenge remains to identify the most efficient members of the signaling cascade to target and drugs which might be bioavailable with negligible toxicity-related side effects. By elucidating those unique profiles and using an appropriate combination of therapeutic agents we will impact survival in melanoma.

ABBREVIATION

MAPK: Mitogen Activated Protein Kinase, AML: Acute Amyloid Leukemia, PI3K: phosphatidylinositol-3-kinases (PI3K), AKT: Protein Kinase B, BRCA-1: Breast Cancer gene, RTKs: Receptor Tyrosine Kinases, (EGFR): Epidermal Growth Factor Receptor, (PDGFR): Platelet-Derived Growth Factor Receptor, (VEGF): Vascular Endothelial Growth Factor Receptor, (FLT-3): fms-Related Tyrosine Kinase-3, (SH2): Src Homology 2, (PTB): Phosphotyrosine Binding, (GRB2): Growth Factor Receptor-Bound Protein 2.

AUTHORS CONTRIBUTIONS

All the author have contributed equally

CONFLICT OF INTERESTS

Declared none

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