


MAP3K1: Genomic Alterations in Cancer and Function in Promoting Cell Survival or Apoptosis

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Trang T. Pham^{1,*}, Steven P. Angus^{1,*}, and Gary L. Johnson¹

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Abstract

MAP3K1 is a member of the mitogen-activated protein kinase kinase kinase (MAP3K) family of serine/threonine kinases. MAP3K1 regulates JNK activation and is unique among human kinases in that it also encodes an E3 ligase domain that ubiquitylates c-Jun and ERK1/2. Full length MAP3K1 regulates cell migration and contributes to pro-survival signaling while its caspase 3-mediated cleavage generates a C-terminal kinase domain that promotes apoptosis. The critical function of MAP3K1 in cell fate decisions suggests that it may be a target for deregulation in cancer. Recent large-scale genomic studies have revealed that MAP3K1 copy number loss and somatic missense or nonsense mutations are observed in a significant number of different cancers, being most prominent in luminal breast cancer. The alteration of MAP3K1 in diverse cancer types demonstrates the importance of defining phenotypes for possible therapeutic targeting of tumor cell vulnerabilities created when MAP3K1 function is lost or gained.

Keywords

MAP3K, MEKK, protein kinase, apoptosis

Mitogen-activated protein kinases (MAPKs) are key mediators of evolutionarily conserved signaling networks that play an essential role in multiple aspects of cell physiology.^{1,2} Activated by diverse stimuli, these signaling networks involve 3 sequential phosphorylation steps from MAPK kinase kinases (MAP3Ks, MEK kinases, or MKKKs) to MAPK kinases (MAP2Ks, MEK, or MKKs) to MAPKs. MAPKs are categorized into 4 subfamilies, extracellular signal response kinase 1/2 (ERK1/2), extracellular signal response kinase 5 (ERK5), c-Jun NH2-terminal kinase (JNK) or stress-activated protein kinase (SAPK), and p38. Twenty MAP3Ks, 7 MAP2Ks, and 11 MAPKs constitute an integrated signaling network responding to diverse stimuli to control critical functions in virtually all cells.³

MAP3K1 or MEKK1 (MEK kinase 1) is a 196-kDa serine-threonine kinase that belongs to the MAP3K family and the STE superfamily.^{2,4} MAP3K1 was originally identified as the mammalian homolog of the yeast MAP3Ks Ste11 and Byr2 that function in pheromone responsive signaling.⁵ In addition to the conserved kinase domain, MAP3K1 has several unique structural characteristics that mediate its specific activities compared with other MAP3Ks. Studies have demonstrated that MAP3K1 functions in cell survival, apoptosis, and cell motility/migration in multiple normal and tumor cell types. Genetic alterations in the *MAP3K1* gene have been recently found in comprehensive genomic

analyses of cancers. As discussed herein, the function of MAP3K1 in apoptosis suggests why it is deleted or mutated in specific cancers.

MAP3K1 Is a Serine/Threonine Protein Kinase, E3 Ubiquitin Ligase, Scaffolding Protein, and Caspase-3 Substrate

The kinase domain of MAP3K1 is located at the C-terminus (residues 1243-1508) (Fig. 1A). MAP3K1 selectively phosphorylates and activates MAP2K4, which in turn phosphorylates and activates JNK.^{6,7} MAP3K1 can also phosphorylate MAP2K1/2 and MAP2K7 that phosphorylate and activate ERK1/2 and JNK, respectively.^{5,8} Following its oligomerization, MAP3K1 can also trans-autophosphorylate, which

¹Department of Pharmacology, Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, Chapel Hill, NC, USA

*These authors contributed equally to this work.

Corresponding Author:

Gary L. Johnson, Department of Pharmacology, Lineberger Comprehensive Cancer Center, University of North Carolina School of Medicine, 4016 Genetic Medicine Bldg., Campus Box 7365, Chapel Hill, NC 27599, USA
(Email: glj@med.unc.edu).

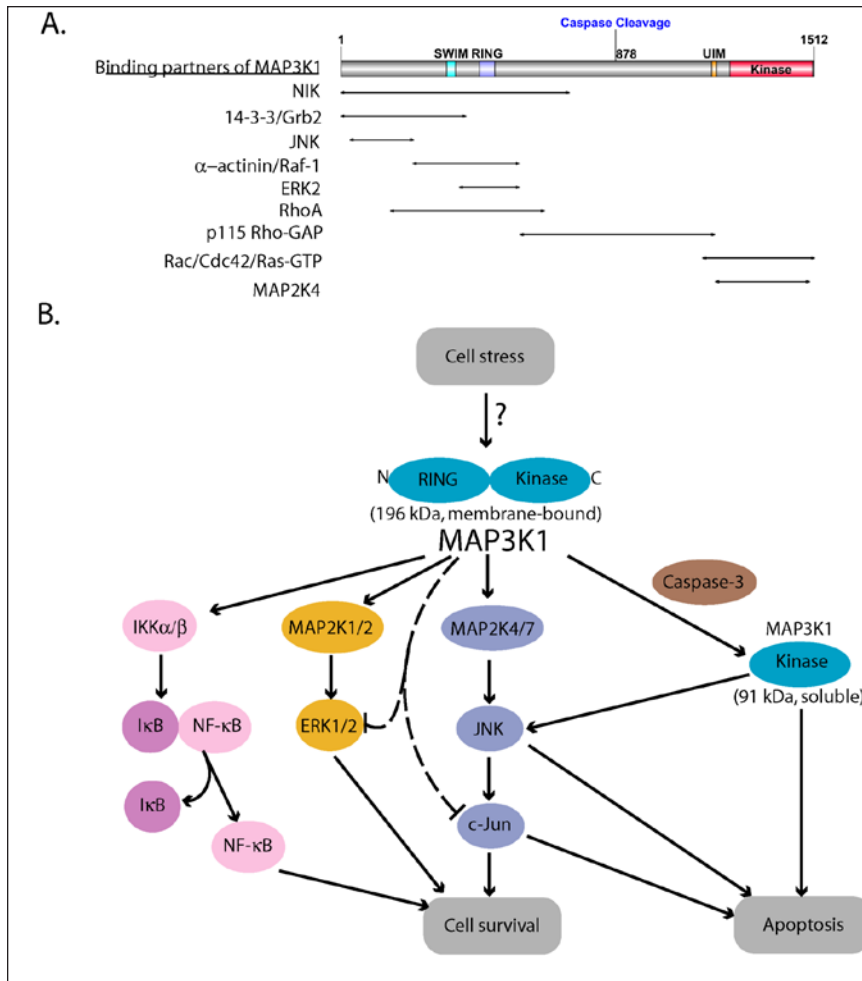


Figure 1. MAP3K1 domain organization and dual roles in cell survival and apoptosis. **(A)** Schematic structure of MAP3K1. It contains a SWIM and a RING zinc finger domain near the N-terminus and a serine/threonine kinase domain at the C-terminus. Caspase-3 cleavage occurs at the aspartate 874 of the mouse MAP3K1, which is equivalent to residue 878 of the human homolog. The protein domain structure was created using DOG 1.0 program.¹⁷ MAP3K1 also harbors binding sites for multiple upstream and downstream proteins indicated by the arrows. **(B)** MAP3K1 has a switch-like function that determines cell fate. Activation of MAP2K4/7-JNK-c-Jun, MAP2K1/2-ERK1/2, and NF- κ B mediated by full length MAP3K1 promotes cell survival while caspase cleavage, which generates the soluble active kinase domain, induces apoptosis. MAP3K1 also ubiquitinates c-Jun and ERK1/2, leading to their degradation.

presumably regulates its activation.^{9,10} Located upstream of the kinase domain are a conserved caspase-3 cleavage site (⁸⁷⁵DTLDG⁸⁷⁹ in the human homolog) and an Ubiquitin Interacting Motif (UIM).¹¹ Although the role of the UIM is unclear, caspase-3 cleavage separating the C-terminal kinase domain from the regulatory N-terminus is required for apoptosis induction by MAP3K1. Unique among MAP3Ks, the N-terminus of MAP3K1 has 2 zinc-finger domains, a SWIM domain (residues 338-366), and a RING domain (residues 443-492), which are characterized by specific spacing of the conserved metal chelating cysteine and

histidine residues.^{12,13} SWIM domains are ancient domains found in several functionally unrelated proteins such as the bacterial SWI2/SNF2 ATPase and the plant MuDR transposases, among others.¹³ Residues 443 to 492 of MAP3K1 show the typical pattern of the plant homeodomain (PHD) and its closely related RING domain. Like many RING-domain-containing proteins, MAP3K1 exhibits E3 ubiquitin ligase activity. MAP3K1 catalyzes the poly-ubiquitylation of c-Jun and ERK1/2, resulting in their degradation by proteasomes.^{12,14} The SWIM domain directly binds c-Jun and is required for MAP3K1-mediated c-Jun ubiquitylation.¹⁵ MAP3K1 can also ubiquitylate itself without causing its degradation, and this auto-ubiquitylation leads to the inhibition of downstream signaling.¹⁶ MAP3K1 ubiquitylation requires both functional RING and kinase domains.¹²

As an additional facet of dynamic signaling control, MAP3K1 has a scaffold function, capable of binding multiple signaling proteins. MAP3K1 binds MAP2K4 and JNK via sequences within its kinase domain and N-terminus.^{18,19} GTP-bound Ras, Raf-1, MAP2K1, and ERK2 were also purified in complexes with MAP3K1.^{20,21} MAP3K1 binds GTPases of the Rho superfamily controlling the actin cytoskeleton and cell migration. Rac and Cdc42 bind the C-terminal kinase domain of MAP3K1,²² while RhoA and Rho-GAP binding sites are N-terminal of the kinase domain.²³ RhoA binding requires the GTP bound state of the

GTPase and increases MAP3K1 kinase activity.

MAP3K1 integrates signals from multiple upstream stimuli by interacting with several different kinases, including c-Abl,²⁴ NIK,²⁵ GCK,⁹ HPK1,²⁶ RIP,²⁷ PKC β ,²⁸ and GSK3 β .²⁹ MAP3K1 can also bind several adaptor proteins such as TRAF2,³⁰ Grb2,³¹ and Axin.³² Interaction of MAP3K1 with different proteins controls the subcellular localization of MAP3K1 signaling complexes. For example, FAK and α -actinin tether MAP3K1 to actin stress fibers entering focal adhesions.^{33,34} Caspase cleavage of MAP3K1 releases the kinase domain into the cytosol.³⁵ Thus, the

specific signal output emanating from MAP3K1 depends on its specific posttranslational modifications, cellular binding partners, and subcellular localization.

MAP3K1 Has Both Anti- and Pro-Apoptotic Functions

MAP3K1 is activated by a variety of stimuli such as growth factors, proinflammatory cytokines, microtubule disruption, cell shape disturbance, cold temperature, mild hyperosmolarity, and other cell stresses.^{36,37} Activation of full length MAP3K1 stimulates both MAP2K4/7-JNK and MAP2K1/2-ERK1/2 pathways.^{4,5,38} MAP3K1 has also been reported to activate the p38 pathway possibly due to the ability of MAP2K4 to phosphorylate p38.^{4,18,39} However, the effect of MAP3K1 on ERK and p38 is modest compared with its ability to activate JNK. *MAP3K1* gene knockouts defined the essential role of MAP3K1 for JNK activation in response to several of the stress stimuli listed above.^{36,40} In response to proinflammatory cytokines and specific stresses, MAP3K1 may phosphorylate and activate I κ B kinase α and β (IKK α/β), leading to NF- κ B activation.^{41,42} ERK and NF- κ B pathways promote cell survival while JNK, acting through the AP-1 transcription factors (particularly c-Jun), has both pro- and anti-apoptotic effects depending on the cell type and stimulus.⁴ MAP3K1 deletion further substantiated its pro-survival function, as demonstrated by increased cell death in response to mild hyperosmotic stress, cold temperature, and microtubule disruption in mouse embryonic stem cells and to oxidative stress in stem cell-derived cardiac myocytes.^{37,43}

Confirming its critical role in balancing cell fate decisions, MAP3K1 has been shown to be important in mediating apoptosis in response to multiple cell stresses that include genotoxins,¹¹ anoikis,⁴⁴ growth factor withdrawal,⁴⁵ and Fas ligand.⁴⁶ Following an apoptotic signal, MAP3K1 is cleaved by activated caspase-3 to generate a 91-kDa C-terminal fragment that contains the kinase domain.¹¹ The soluble C-terminal fragment of MAP3K1 is cytosolic, and the kinase activity induces apoptosis. This catalytic fragment exhibits selectivity toward JNK with little activation of ERK1/2.^{5,35,38,46} The strong activation of JNK promotes stabilization of p53 and phosphorylation of Bcl family proteins.^{35,46-51} MAP3K1 cleavage also suppresses activation of the pro-survival NF- κ B pathway.⁴⁶ Ectopic expression of MAP3K1 often leads to apoptosis because of its susceptibility to cleavage, leading to the generation of the constitutively active 91-kDa fragment.^{45,52} Mutation of the caspase cleavage site, expression of kinase-inactive MAP3K1, or tethering the kinase domain to membranes strongly suppresses MAP3K1-induced apoptosis.^{35,53} Loss of caspase-3 cleavage of MAP3K1 confers resistance to the chemotherapeutic agent cisplatin.⁵⁴ Functionality of the PHD/RING domain was reported to be involved in MAP3K1-mediated

JNK activation and apoptosis in response to cytoskeleton-disrupting drugs, but the mechanism is unclear.⁵⁵ The cumulative properties determined for MAP3K1 suggests it has an unusual molecular switch that changes its function in regulating cell fate controlled by caspase 3 and subcellular localization (Fig. 1B).

Role of MAP3K1 in Regulation of Cell Migration

In addition to promoting survival or apoptotic decisions, MAP3K1 regulates the motility and migration of various cell types. Newborn *MAP3K1* knockout mice fail to close their eyelids because of a defect in epithelial sheet migration.⁴⁰ Embryonic fibroblasts and stem cells lacking MAP3K1 have impaired motility while overexpression of MAP3K1 induces lamellipodia-like structures.^{36,40} MAP3K1 regulation of cell motility is, in part, mediated by the protease calpain localized at focal adhesions.³⁴ ERK1/2 activation by MAP3K1 at the site of actin fibers associating with focal adhesions leads to calpain activation. Calpain catalyzes the cleavage of several focal adhesion proteins involved in the control of rear-end detachment during migration. MAP3K1 also regulates the expression of urokinase-type plasminogen activator (uPA) by modulating the expression, activity, and stability of AP-1 transcription factors.^{56,57} uPA catalyzes the proteolysis of plasminogen to produce active plasmin, leading to extracellular matrix degradation. Loss of MAP3K1 expression reduces cell migration and invasion and delays tumor metastasis.^{57,58}

Additional Functions of MAP3K1

MAP3K1 has been implicated in Wnt signaling. Wnt stimulation of cells was shown to induce association of MAP3K1 and Axin, a negative regulator of Wnt signaling.⁵⁹ Knockdown of MAP3K1 impairs the expression of Wnt target genes. Interestingly, the integrity of the PHD/RING domain, but not the kinase domain, is essential for MAP3K1 regulation of Wnt signaling. The association of Axin and MAP3K1 also activates JNK via MAP3K1-MAP2K4 when Axin is overexpressed.³²

Crosstalk between MAP3K1 and hormone receptor signaling has also been reported. MAP3K1 can activate the transcriptional activity of estrogen receptor (ER) in endometrial and ovarian cancer cells⁶⁰ as well as androgen receptor in prostate cancer cells,⁶¹ possibly through JNK and p38 pathways. In endometrial and ovarian cancer, overexpression of kinase active MAP3K1 shifts 4-hydroxytamoxifen from an antagonist to an agonist of ER signaling, indicating that the agonistic activity of this endocrine therapeutic compound can be modulated by MAP3K1.⁶⁰ In prostate cancer cells, apoptosis induced by overexpression of the constitutively active MAP3K1 is dependent on

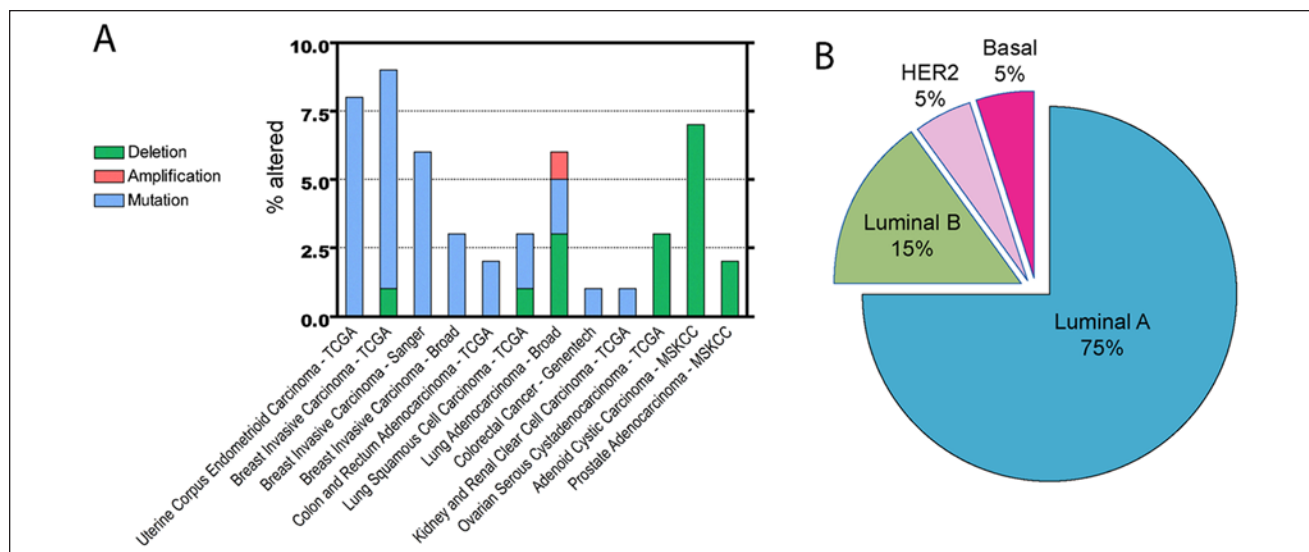


Figure 2. Alterations in *MAP3K1* genes are observed at relatively low frequency in multiple cancers with prevalent occurrence in luminal A breast cancer. **(A)** Histogram illustrating the percentage of *MAP3K1* alterations for each cancer study: deletion (green), amplification (red), mutation (blue). **(B)** Alterations of *MAP3K1* in the TCGA Breast Invasive Carcinoma data set are predominant in the luminal A, followed by luminal B, breast cancer subtypes. The data were obtained and analyzed by cBioPortal (<http://www.cbioportal.org/public-portal/>).^{64,65}

the presence of androgen receptor.⁶¹ Another transcription factor, Stat3, which is the effector of interferon and cytokine signaling, is phosphorylated and activated by MAP3K1 following EGF stimulation, and MAP3K1 activation of Stat3 requires the known upstream activators of Stat3, Jak, and Src.⁶²

Genetic investigation of familial and sporadic cases of the 46,XY disorder of sex determination revealed that the disease is associated with mutations of *MAP3K1* that increase ERK and, to a lesser extent, p38 phosphorylation in lymphoblastoid cell lines isolated from patients.⁵³ The mutations occur in the Grb2, FAK, and RhoA binding regions, causing an enhancement of RhoA binding, which potentially up-regulates MAP3K1 activity. This observation emphasizes a role of MAP3K1 in human development of sexual organs and sex determination.

MAP3K1 and the Cancer Genome

Gene expression, whole exome sequencing, and copy number variation analyses of substantial numbers of multiple human tumor types hold the promise of deciphering the genomic heterogeneity of cancer. While deregulation of the MAPK signaling architecture has long been appreciated as a frequent event in tumorigenesis, recent efforts to systematically categorize the human cancer genome have illuminated the potential contribution of specific gene alterations. In a study aimed at identifying novel somatic mutations, Kan *et al.* utilized >400 tumor samples from breast, lung, ovarian, pancreatic, and prostate cancers.⁶³ *MAP3K1* copy

number loss and somatic missense or nonsense mutations were observed in a significant fraction of the tumor data set. *MAP3K1* mutations were more frequently observed in hormone receptor positive (HR+) breast cancer as compared with other tumor types and additional breast cancer subtypes, including HER2+ and triple-negative. Subsequent to this study, increasing numbers of cancer genome analyses from a multitude of tumor types have enriched our appreciation of alterations that occur at lower frequencies but may have significant impact on tumor characteristics and patient outcomes. This is certainly true for the deletion or mutation of *MAP3K1* found at a low to modest frequency in many tumor types (Fig. 2).

Assessment of *MAP3K1* alteration frequency across multiple data sets using the cBio Cancer Genomics Portal^{64,65} further substantiates the notion that *MAP3K1* loss-of-function contributes to specific cancer phenotypes (Fig. 2A). Ovarian, prostate, and adenoid cystic carcinoma exhibit copy number loss of *MAP3K1*, while uterine and breast carcinomas predominantly harbor somatic mutations. The extensive genomic analyses of breast cancer samples have been augmented by the classification of distinct subtypes (luminal A, luminal B, HER2, and basal) based on their mRNA expression patterns. Estrogen receptor (ER) positive breast cancer includes the luminal A and luminal B subtypes, differentiated generally by higher grade and poorer prognosis in luminal B as compared to luminal A. Amplification of the *HER2/ERBB2* gene typifies the HER2 subtype, while the basal subtype (also termed triple-negative breast cancer, TNBC) lacks expression of ER, progesterone

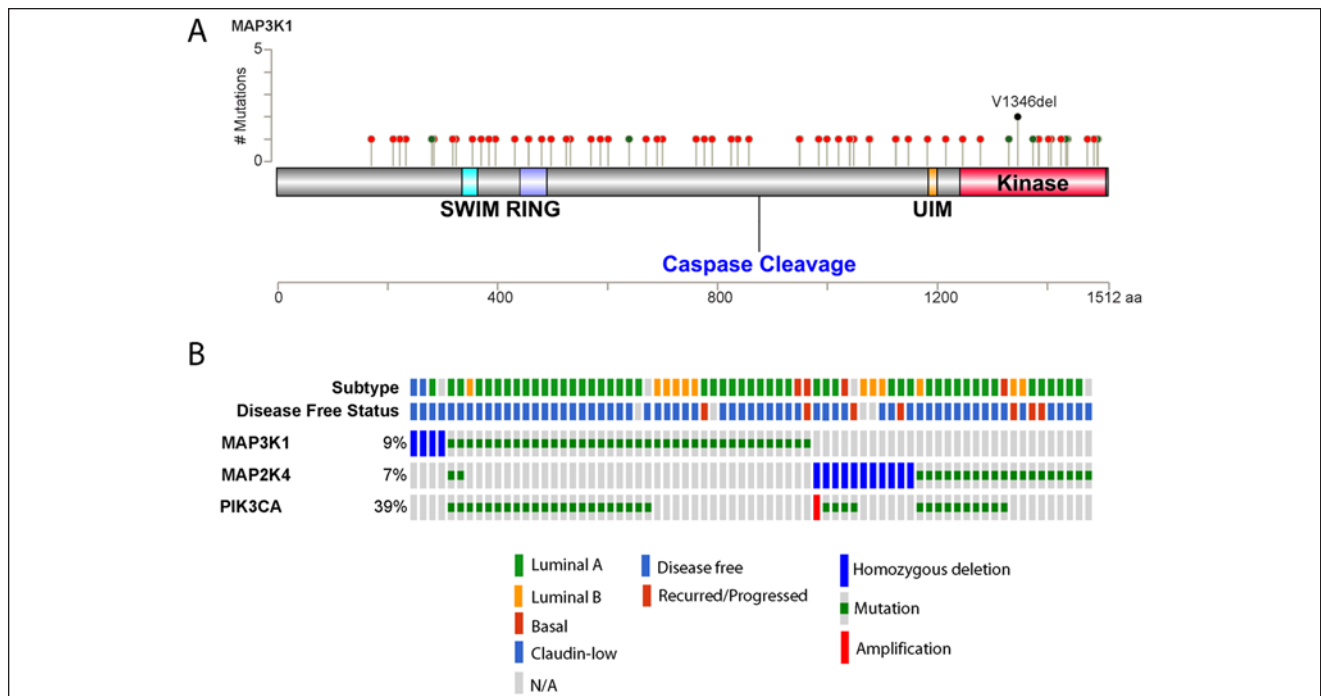


Figure 3. Characterization of *MAP3K1* alterations in breast cancer. **(A)** Somatic mutations found in *MAP3K1* gene by the TCGA Breast Invasive Carcinoma study are predicted to be loss-of-function. The mutations are represented by circles: nonsense, splicing mutations, frameshift deletion, and insertion (red), missense mutations (green), and inframe deletion (black). **(B)** *MAP3K1* alterations in breast cancer are mutually exclusive with those of *MAP2K4* and partially overlap with those of *PIK3CA*. Shown is the oncoprint of *MAP3K1*, *MAP2K4*, and *PIK3CA* genes, in which individual samples are represented as columns and individual genes are represented as rows. Subtype assignment and disease-free status of the patients are shown in the first 2 rows. The data were obtained and visualized by cBioPortal database using the TCGA Breast Invasive Carcinoma data set with modifications.

receptor, and HER2. Somatic mutations in the Cancer Genome Atlas study of breast invasive carcinoma in the context of mRNA expression subtypes revealed that *MAP3K1* alterations were enriched in the luminal A subtype (Fig. 2B).⁶⁶ Strikingly, the occurrence of mutations in *MAP3K1* among luminal A samples was second only to mutations in *PIK3CA*. While the observation of copy number loss may suggest a putative tumor suppressor function of the gene product, the interpretation of somatic mutation data requires more rigorous analysis. Visualization of the mutations in *MAP3K1* from the TCGA breast cancer data set reveals the absence of any hotspots or domain-specific alterations (Fig. 3A). Nonsense mutations were observed (19% of observed mutations) but the majority of mutations result in a frame-shift deletion or insertion (59%) and are predicted to be inactivating. Only 1 specific somatic mutation (an in-frame deletion) occurred in 2 unique samples and 4 of the 6 missense mutations observed occurred in the kinase domain of *MAP3K1*. Down-regulation, loss of function mutations, and homozygous deletion of the *MAP3K1* downstream target *MAP2K4* have also been reported in clinical samples from several types of cancer such as prostate, pancreatic, and ovarian cancer.^{50,66-69} Comparable portraits of *MAP3K1* and *MAP2K4*

alterations were observed in separate large-scale breast cancer data sets.^{67,70}

Functional Consequences of *MAP3K1* Perturbation in Cancer

The significance of specific genetic alterations in cancer genome data sets can be bolstered by the concurrent analysis of additional components that constitute a given pathway or functional complex. Alterations of 2 genes that affect the same pathway are typically not seen in a single individual. For example, samples with *MAP3K1* alterations in the TCGA breast invasive carcinoma data set are notably distinct from those with alterations in *MAP2K4* (Fig. 3B). Given the interplay of *MAP3K1* and *MAP2K4* in cell fate decisions (Fig. 1B), it can be hypothesized that the loss of function of either gene product is sufficient to deregulate the pathway in predominantly luminal A breast cancer (Fig. 3B). In contrast, activating mutations in *PIK3CA* are observed in approximately 50% of samples harboring *MAP3K1* or *MAP2K4* alterations. While it is known that PI3K-AKT signaling negatively regulates *MAP3K1*-JNK, the interplay between these 2 pathways in luminal A breast cancer deserves more study.

The majority of breast cancers are luminal, and the luminal A subtype is characterized by better prognosis and lower rate of recurrence than the other subtypes (Fig. 3B).⁶⁷ The reconciliation of MAP3K1 alterations in cancer, and the luminal A breast cancer subtype specifically, with known MAP3K1 function remains an open question. Mammary gland involution is a complex process of coordinated apoptosis and tissue remodeling that occurs postlactationally and, in a related but distinct process termed lobular involution, occurs with age.^{71,72} Delay or failure to undergo both types of involution has been identified as a breast cancer risk factor.⁷¹ One hypothesized explanation for this correlation is that involution removes the pool of epithelial cells that may represent cancer progenitors. Given the strong association between caspase activation and mammary gland involution, it is reasonable to hypothesize that MAP3K1 might play an important role in the process of involution. Loss of MAP3K1 function causes insensitivity to cell death, potentially promoting the emergence of cancer cells. Moreover, with roles in the degradation of focal adhesion and extracellular matrix as discussed above, MAP3K1 might be essential for the detachment of epithelial cells from alveolar cells, causing the cells to die during anoikis.⁷² MAP3K1 is also capable of activating NF- κ B, Stat3, and calpains, all of which have been reported to induce the lysosomal pathway of cell death during involution.⁷² Future studies dissecting the role of involution as a determinant of breast cancer subtype and outcome with regard to MAP3K1 functioning will be enlightening.

Perturbation of MAP3K1 signaling affects multiple pathways—IKK-NF κ B, ERK1/2, and JNK. Thus, the impact of MAP3K1 mutation and deletion on cancer cell signaling networks demand further investigation. For example, TCGA cases of uterine corpus endometroid carcinoma with observed MAP3K1 alterations trend toward better overall survival ($P = 0.071$) when compared with unaltered MAP3K1 cases.⁷³ Concomitantly with defective apoptosis, MAP3K1 impairment can result in defects in pro-survival signaling and cell migration, which have the potential to reduce tumor growth and metastasis. Whether this is related to the better prognosis of luminal A breast cancer and uterine corpus endometroid carcinoma with MAP3K1 mutations needs evaluation. Contrary to this hypothesis, MAP3K1 and MAP2K4 were identified as metastasis suppressors in ovarian, prostate, and gastric cancer through both clinical studies and experimental models.⁵⁰ Therefore, it will be of interest to determine if other cancer types with functional loss of MAP3K1 are similar to the luminal A breast cancer subtype in molecular phenotype and clinical outcome. In a similar manner, elucidation of the specific contribution of MAP3K1 signaling (and possibly MAP2K4) to the luminal A mRNA signature may provide insight into the subtype-specific phenotype.

Recently, an *in vivo* mutagenesis screen in mice identified MAP3K1 as a driver in melanomagenesis. Ni *et al.* utilized a *piggyBac* transposon strategy that identified multiple activating insertions in introns 9 and 10 of the *MAP3K1* gene.⁷⁴

These insertions were shown to result in the production of truncated MAP3K1 products lacking the N-terminal SWIM and RING domains (Fig. 1A). On ectopic expression, these aberrant forms of MAP3K1 activated ERK1/2 that was sustainable even in the presence of a BRAF inhibitor and promoted proliferation in the absence of growth factors in melanocytes. Thus, constitutively active MAP3K1 functioned as a driver of melanoma in their model. To support this observation, the TCGA melanoma data set available on cBioPortal database showed *MAP3K1* amplification and mRNA up-regulation in approximately 5% of human melanomas.^{64,65} Taken together, these data suggest that MAP3K1 may function as a driver in some cancer types (e.g., melanoma) and is inactivated in other types (e.g., luminal A breast cancer). This dichotomy is consistent with MAP3K1 having a molecular switch-like function for cell survival or apoptosis controlled, in part, by caspase 3 and subcellular localization.

Future Directions/Concluding Remarks

At present, a signaling network displaying MAP3K1 as the central hub illustrates the complex and intricate nature of its function *in vivo*. Undoubtedly, cell type-specificity and even tissue environment are critical determinants of the cell fate decisions mediated by MAP3K1. Recently, the comprehensive nature of genomic efforts to categorize human cancer has provided further insight into the molecular contribution of heretofore unappreciated and understudied gene products. Certainly, MAP3K1 is an understudied kinase in relation to its function in cancers. Given the relative frequency of MAP3K1 mutations in many tumor types, ongoing investigation of MAP3K1 function will be important for providing answers to questions surrounding specific cancer phenotypes and their vulnerabilities when MAP3K1 is mutated or deleted.

Declaration of Conflicting Interests

The author(s) declared no potential conflicts of interest with respect to the research, authorship, and/or publication of this article.

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