MEKK2: A Potential Target for Cancer Cell Migration and Metastasis

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A critical need exists for cancer therapies that block metastasis. Cancer metastasis requires that tumor cells move from the primary tumor to other tissues, and release of cell adhesion to the extracellular matrix is necessary for cell migration. Cellular signaling networks control tumor cell functions essential for metastasis, including migration and adhesion. For example, the mitogen-activated protein kinases (MAPK) regulate cell migration, adhesion, survival and proliferation, as well as other functions essential for tumor progression. We have demonstrated that silencing expression of the MAPK regulator MEKK2 inhibits breast tumor growth and metastasis in vivo, and decreases breast tumor cell migration in vitro. We discovered that tumor cell attachment to the matrix protein fibronectin induces MEKK2 activation and localization to protein complexes called focal adhesions that form at the cellular interface with extracellular matrices to control cell attachment. Furthermore, we found that MEKK2 controls the composition of focal adhesions, at least in part, by promoting modification of focal adhesion components leading to focal adhesion disassembly and turnover. We propose that the MEKK2 signaling network may contain novel therapeutic targets for blocking cancer metastasis. Herein we provide an update on our current work and highlight future research that will focus on defining the mechanisms by which MEKK2 and its effectors influence tumor cell function.

Keywords: MEKK2; MAP3K2; MAPK, kinase; focal adhesion; fibronectin; paxillin

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Introduction

Breast cancer progression from primary tumor to distant metastases dramatically reduces survival. For example, Kennecke et al. reported that the 10-year survival estimate for patients with an aggressive subcategory of breast tumors was 62.6% whereas the median duration of survival from the time of first distant metastasis was only 0.9 years. This severely worsened prognosis underscores the need for effective treatments for metastatic cancer. Cancer therapeutic options for effective treatment of metastatic cancer are very limited, nonspecific, and often ineffective at blocking disease progression. Therefore, we are interested in determining whether specifically targeting genes essential for tumor progression and metastasis could be an effective and specific approach to develop novel...
therapeutics for treating metastatic breast cancer.

The process of metastasis involves cancer cell movement from the primary tumor into nearby blood or lymph vessels where they may travel to distant tissues, extravasate from the vasculature/lymphatics, and ultimately form new tumors [2]. As tumor cell migration is required for these metastasis steps, cell migration is a logical target for the development of novel therapies against cancer metastasis. Cell migration is a tightly regulated and dynamic process that depends on coordinated spatiotemporal control of cell engagement and release of adhesion to the extracellular matrix. This cell-matrix adhesion is controlled by membrane-bound matrix receptors called integrins, and by integrin-associated protein complexes called focal adhesions that are involved in each step of cell migration including attachment of the leading edge and the formation of immature focal adhesions, followed by focal adhesion maturation and cell body translocation, and finally focal adhesion disassembly and trailing edge retraction [3]. Specifically, protein components of focal adhesions assemble on the intracellular side of matrix-bound integrins and transduce signaling between the extracellular matrix and the cell. Structurally, the integrin focal adhesome consists of cytoskeletal, adaptor, and signaling proteins, which Zaidel-Bar et al. most recently reported to total 180 proteins; a number that has been growing over recent years as more are identified [4, 5]. Functionally, focal adhesions mediate the necessary cell signaling and “traction” of the cell onto a substrate for myosin II motors to generate the force, by sliding on actin filaments, for cell translocation. The necessity of focal adhesions to be both strong and durable as well as capable of undergoing continuous assembly and disassembly requires an elaborate regulation system including changes in components, conformations, and signal transduction [3, 6]. For example, the role of MAPKs in cell migration was reviewed by Wu and colleagues and others suggesting that several migration signaling pathways may converge on c-Jun amino-terminal kinase (JNK)-mediated phosphorylation of paxillin, a key focal adhesion scaffolding protein [7, 8]. However, the precise mechanisms that regulate focal adhesion protein expression, the identity of regulators, trafficking and order of component assembly and disassembly, remain largely unknown [6]. Collectively, cell migration is an essential component of cancer metastasis, and cell migration depends on focal adhesion turnover that is controlled by signal transduction regulators,
including JNK. Further investigation of signal transduction pathways involved with focal adhesions and cell migration may provide potential targets to inhibit cancer cell migration and metastasis.

MAPks and MEKK2

MAPK signal transduction encompasses a network of pathways that regulate multiple cellular processes including growth, differentiation, stress response, migration and survival [10]. MAPK pathways are activated in response to diverse cell stimuli ranging from growth factors to stress and mechanical force. In the canonical MAPK signaling module, a MAP kinase kinase kinase (MAP3K) phosphorylates activation loop residues of a MAP kinase kinase (MAP2K), which becomes activated and in turn phosphorylates and activates a MAPK. Due to their importance in both homeostasis and disease, the signaling pathways that activate the ERK1/2, JNK1/2/3, and p38/α/β/γ pathways have been extensively studied. Given that MAP3Ks outnumber MAPKs (22 MAP3Ks and 12 total MAPK isoforms), we have previously proposed that the MAP3K signaling level provides substantial specificity for transmitting upstream signaling onto downstream MAPK pathways [11]. Importantly, some MAP3Ks can activate multiple MAP2Ks to regulate at least 2 MAPks. For example, MEKK2 can activate MKK4 and MKK7, as well as MEK5, to activate JNK and ERK5/BMK1, respectively [11, 13]. MEKK2 has been shown to regulate MAPK signaling in response to multiple upstream cell stimuli including EGF, FGF, sorbitol, UV, and others [12, 13].

MEKK2 is a ~70 kDa protein with an amino-terminal phox and Bem1p (PB1) domain and a C-terminal kinase domain. Cheng et al. reported that MEKK2 homodimerizes via its kinase domain thereby activating the kinase likely via transphosphorylation [14]. Furthermore, MEKK2 can heterodimerize with MEK5 via the PB1 domains of both proteins to increase MEK5 activation [15], which may also be facilitated by MEKK2 association with the scaffold protein Lad/RIBP [16]. In the JNK pathway of MEKK2 signaling, Hammaker et al. reported that MEKK2 phosphorylation of MKK4/7 was increased by IL-1 and that downstream activation of c-Jun through JNK was dependent on IL-1. This finding was particularly relevant to inflammatory syndromes, including rheumatoid arthritis [17]. Although several protein interactions have been identified for MEKK2, there remains a significant gap in knowledge of MEKK2 structure, function, and regulation including upstream components and mechanisms of activation, intracellular trafficking, substrates (if any) other than MAP2Ks, and negative regulation following an activating stimulus (e.g. by ubiquitin ligases or phosphatases). Defining these and other aspects of MEKK2 will be important for basic understanding of its functioning as well as for its relevance to diseases.

Interestingly, several MAP3Ks have activating mutations or altered expression in different cancer types including most notably B-Raf [18, 20], but also A-Raf, Tpl2, and MEKK3 [21, 22]. Strikingly, MEKK1 is the 2nd most mutated gene in luminal A breast cancers from the Cancer Genome Atlas study of breast invasive carcinoma [23]. Additionally, MEKK1 copy number loss and somatic or nonsense mutations were observed in a significant fraction of over 400 tumor samples examined by Kan and colleagues [24]. As with other MAP3Ks, MEKK2 may play a role in cancer development and/or progression. For example, the CBio Cancer Genomics Portal shows that MEKK2 is mutated, deleted, or amplified in many types of human cancer, including breast, prostate, and others. Furthermore, several downstream MEKK2 effector components are also altered in human cancer including ERK5, M KK4, AP-1, MEF2, and others. Therefore, in addition to MEKK2 specifically, the MEKK2 signaling network may provide therapeutic targets for cancer. This finding is perhaps not surprising given that the ERK1/2, JNK, p38, and ERK5 pathways have each been reported to play a role in a variety of cancers.

Knockdown of MEKK2 inhibits tumor growth and metastasis in vivo

Given the links between some MAP3K and cancer progression, discussed above, we combined shRNA-mediated gene silencing with whole animal imaging of xenograft tumor progression to directly examine the role of 9 MAP3Ks, including MEKK2, in tumor growth and metastasis. We silenced individual MAP3K genes in triple negative (ER-, PR-, HER2-) MDA-MB-231 claudin-low human breast cancer cells, and then orthotopically injected the cells into mouse mammary glands and monitored the resultant tumors for growth and metastasis [25, 26]. Blocking MEKK2 expression significantly reduced tumor growth and metastasis in vivo. Interestingly, although MEKK2 silencing reduced tumor growth in vivo, MDA-MB-231 cells with stable shRNA-mediated knockdown of MEKK2 do not exhibit significantly different proliferation rates or viability compared to wild-type MDA-MB-231 cells in growth conditions in vitro. This surprising distinction underscores the importance of MEKK2 expression in tumor cells within the tumor microenvironment. Therefore, one research topic of great interest to our lab is this different role of MEKK2 in proliferation in vivo and in vitro and identifying specific component(s) of the microenvironment that influence MEKK2 function. In addition to its impact on xenograft growth, we discovered that MEKK2 knockdown inhibits MDA-MB-231 cell migration. In the following sections we highlight our findings from our recent investigation of the importance of MEKK2 in tumor cell migration.
MEKK2 regulates focal adhesion stability and motility in invasive breast cancer cells

Our in vivo discovery led us to ask which tumor cell functions were under the control of MEKK2. We again utilized MEKK2 shRNA vectors to silence or “knock down” MEKK2 expression in MDA-MB-231 cell lines, and then performed cell function assays comparing the parental cell line to MEKK2-deficient cells to define which metastasis-related functions are regulated by MEKK2. These genetic tools gave us the means to probe MEKK2 regulation and function related to MEKK2 subcellular localization and its effects on cell attachment, spreading, focal adhesion stabilization, migration, and MAPK signaling [22].

During the course of our investigations, we developed a novel and extremely valuable technique wherein we utilized IMARIS software (Bitplane Inc.) to reconstruct cellular structures in three dimensions using fluorescent microscopy images, with the goal of quantifying the spatial and temporal regulation of individual proteins. In this technique, Z-stack fluorescent images are first captured and deconvoluted using softWoRx software (Applied Precision) and rendered in 3D using IMARIS. Our imaging technique is used to calculate the intensity of the desired “target marker” specifically within the volumes created by the surfaces of the surface-defining marker (in intensity units/µm³). For example, in our initial experiments we used vinculin, a hallmark focal adhesion protein, as the surface-defining marker to construct the 3D volumes of focal adhesions, and MEKK2 as the target marker. Using this technique we determined that the extracellular matrix molecule fibronectin induced MEKK2 recruitment into focal adhesions over time when MDA-MB-231 cells were attached to fibronectin.

Importantly, we determined that silencing MEKK2 expression resulted in increased numbers and volumes of focal adhesions with an overall increase in cell spreading. Furthermore, this increased cell adhesion induced by MEKK2 knockdown translated into a decrease in MDA-MB-231 cell migration compared to control cells. Our subsequent investigation into the mechanisms by which MEKK2 regulates adhesion number and size revealed that tumor cell attachment to fibronectin induced both MEKK2 translocation to focal adhesions and MEKK2 kinase activation, resulting in increased signaling to downstream effectors MEK5 and ERK5. Furthermore, blockade of either MEK5 or ERK5 kinase activity abolished MEKK2’s effects. Interestingly, fibronectin-stimulated MEKK2 also lead to increased phosphorylation of focal adhesion kinase (FAK), a key regulator of focal adhesion turnover and persistence. The significance of MEKK2-dependent FAK activity is currently an area of investigation in our lab.

It is possible that MEKK2-dependent MAPK activity controls focal adhesion formation or disassembly. MAPKs have previously been linked to focal adhesion regulation by our group and others. For example, JNK is an MEKK2 effector that was reported to increase focal adhesion turnover and migration. Indeed, Huang and colleagues reported that JNK1 phosphorylated the focal adhesion adaptor protein and scaffold paxillin on serine 178 in multiple cell types, which, in turn, influenced focal adhesion stability [31]. In a second example, we found that MEKK1, a related MAP3K protein, was necessary for membrane-proximal ERK1/2 activity that promotes function of the protease calpain, which subsequently cleaves the focal adhesion structural components spectrin and talin [29]. A third example of MAPK function in focal adhesions was provided by Sawhney and colleagues, who demonstrated that ERK5 could promote autophosphorylation and activation of FAK [29]. Given these links between MAPKs, focal adhesion turnover, and cell migration, we are currently exploring mechanisms by which MEKK2 might regulate focal adhesion stability.

Future directions

Given our findings described above detailing at least part of MEKK2’s mechanistic role in cancer cell metastasis, migration, and focal adhesion turnover, multiple important potential directions for our future research emerge. For example, our understanding of the upstream mechanisms by which ligation of receptors (e.g. the EGFR or integrins) lead to activation of MEKK2 is poorly understood and we are keenly interested in defining how MEKK2 becomes activated by these diverse stimuli. However, our current emphasis is to determine whether MEKK2 substrates are limited to those which have previously been reported or whether unidentified substrates remain. Indeed, whereas several binding partners have been identified for MEKK2 including Lad/RIBP [30], CHIP [31], PRK2 [31], and other interacting proteins that we have uncovered thus far (unpublished observations), much less is known about whether any of these represent MEKK2 kinase substrates. Therefore, our laboratory is actively seeking to identify novel MEKK2 kinase substrates, as well as to determine how MEKK2 forms functional complexes with these candidate effectors. Once substrate proteins have been identified, we will investigate the functional relevance of their phosphorylation by MEKK2, with an emphasis on defining the mechanisms by which these modifications influence tumor cell migration and metastasis.

Finally, given our observation that MEKK2 ablation blocks metastasis, we recognize that the long-term goals of this research would be greatly enhanced by the development of specific MEKK2 inhibitors. To that end, some initial drug discovery efforts have yielded encouraging results [32], and we anticipate that this will be
an important part of our future research efforts.

Conclusions

A simplified model of our proposed MEKK2 signaling network, including binding partners, kinase substrates, and effectors is provided in Figure 1. MEKK2 is a MAP3K that is required for mammary xenograft metastasis. Our discoveries indicate that MEKK2 regulates breast cancer cell migration, and we propose that this function is mediated at least partly through control of focal adhesion components and turnover. Furthermore, we propose that the MEKK2 signaling network contains potential novel target proteins for the development of cancer metastasis therapy. Our ongoing research is directed toward further defining the MEKK2 network and we will use this important information to develop novel therapies to treat diseases in which MEKK2 plays an important role, including breast cancer metastasis.

Conflict of interest

The authors declare that they have no Conflicting interests.

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