A ROLE FOR MEK3 IN THE ONCOGENESIS OF ACUTE LYMPHOCYTIC LEUKEMIA: INACTIVATION OF MAPK P38 PROMOTES CELL PROLIFERATION THROUGH ENHANCED DEGRADATION OF MUTANT MEK3

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Dedication

To my amazing husband:

Emmanuel

To my lovely parents:

Roberto and Iris

To my siblings:

Yamilie and Alex

To my princesses:

Alexandra and Kamila

Por su amor incondicional, oraciones, abrazos y risas. Los amo!!

(For your unconditional love, prayers, hugs, and laughter. I love you all!!)

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Abstract

In the United States, pediatric leukemia has the second-highest cancer mortality rate in Hispanic children, especially Acute Lymphocytic Leukemia (ALL). While treatment of ALL has improved overall the five-year survival rate of ~90 %, not everyone has benefited. Twenty percent of them will experience relapse, and from these, 30 – 50 % will die. Unfortunately, the cause behind these dreadful statistics is poorly understood due to the complex etiology of this disease. Thereby, it is essential to identify potential oncogenic proteins that promote ALL so that new strategies can be developed to diagnose and treat this cancer. Whole Exome Sequencing (WES) coupled with OncoMiner Pipeline sorting identified five Single Nucleotide Polymorphisms (SNP's) on the Mitogen-Activated Protein Kinase Kinase 3 (MEK3) (MAP2K3) gene in El Paso del Norte ALL cancer patient library. MAP2K3 mutations were recreated using site-directed mutagenesis and transfected into HEK293 cells to study their impact on cell function. Three mutations were located within the kinase domain and two others located to the MEK3 amino domain. Transfection of HEK293 cells revealed these variants impact protein stability by inducing increased degradation of MEK3. Data shown here further suggest that they serve to block the auto-phosphorylation of MEK3, and loss of kinase activity towards p38. MEK3 is responsible for the activation of MAPK p38 to mediate growth-inhibitory and pro-apoptotic signals. Thus, inhibition of p38 activity through enhanced degradation of MEK3 mutants renders this pathway nonfunctional contributing to tumor cell proliferation. These findings indicate MEK3 represents a therapeutic target for controlling and treating ALL.

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1.1 The Mitogen-Activated Protein Kinase (MAPK) Signaling Pathway

The Mitogen-activated Protein Kinase (MAPK) signaling pathway is comprised of a group of four serine/threonine MAPKs: Extracellular Signal-Regulated Kinases (ERK1/2), Jun-N-terminal kinases (JNK1/3), p38 (α, β, γ, δ) and ERK5 [1-3]. Each group consists of three kinases activated in a cascade, including MAPK kinase kinase (MAPKKK), MAPK kinase (MAPKK), and MAPK leading to several cellular activities including cell proliferation, differentiation, and apoptosis [3]. Conserved across yeast and mammals, dysregulation in the MAPK pathway are associated with neurodegenerative diseases and cancer [4].

The ERK pathway

ERKs consist of a group of five serine/threonine kinases (ERK 1, 2, 3, 5, and 6) that are localized to the cytoplasm. Once activated, they translocate to the nucleus and regulate transcription factors involved in biological processes including cellular proliferation, apoptosis, and cell cycle regulation [5]. A-Raf, c-Raf1, and B-Raf are the MAPKKKs responsible for the activation and phosphorylation of MEK1 and MEK2 (MAPKKs) [6] which then catalyze the phosphorylation of ERK1 and 2 at their Thr-Glu-Tyr motif [7]. ERK1 and 2 share 84 % of homology with a 44 and 42 kilodalton (kDa) molecular weight, respectively, and are the most studied. These enzymes are widely expressed and activated in response to mitogenic stimulation, viruses, and oncogenes [8]. ERK1/2 have the same substrate specificity; however, mice lacking ERK1 are viable in contrast to $ERK2^{-/-}$ mice. Both proteins are components of the Ras/Raf/MEK/ERK

signaling pathway involved in several diseases including cancer. Lastly, Ras kinase mutations are present in 30 % of all cancers, while only 7 % for Raf [8].

JNK pathway

The JNK pathway includes JNK1, 2, and 3, which are activated by stress and inflammatory signals, leading to the activation of transcription factors such as c-Jun, Elk-1, Activated Transcription Factor 2 (ATF2), which are involved in apoptosis, differentiation, and proliferation [7]. They are ubiquitously expressed except for JNK3 that is restricted to the testis, brain, and heart [7]. MEK4 and MEK7 are kinases responsible for phosphorylating JNKs conserved Thr-Pro-Tyr motif in their activation loop [7]. Mice deficient in JNK1 and 2 are viable; however, T-cell differentiation is affected [9].

The p38 pathway

MAPK p38 is made up of a group of four serine/threonine kinases. p38α (MAPK14) is widely expressed whereas the other three isoforms' expression is more restricted. p38β (MAPK11) is abundant in the brain, p38γ (MAPK12) in skeletal muscle, and p38δ (MAPK13) in testis, small intestine, and pancreas [10]. p38α and β are most homologous to each other, at to 75 % [11]. Mice lacking p38α are embryonically lethal while knockout mice for p38β, p38γ, or p38δ maintain normal development [10].

Downstream targets of p38 involve activation of MAPK-activated protein kinase 2 and 3 (MAPKAP-K2/3), mitogen-activated protein kinase-interacting protein MKN1/2 and MSK1/2; *transcription factors* such as the myocyte enhancing factor 2 (MEF2), C/EBP Homologous Protein (CHOP), ATF2, signal transducer and activator of transcription 1 (STAT1), p53, serum response factor accessory protein 1 (SAP1), nuclear factor of activated T-cells (NFATp), CDX3, peroxisome proliferators activated receptor γ

coactivator (PGC-1) and USF-1; and *cytoskeletal proteins* [11]. Negative regulation of p38 can occur by tyrosine specific MAPK phosphatases (TS-MKPs), serine/threonine-specific MKPs (SS-MKPs), and tyrosine/threonine dual-specificity phosphatases (DS-MSKPs) such as PTP-SL, PP2A, and MKP1, respectively [10]. Additionally, evidence indicates that microRNAs (such as the miR-17 ˜ 92 cluster, the miR-200 family, miR-196a, miR-124 and -128) play an important role to directly, or indirectly, promote negative regulation of p38 [10].

Auto-activation of p38 is possible under a non-canonical process, through T cell receptor (TCR) stimulation and binding of p38 to TAB1 (TAK1-binding protein 1) [12]. Activation of p38 by TCR is LCK/Zap70 dependent and induces phosphorylation on tyrosine residue 323, triggering autophosphorylation of p38 in its Thr180/Tyr182 motif. [13]. Still, the exact mechanisms for this regulation are not well understood.

1.2 Mitogen-Activated Protein Kinase Kinase (MAPKKs) Family

MAPK ERK kinases (MEK) are dual-specificity kinases that belong to the MAPKK family. They are responsible for the phosphorylation of serine/threonine and tyrosine residues of their specific MAPK substrate within their Thr-X-Tyr motif [3]. MEKs are very similar in structure to other kinases in that they possess an amino-terminal domain, a kinase domain and a carboxylic-terminal domain [3]. There are two domains, a docking (D) and a versatile docking domain (DVD), which are essential for binding MAPK and MAP3K, respectively [14]. The DVD domains are found downstream of their kinase domain [14]. Seven MEKs have been identified (MEK1-7) [3]. They share structural homology in their kinase domain, while their amino and carboxylic terminals are distinct [3] (Figure 1.1). MEK1 and MEK2 are specific activators of ERK1/2, MEK3, and MEK6 of

p38, MEK4, and MEK7 of JNK and MEK5 of ERK5 [1, 15, 16]. MEK4 can also activate p38 [1, 15, 16].

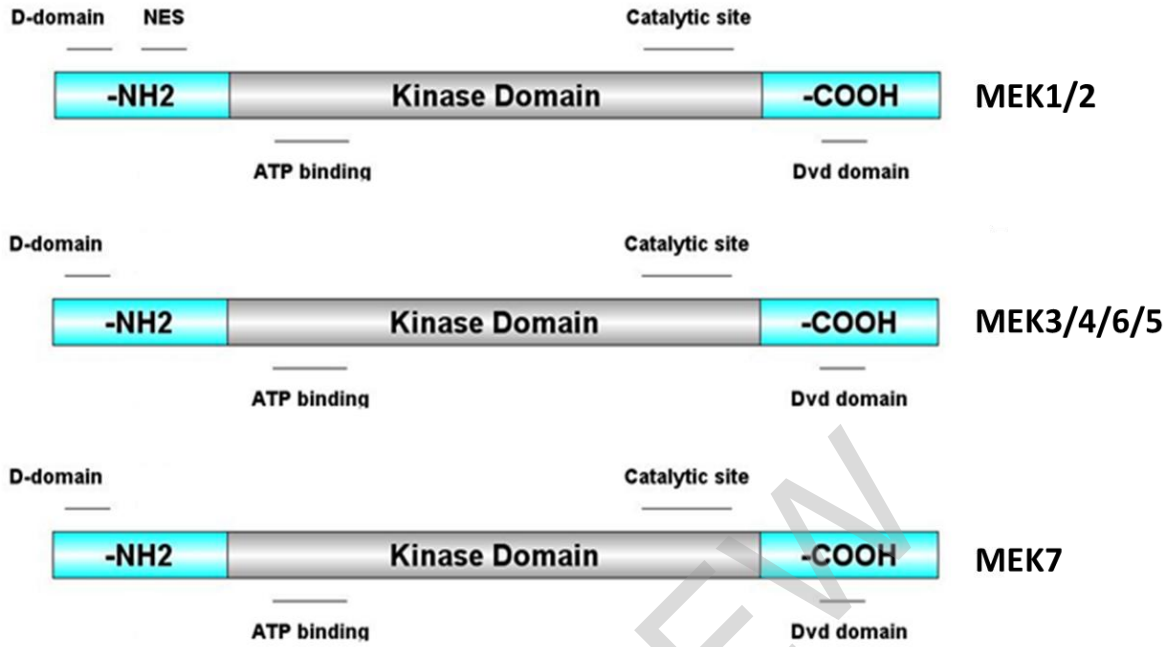

Figure 1.1: Structure of the MEK family. Schematic representation of the MEK family, which is comprised of seven members (MEK1-7). They share structural homology in their kinase domain, while their amino and carboxylic terminals are distinct. The docking (D) and the versatile docking domain (DVD) are essential for binding MAPK and MAP3K, respectively [3].