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THE ROLE OF MITOGEN-ACTIVATED PROTEIN KINASE PATHWAY IN PEDIATRIC ACUTE LYMPHOBLASTIC LEUKEMIA & ITS RELEVANCE FOR THE IN-VITRO CHEMO-SENSITIVITY

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ABSTRACT

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer worldwide. Like other types of cancer, leukemia is a multi-genic, multifactorial, and complex disease. MAPK (Ras) pathway is supposed to be vital in the development of leukemia. Mutations in the Ras gene and consequently high Ras proteins are highly prevalent in leukemia. The Ras pathway remains open as a result of activator mutations. This leads to carcinogenesis by producing uncontrolled proliferation signals in the lymphoblast. Additionally, there is a strong relationship between the high abnormal proteins of Ras pathway in ALL and in vitro cellular resistance to chemotherapeutics, the unfavorable patient's criteria, as well as worse outcome.

INTRODUCTION

Acute lymphoblastic leukemia (ALL) is the most common childhood cancer worldwide, with prevalence up to 25% of cancers in children who are under the age of 15 years. The incidence of ALL varies by age, ethnicity and geographic region, by immunologic and molecular subtypes **(Tulstrup et al., 2017; Inaba & Pui, 2021)**.

Leukemia represents a group of neoplastic disorders in the bone marrow. At a specific differentiation stage of normal lymphoid or myeloid hematopoiesis, the progenitor cells in the bone marrow arrest and undergo clonal growth. Uncontrollably, the bone marrow itself and other organs become infiltrated by the arrested immature white blood cells (blasts). Finally, this disorder results in absolute death when not treated **(Davis et al., 2014)**.

Pathogenesis and risk factors

Like other types of cancer, leukemia is a multi-genic, multifactorial, and complex disease. It is a known fact that most cases of leukemia are result from the cytogenetic disorders and the molecular changes. Less than 5% of leukemia cases have underlying hereditary genetic abnormalities. Conditions such as Dawn syndrome, congential agammaglobulinemia, and ataxia telangiectasia are associated with high incidence of leukemia. For example Dawn syndrome cases have a 10-30 folds increased risk for ALL **(Zwerdling, 2017).**

Mitogen-Activated Protein Kinase (MAPK) pathway

Mitogen-Activated Protein Kinase (MAPK) pathway is one of the most important mechanisms of eukaryotic cell regulation. It is an evolutionarily signal transduction pathway that regulates vital cellular processes such as growth, proliferation, mitosis, metabolism, differentiation and apoptosis by controlling the transcription of many genes **(son et al., 2011)**.

The MAPK pathway can be activated by wide variety of ligands that bind tyrosine kinase receptors, including growth factors [e.g., platelet-derived growth factor (PDGF), epidermal growth factor (EGF) and fibroblast growth factor (FGF)], hormones (e.g., insulin), inflammatory cytokines of tumor necrosis factor (TNF) family and environmental stresses as radiation **(Cargnello & Roux, 2011).**

The MAPK pathway consists of a set of three sequentially acting kinases: an MAPK kinase kinase (MAP3Ks), an MAPK kinase (MAP2Ks), and an MAPK **(Krishna & Narang, 2008).**

MAPK pathway activation implicates successive phosphorylation and activation of a MAP3K then a MAP2K to finally activation of a particular MAPK. MAPK phosphorylates different substrates in the cytosol and nucleus (biological response) **figure (1) (Cargnello & Roux, 2011).**

In mammals, there are three main MAPKs families: JNKs (Jun amino-terminal kinases), p38/SAPKs (stress-activated protein kinases) & ERKs (extracellular-signal-regulated kinases). Members of ERK family include: the ERK1 & ERK2, classic ERKs that encompass mainly a

kinase domain, and the ERK5, larger ERKs that have a much more prolonged sequence carboxy-terminal to their kinase domain. Classic ERK1/2 module has imperative upstream regulators. Cell surface receptors, such as receptor tyrosine kinases (RTKs), G-protein-coupled receptors, integrins, and the small GTPases are examples of these regulators. MAP2K, also known MEK (MAP2K/ERK), is the direct activator of ERK. Seven MEK subtypes have been identified. MEKs for the ERK1/2 module are MEK1 and MEK2 **(Morrison, 2012).**

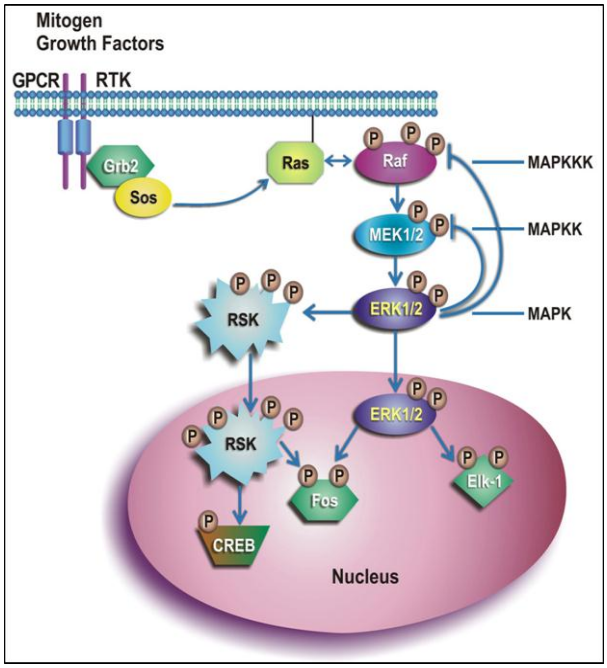

Figure 1: MAPK pathway.

The MAP3Ks include members of the Raf family, Mos, and Tpl2. RAS family contains four protein isoforms (HRas, NRas, KRas 4A, KRas 4B). These proteins are membrane related GTPases. By cycling between an active GTP linked and inactive GDP linked state, they interconnect on or off messages to downstream effector proteins **figure (2)**. All the three members of Raf gene family (A Raf, B Raf, Raf-1) can be triggered by all four isoforms of Ras **(Ward et al., 2012).**

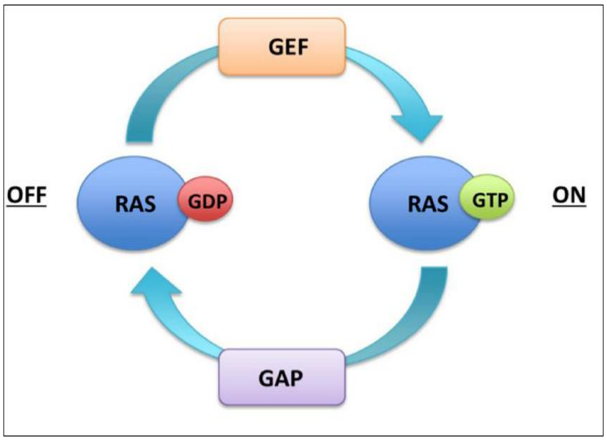

Figure 2: Ras pathway & its regulation.

Activation of the Ras/Raf/MEK/ERK pathway begins by ligand binding to RTK. This encourages the auto-phosphorylation of its intra-cellular SH2 domain. Guanine nucleotide exchange factors (GEFs) such as son of sevenless (SOS) is localized to the membrane by recruited growth factor receptor bound protein 2 (Grb2). The last step induces Ras to exchange GDP for GTP. Ras- Raf phosphorylation results in formation of Raf homo or hetero dimers. Raf consequently triggers MEK1/2, which display restricted substrate specificity for ERK1/2. ERK1/2 is a potent kinase for diverse nuclear and cytoplasmic substrates **(Knight & Irving, 2014).**

One of the downstream substrates of ERK1/2 is p90 Rsk-1. p90 Rsk-1can motivate response element-binding protein transcription factor that affect gene expression. Phosphorylated ERK can stimulate further transcription factors via translocation directly to the nucleus. These factors include bind the promoters of many genes, that are important in encouraging growth and preventing apoptosis in the cells. The crucial apoptotic effectors (Bcl-2, Mcl-1, Bad, Bim, caspase-9 and many others) are also phosphorylated by ERK **(Steelman et al., 2011).**

ERK1/2-mediated inhibitory phosphorylation of its upstream regulators controls most of the feedback mechanisms identified. ERK1/2 can down-regulate MEK1 activity by phosphorylation of MEK1 on Thr292 and Thr386 **(Eblen el al., 2004).** ERK1/2 can also inhibit Raf-1 interaction with Ras by multiple sites phosphorylation of Raf-1 **(Rushworth el al., 2006).**

Furthermore, ERK1/2 can affect the rate of Ras-mediated receptor tyrosine kinase signaling toward Raf/MEK/ERK by phosphorylation of GEFs regulating its interaction with the adaptor protein Grb2. In addition, cellular MEK1 and MEK2 levels can be feedback-regulated by the Raf/MEK/ERK pathway. In different cell types, ERK1/2 knockdown mediated by RNA interference abrogated ΔRaf-1: ER- or B-RafV600E- mediated MEK/ERK activation increased MEK1 but decreased MEK2 levels. MEK1 levels are up-regulated at transcriptional level whereas MEK2 levels are down-regulated at post-translational level **(Hong et al., 2015).**

MAPK pathway: Cancer implications

It is not surprising that abnormal regulation of Ras pathway has been linked to cancer, since this pathway plays a main role in the control of a multitude of critical cellular processes. Important drivers in that regard are the gain-of-function mutations particularly in RAS and RAF. More than 30% of all human cancers have RAS mutations. The N-RAS and H-RAS mutations have the lowest frequency (8% and 2% respectively) among the RAS isoforms while K-RAS mutations have the highest frequency (22%) **(Mendoza & Blenis, 2011)**

Mutations that activate B-Raf, have been detected in breast cancer, colorectal carcinomas, melanoma and

others. Approximately 50-60% of melanomas harbor B-RAF mutations. Approximately one percentage of cancers has C-RAF mutations. The aberrant activation of upstream RTKs also affected the RAS-RAF-MEK-ERK cascade. Increased ligand binding, increased dimerization or structural changes occur as a result of genetic mutations in these RTKs. Consequently, increased signaling through the pathway happens **(McDermott & Qin, 2015).**

The levels and activities of Bcl-2 family proteins (the pro-apoptotic protein BIM & anti-apoptotic protein MCL-1) are regulated by ERK1/2. Expression of MCL-1 is increased in several types of cancer and is associated with poor prognosis and resistance to chemotherapy. ERK1/2 phosphorylates MCL-1, resulting in stabilization of it and promoting the survival of tumor cells. Proteins like myosin light chain kinase, calpain, and focal adhesion kinase are phosphorylated by ERK and this promotes cancer cell migration. Tumor invasion is promoted by ERK pathway via the induction of expression of matrix metalloproteinases (degrade of extracellular matrix). All these effects make the ERK signaling pathway a prominent therapeutic target for the development of anticancer drugs **(Kim & Choi, 2010).**

The MEK, the second most important component in the series, is crucial for mediating the oncogenic effects of RAS signaling. This is demonstrated by the efficacy of MEK inhibitors in RAS-mutated cancers **(Caunt et al., 2014).** Many MEK inhibitors have reached clinical trials. The first MEK1/2 inhibitor to be tested in humans was CI-1040 (PD-184352). Some other MEK inhibitors that followed include Selumetinib, Refametinib, Cobimetinib, Trametinib. These inhibitors advanced to clinical trials **(Lorusso et al., 2005; McDermott & Qin, 2015).**

Selumetinib, for example, show MEK inhibition in B-RAF & RAS mutated tumor cell lines. In 2004, it was studied on patients with advanced cancers in a phase I trial. Numerous studies were done that intended to assess it, as a single agent or in arrangement with others anticancer. Some clinical studies reported clinical activity for the combination of selumetinib with paclitaxel in patients with K-RAS mutated non-small cell lung cancer (NSCLC). it also showed activity, as a single agent, in patients with low grade serous ovarian carcinoma and biliary **(Jänne et al., 2013; Farley et al., 2013).**

MAPK pathway in ALL

MAPK pathway is supposed to be vital in the development of leukemia. Deteriorations in components of the Ras pathway signaling as a result of mutations are among the causes of leukemia pathogenesis **(Akin-Bali et al., 2021).** Mutations in Ras (NRAS & KRAS), BRAF and genes encoding proteins which modulate the pathway (such as mutations in the Shp2 phosphatase, a.k.a. PTPN11) present in pediatric ALL. About 35% of an unselected cohort of pediatric ALL patients contained mutations in NRAS/KRAS2/PTPN11. Mutations make Ras pathway remains activated as a result of the permanent attachment of GTP to the Ras. This leads to carcinogenesis by producing uncontrolled proliferation of the lymphoblast. These mutations were exclusive, this points to their importance in leukemogenesis **(Armstrong et al., 2004; Perentesis et al., 2004; Al-Kzayer et al., 2015).**

In a study by **Kim and co-workers (1999),** the activation of ERK protein in Ras pathway was tested in about 80 human acute leukemia samples by in vitro kinase assay and immune-blot analysis. Their results suggested that ERK and MEK were constitutively activated in acute leukemia. Also, the hyper-expression of ERK was reported by RT-PCR. In addition, one of possible mechanisms underlying ERK constitutive activation was the significant decrease in a specific ERK phosphatase gene expression (PAC1 gene).

Ras pathway mutations also have been connected to BCP-ALL relapse and chemotherapy resistance. Nevertheless, the rate of occurrence & the predictive value of sub-clonal mutations in this pathway in certain subgroups are lacking **(Ward et al., 2012).**

Thirteen RAS pathway genes were sequenced by directed deep sequencing in cases with pediatric BCP-ALL. This was done at initial diagnosis and in relapse. In about half of patients, mutations were reported. Mutation frequencies were highest for example in high hyper-diploid, t (4; 11)-rearranged, BCR-ABL1-like. RAS pathway-mutated cells were more resistant to prednisolone and vincristine ex vivo. Unfavorable risk features and outcome in newly diagnosed pediatric BCP-ALL are linked with Ras clonal mutations. RAS mutant cells were sensitive to a MEK inhibitor in vitro; in addition inhibition of MEK enzyme sensitized resistant cells to prednisolone **(Jerchel et al., 2018).**

The response to glucocorticoids (GC), either in vitro or in vivo, is a major prognostic factor in childhood ALL**.** Polak and co-workers, in a study in 2016, tried to reveal the molecular causes of GC resistance in B-ALL and illustrate the probability of targeted intervention in these mechanisms. Significantly higher expression of MAPK pathway components was showed in the resistant cells. In addition, ALL cell lines had markedly higher baseline activity of MEK. Inhibition of MEK1/2 increased GCs-induced cell death. This inhibition increased in vitro dexamethasone activity in primary ALL blasts from about 90 % of tested patients. In attempt to approve these observations, the constitutively active MEK mutant was overexpressed in GC sensitive cells. As a result, this forced MEK activity induced resistance to dexamethasone in these cells **(Kaspers et al., 1998; Polak et al., 2016).**

A genome-scale short hairpin RNA screen was done in a study in 2015 to recognize mediators of prednisolone

sensitivity in pALL cell lines. The study identified the MAPK pathway as a mediator of prednisolone resistance in pALL. Increased cellular sensitivity to prednisolone was showed with the knockdown of the definite MAPK pathway members MEK2 and MEK4 through different mechanisms. Through increasing the levels of the glucocorticoid receptor, the knockdown of MEK4 increased sensitivity specifically to prednisolone. While the knockdown of MEK2, via increasing the levels of p53, increased the sensitivity to all chemotherapy agents. Besides, relapse samples have an enhanced response to MEK inhibition compared to matched diagnosis samples in xenograft models. The data from this study indicated that the MAPK pathway is an attractive target for prevention and/or treatment of relapsed disease **(Courtney et al., 2015).**

Moreover, the MAPK cascade may be induced by chemotherapeutic drugs used in leukemia therapy. This could contribute to chemotherapy resistance. ERK1/2 stimulation is induced by oxidative stress and this is reported in a diversity of cell types. ROS can activate the MAPK signaling series at several points. In some settings, ROS can act directly on cytokine and growth factor receptors to induce the Ras pathway. Thus, this pathway is important player in resistance of neoplastic cells. Doxorubicin, a commonly anti-leukemia drug, exerts its chemotherapeutic influence through more than one mechanism. It interacts with DNA and inhibits topoisomerase II. Another mechanism is the generation of reactive oxygen species (ROS). Anti-oxidants can decrease the capability of doxorubicin to induce apoptosis by scavenging oxygen radical. However, ROS are also the cause of some undesirable side effects of this drug. Accordingly, this pathway is a future target as a novel therapeutic approach for drug resistant leukemia, which is often multi-resistant to chemotherapeutic agents **(McCubrey et al., 2006; Steelman et al., 2011).**

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30. Zwerdling, T. Pediatric Acute Lymphoblastic Leukemia. Hematol Transfus Int J., 2017; 5(3): 00118. DOI: 10.15406/htij. 2017.05. 00118 Pediatric Acute Lymphoblastic Leukemia 2/8 Copyright: 2017 Zwerdling used as better and more objective methodologies have become clinical standard for classification (Doctoral dissertation, These newer schema are based on immunologic analysis of leukemic cells using flow cytometry and molecularly based characteristics of cancer cells. The WHO recognizes the following distinct types of leukemia [24].