

A molecular look at the RAS/RAF/MEK/ERK pathway in pediatric acute lymphocytic leukemia (ALL)

Abstract

Pediatric leukemia is a multifactorial disease with an unknown etiology but can be treated. Just like many other types of cancer, genetic changes take place in leukemia. These genetic lesions, which are effective in the activation of oncogenes or inactivation of tumor suppressor genes; could lead to the development of leukemia via disruptions in the mechanisms regulating cell death, cellular differentiation or division. Studying the genetic anomalies that have not yet been determined allows treatment options that affect these steps, and thus the development of personalized treatment methods to treat chemotherapy-resistant and recurrent leukemia. This review aims to evaluate the role of RAS/RAF/MEK/ERK pathway, which was reported by previous studies to be important in development of cancer, in a pediatric leukemia subtype namely acute lymphoblastic leukemia (ALL).

Keywords: leukemia, pediatrics, biomarker, gene, polymorphism

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Abbreviations: ALL, Acute Lymphoblastic Leukemia; MLL, Mixed Lineage Leukemia; GAP, GTPase Activating Protein; GEF, Guanine Exchange Factor; JMMLs, juvenile myelomonocytic leukemias

Introduction

Leukemia is a group of malignant diseases with an unknown etiology in which the progenitor cells in the bone marrow stall at a certain differentiation stage and undergo clonal expansion uncontrollably, infiltrate the bone marrow itself and all the other organs, and finally results in absolute death when not treated.^{1,2} Leukemia was first described by French physician Alfred Velpéau in 1827. In 1847, Virchow named the disease “leukemia” which consists of the Greek words “leukos” meaning white and “haima” meaning blood.^{3,4} Leukemia is a multigenic, multifactorial, complex and heterogeneous disease, just like the other types of cancer. It is a known fact that cytogenetic disorders and the molecular changes they cause are determinants in the pathogenesis and prognosis of leukemia. Knowledge about some of these cytogenetic disorders and the associated molecular changes enables the treatment options that affect these steps and thus the treatment of resistant and recurrent leukemia to chemotherapy. However, these genetic anomalies are insufficient to explain the biological basis of leukemia and the differences in the response to treatment or the underlying cause of leukemia in individuals who do not have any genetic anomaly. Therefore, gene expression profiling at the level of transcriptomics is commonly used to identify the anomalies of the leukemic cell, allowing them to be utilized as tools in diagnosis.⁵ Studies in the field aim to identify novel genetic variations within tumor suppressors, oncogenes or genes involved in lymphocyte differentiation and apoptosis as well as important cellular pathways that could help better understand the pathogenesis of leukemia.^{6,7} At the same time, efforts focus on pinpointing prognostic biomarkers which can enable development of personalized treatment protocols and most importantly improving the prognosis of the disease. Leukemia are the leading cause of pediatric cancers in the world. Improved risk assessments of pediatric leukemia, use of new chemotherapeutic drugs, especially targeted drugs, and improved support therapies have resulted in a significant increase in survival rates. However, the molecular basis of pediatric

leukemia is still not fully understood and scientists believe that there are still many leukemia subgroups that have not yet been classified.

When a cell undergoes malignant changes; it gains properties such as self-growth, insensitivity to anti-growth signals, unlimited proliferation, protection against signals for cell death, tissue invasion, metastasis as well as the ability to stimulate angiogenesis. The abilities to stop cellular differentiation and escape from the host immune system are suspected but yet to be proven.^{6,8} Proto-oncogenes, which are known as genes capable of controlling cell growth and vital activities, are transformed into oncogenes when a mutation capable of causing cancer takes place. This malignant change can be due to point mutations, gene amplifications, rearrangements and insertions. When oncogenes are classified according to their functional and biochemical properties of the proteins they encode; they could be grouped under transcription factors, growth factors and their receptors, and the factors involved in signal transduction mechanisms.⁸⁻¹⁰ Tumor suppressor genes, which play an important role in carcinogenesis and function in the opposite direction of oncogenes, have the ability to inhibit proliferation and activate apoptosis in the cell. As a result of loss or inactivation of these genes, an increase in cell proliferation and disruptions in the cellular death mechanisms are observed. These genes that are active in different pathways are known as transcription factors and regulators, kinase inhibitors and structural cellular components.⁷⁻¹³

RAS/RAF/MEK/ERK pathway

RAS/RAF/MEK/ERK pathway, which is believed to be important in the development of leukemia, affects many important cellular processes such as cell growth, division, transformation, metabolism control, cell migration, inflammation and apoptosis by controlling the transcription of many genes in almost all eukaryotic organisms. In other words, it is an evolutionarily conserved signaling pathway that connects the signaling system regulating important cellular tasks such as growth, proliferation, differentiation and apoptosis with extracellular signals.^{14,15} This pathway involves serine/threonine protein kinases and it is active in almost all cell types. Several growth factors could activate the RAS/RAF/MEK/ERK pathway as mitogens. RAS family proteins take on highly critical roles in all aspects of cell

biology, serving as keys to intracellular signal transduction including cell division, differentiation, intracellular protein transport, and the organization of the cell skeleton. Point mutations, deletions and

chromosomal translocations, which cause deteriorations in the RAS/RAF/MEK/ERK pathway signaling is among the causes of Leukemia pathogenesis¹⁶⁻²⁰ (Table 1).

Table 1 Chromosomal localization of RAS/RAF/MEK/ERK pathway genes, the detected anomalies in different types of cancer and their frequencies

Location	Gene symbol	Gene name	Exons	Pathophysiological and clinical outcome in leukemia	Frequency in leukemia	Associated cancers
12p12.1	KRAS	KRAS Proto-Oncogene, GTPase	Exon	Poor prognosis	%6-20	All cancers %30
1p13.2	NRAS	NRAS Proto-Oncogene, GTPase	Exon	Poor prognosis	15%	All cancers %30
7q34	BRAF	B-Raf Proto-Oncogene, Serine/Threonine Kinase	Exon	Unknown	%10-20	All cancers % 28 - 37, Melanoma %60-70
15q22.31	MAP2K1-MEK1	Mitogen-Activated Protein Kinase Kinase 1	Exon	Unknown	Unknown	% 6 - 7 Melanoma
19p13.3	MAP2K2-MEK2	Mitogen-Activated Protein Kinase Kinase 2	Exon	Unknown	Unknown	% 6 - 7 Melanoma
16p11.2	ERK1-MAPK3	Mitogen-Activated Protein Kinase 3		Unknown	Unknown	All cancers %30
22q11.22	ERK2-MAPK1	Mitogen-Activated Protein Kinase 1	Exon	Unknown	Unknown	All cancers %30
12q24.13	PTNP11	Protein Tyrosine Phosphatase, Non-Receptor Type 11	Exon	Poor prognosis	%35 in JMML	Noonan Syndrome %50
13q12.2	FLT3	Fms Related Tyrosine Kinase 3	1 T D mutations	Poor prognosis	%4-17 in AML	

RAS gene family

RAS proteins are called small GTPases due to their small size (about 21 kD) and enzymatic activities. Ras activity is controlled by GTP hydrolysis; while the GDP-dependent form is ineffective, the GTP-dependent form acts as an active enzyme and initiates the first signal of a pathway leading to gene expression activity. GTPase Activating Proteins (GAPs) and Guanine Exchange Factors (GEFs) are involved in the regulation of this signaling. GAPs and GEFs are activated by various ligands in different cell types. Resulting interactions cause effector protein activation by phosphorylation and set the signal transduction pathways in motion. The most well-known pathways among these are MAPK/ERK and PI3-Kinase signal transduction pathways (Figure 1). Activating mutations in RAS proto-oncogenes are the most common genetic change in human cancers. Oncogenic mutations leading to activation of RAS genes trigger cell proliferation, and a comprehensive analysis of the literature indicates that such mutations occur in 30% of all human cancers.¹⁴⁻²⁰ RAS is the key step that activates the RAS/RAF/MEK/ERK pathway. While the GDP-bound RAS is inactive, GTP-bound RAS is in active form.

Mutations in the RAS gene cause changes in the RAS protein. Activator mutations (gain of function) cause the GTP to remain permanently attached to RAS. As a result of this, the RAS-MAPK pathway remains open and leads to carcinogenesis by producing uncontrolled proliferation signals in the cell as a result of an abnormal pathway activation. In most studies on human tumors, 12nd, 13th and 61st codons of RAS were identified as oncogenic mutation regions that get mutated very often. N-K and H-RAS mutations have been reported in 15% of all human cancers. In the case of leukemia, it is

known that the 6-20% of mutations in ALL cases are in this protein family and they are most commonly mutated in N-RAS cancer patients.^{17,18} Lubbert et al.²¹ indicated in their study on ALL patients that all RAS mutations were detected in the NRAS protein coding gene. They also claimed that carrying RAS mutations was not related to age and gender but was associated with increased risk of relapse. In a study of 109 infants with Mixed Lineage Leukemia (MLL), RAS gene mutations were detected in 14% of the study group. RAS mutant infants have been reported to possess high leukocyte count and develop glucocorticoid resistance. It is reported that the presence of RAS mutations alone can be associated with poor prognosis for infant leukemia and therefore it was suggested that abnormal RAS pathway activators and inhibitors should be identified when risk classification is performed.²² Jerchel et al.¹⁹ reported the frequency of RAS mutations in 460 newly diagnosed pediatric precursor B-ALL as 15%. However, these studies are restricted to N-RAS and K-RAS and hence, do not include genes encoding other proteins involved in the RAS/RAF/MEK/ERK kinase pathway. The clinical significance of RAS mutations is controversial due to lack of scientific data. On the other hand, some believe that the clonal mutations of NRAS, KRAS, PTNP11 and FLT3 may be related to the development of resistance to chemotherapy.²³ It is suggested that mutations in RAS pathway genes proteins could potentially be biomarkers for MEK/ERK targeted therapies.²⁴⁻²⁶

RAF gene family

RAF is another serine/tyrosine kinase acting in the second step of the RAS/RAF/MEK/ERK pathway and has 3 different isoforms: ARAF, BRAF and CRAF. The activated RAF activates MEK that

functions in the next step of the pathway. Among the RAF isoforms, BRAF has been associated with cancer. The frequency of BRAF mutations is known to be high in melanoma and thyroid cancers, but these mutations are also encountered in hematological malignancies. 80% of BRAF mutations detected in solid tumor cases are V600E mutations. Although the BRAF V600E mutation is frequently associated with hairy cell leukemia in hematological malignancies, it is associated with many types of leukemia and myeloma, such as plasma cell myeloma, CLL and ALL. Mutations in the P-Loop domain and the activation segment of BRAF cause conformational changes and inactivation of the protein which results in the uncontrolled activation of the RAS/RAF/MEK/ERK cascade.²⁷⁻²⁹ Therefore, there are studies reporting that BRAF mutation-targeted leukemia therapies can be used in acute leukemia. It is predicted that the presence of BRAF mutations and other genetic anomalies within the RAS/RAF/MEK/ERK pathway could be capable of altering AML subtyping.²⁷⁻³²

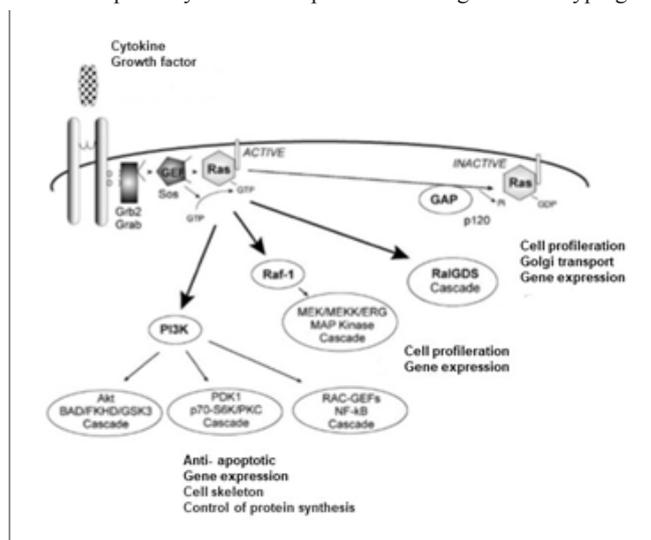


Figure 1 RAS/RAF/MEK/ERK signaling pathway.

MEK1/2 and ERK1/2 gene family

MEK1 and MEK2 which are activated by RAF proteins activate their target proteins ERK1/2. Although genetic variations are very rare in MEK1 and MEK2 genes, increase in their expression levels is observed in some types of cancer. Once activated, ERK1/2 activates downstream target proteins located in the cytoplasm. It translocates to the nucleus to further activate the corresponding genes. There are 5 different isoforms of the ERK family proteins, among which ERK1/2 are the most known isoforms. ERK proteins enable S0/G1 cells to move on to the S phase during cell cycle which allows the cell to continue to divide. ERK proteins activate the cell-cycle activating genes, whereas they silence the genes that inhibit cell cycle progression.³³⁻³⁴ A study reported the frequency of ERK1/2 mutations in the newly diagnosed ALL patients as 34.5%. Relapse may occur in ALL patients associated with poor prognosis during classification for treatment and patients with relapse would most certainly need new treatment regimens. There are studies in which KRAS mutations are associated with decreased survival. It is thought that selumetinib administration, which is a MEK 1/2 inhibitor, could improve the prognosis of the disease in relapsed ALL patients carrying RAS pathway mutations.³⁵

PTPN11 and FLT3 Genes

Mutations in the PTPN11 gene, one of the most important genes

in the pathway, were detected in 35% of JMMLs, 10% of childhood myelodysplastic syndromes, 7% of acute lymphoblastic leukemia and 4% of acute myeloid leukemia. The majority of these cases are in the pediatric age group. Yamamoto et al. identified SHP2 protein tyrosine phosphatase which is encoded by PTPN11 as a protein that plays an important role in cell signaling. Somatic PTPN11 mutations are frequently described in hematological malignancies. PTPN11, RAS, and FLT3 mutations were investigated in 95 pediatric ALL patients and it was concluded that PTPN11 plays an important role in the development of leukemia. The incidence of PTPN11 in precursor B-ALL was determined as 9.5%. All mutations were identified to be mis-sense mutations and were located on the SH2 domain (23, 36). FLT3, one of the important receptor tyrosine kinases of the RAS pathway family, is regarded as a promising target for the development of new chemotherapy drugs. The frequency of FLT3 mutations in pediatric patients with AML has been reported to be 4-15%. Study by Akin et al. determined FLT3 as an important biomarker for the diagnosis and treatment of pediatric ALL patients, and detected FLT3 ITD and point mutations in 22.2% of this patient group. Since all of these mutations cause changes in the amino acid sequence of the protein, FLT3 was suggested to induce ligand-independent activation.^{37,38}

Conclusion

The complexity of the development of leukemia is due to the fact that it does not rely only on a single factor, but it is an interplay between both environmental and genetic factors. Cytogenetic and molecular genetic disorders are known to be determinant in the pathogenesis and prognosis of leukemia. Conducting research studies based on cytogenetics and molecular genetics is important for improving the diagnosis of leukemia, determining the prognosis, the decision of the treatment option and during the follow-up. Genetic changes are important prognostic factors in leukemia and their effects on leukemia-free survival and treatment options are clearly demonstrated by several previous studies. In addition, despite the increase in the percentage of success in the treatment of leukemia, the occurrence of relapse is still a serious problem. The presence of cancer cells that escape the treatment (radiotherapy, chemotherapy, bone marrow transplantation) and cause, relapse which is defined as minimal residual disease (MRD), clearly plays an important role in the completion of the treatment of the disease. With Detection of these residual cells that escape treatment with the development of new technologies has allowed significant advances towards understanding the clinical significance of their presence as well as their quantity. With each new study, it will be possible to identify genetic subgroups of this disease that are yet to be characterized, prevent relapse and uncover the intricate details underlying the disease.

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None.

Conflict of Interest

The authors declare no conflict of interest.

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