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GENOMIC CHARACTERIZATION OF MIXED LINEAGE LEUKEMIAS

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Abstract – *Developing new technologies and genomic techniques generate big data, which is very helpful to evaluate extensively hematologic malignancies. Besides wet lab results, such as gene and microRNA expression, methylation profile, next-generation sequencing data, whole-exome and RNA-seq, FISH analysis, SNP arrays, new computer algorithm, and programs have allowed for improving the understanding transformation oncohematological disorder mechanism. In addition to that, the whole wet and dry lab results permit us to develop a new prognostic algorithm, which can assist to decide the most appropriate treatment. Furthermore, finding and analyzing new biomarkers drive the geneticists to produce therapy target and personalized medicine. The novel and effective contribution of the new technologies in the context of leukemia of both AML, Acute Myeloid Leukemia and ALL, Acute Lymphoblastic Leukemia and genomic characterization of de novo mixed-lineage leukemia are described in this review.*

KEYWORDS: *MLL, Genomic MLL, Gene expression and methylation of MLL, Characteristics of MLL.*

INTRODUCTION

The last 30 years using new technologies and techniques have modified so much genetics and genomics knowledge. Identification of new subtypes of leukemia, prognosis, and therapy target medicine have been discovered by gene and microRNA expression, methylation profile, next-generation sequencing data, whole-exome and RNA-seq, FISH, florescence *in situ* hybridization, analysis, SNP, Single nucleotide polymorphism, arrays, novel computer algorithm and programs. Leukemia is blood cancer, which includes some different subtypes. Cytogenetics and molecular genomics reveal that the subtypes of leukemia have a different prognosis, diagnosis, treatment, and genomic profiles. The mostly observed genomic profiles include gene expression, chromosomal translocation, mutation and methylation that they are the main research area to distinguish the differences among leukemia types. Classification of recurrent chromosomal translocations firstly was distinguished by cytogenetics and then gene expression profiling has been defined abnormally regulated transcript in given subgroups. These cryptic changes identify how many genes are overexpressed and underexpressed and their effect on the transformation of onchematological disorder. Moreover, a large set of point mutations which some of them have been already explained still working for some of them are analyzed by NGS, next-generation sequencing data. Blood cells are responsible for oxygen transportation, immunity and coagulation. The cells are produced and differentiated as myeloid and lymphoid lineages in the bone marrow then released into the periphery (blood vessels). The whole maturation process of blood cells is called hematopoiesis. The excess production of immature or undifferentiated blood cells is called leukemia or blood cancer that is diagnosed by a taken sample from bone marrow or peripheral blood. The existence of aberrant white blood cells (blasts) is analyzed under a light microscope. Besides cytogenetical result, genomic characterization is needed to decide the type of leukemia. Therefore, the myeloid and lymphoid

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leukemia involving with different chromosomal rearrangements result from abnormal gene expression, mutation and methylation profiles, which have a different prognosis, diagnosis and treatment. Although MLL type leukemia is observed both AML and ALL it is accepted as unique and different leukemia. This review has been written to present the genomic differences of MLL type leukemia.

MLL (MIXED LINEAGE LEUKEMIA)

MLL is a type of leukemia that is observed in both AML and ALL and represents a gene that is known as *KMT2* lysine (K)-specific methyltransferase 2A. The gene was firstly defined in 1970 from ALL patients and named *MLL* (mixed-lineage leukemia) based on the 11q23 translocation, which is distinguished in both myelogenous and lymphoblastic lineages of leukemia[1]. MLL has direct and indirect roles to regulate expression of target genes positively, epigenetic regulation of transcription, embryonic development and hematopoiesis[2]. The location of MLL gene, 23.3 q arm of 11th chromosome, is active for chromosomal translocations. The gene consists of 90.156 bases and encodes histone-lysine N-methyltransferase protein[3].

MLL LEUKEMIA

MLL is a malignant disorder that results from an accumulation of a series of genetic abnormalities that lead to impaired maturation by arresting blood cell maturation, differentiation and abnormal proliferation[4]. Finally, the increasing number of leukemic cells which occurs in both bone marrow and peripheral blood cell. MLL type hematologic malignancies are a combination of some genomic abnormalities simultaneously such as; chromosomal translocations, internal tandem duplications, internal deletions and amplifications[5]. *MLL* gene is a frequent target for repeated translocations that can be identified in acute myeloid leukemia (AML) and acute lymphoblastic leukemia (ALL). Because of its unique molecular, biological, clinical characteristics, it is often correlated with poor prognosis, observed in more than 70% of infant leukemia and overall survival is very low and effective target treatment. MLL usually regulates hematopoietic development by altering epigenetic profiles which affect gene expression globally. The gene is accompanied by some fusion proteins which mediate activation of preleukemic target genes for leukemogenesis, such as *HOXA9, MEIS*. Association of the proteins with a transcriptional elongation complex, including *DOT1L* and *p-TEFb*, deregulates transcription activity. The therapeutic target chemicals disrupt MLL fusion protein super complex effectively and inhibit the growth of MLL-associated leukemic cells.

Pediatric and adult AML show common properties, but the age of leukemia is different in the MLL patient[6]. Involving 11q23 translocation is the most frequent aberrant one, including more than 70% of acute lymphoid leukemia in infants under the age of one year and 35%-50% of infant acute myeloid leukemia[7]. The five-year survival rates show differences among old patients and pediatric patients 30%-40% respectively and it may reach 65%[8]. 3-4% of all AML cases, including *MLL* translocation (11q23/*MLL*+AML), occurs most commonly in young people with new type AML (5-7%) and in treatment-induced AML (t-AML) patients (10-15%). The MLL translocation is hardly ever observed in older patients aged 60 and up[9]. Because of MLL patients presenting a very bad prognostic factor, 11q23 abnormalities are diagnosed with high-risk protocols[10].

The disease outcomes vary greatly between adults and children. MLL type leukemia is a very aggressive one and mostly observed 90% in infants aged < 1 year and (2-5%) children and adults. Cytogenetics often diagnose the leukemia type, risk stratification and therapeutic implications, but there is a need to develop the molecular examination of subtypes, identifying genetic abnormalities which guide the clinicians and hematologist to predict the risk of treatment and developing novel and targeted therapies[4]. As a result of the survival rate MLL rearrangements are linked with a very poor outcome.

MLL REARRANGEMENTS

The genetic lesion is one of the typical characteristics of leukemia. E2A/PBX1, BCR/ABL, ETV6, RUNX1 and MLL rearrangements are the main examples that represent different prognosis, therapy, age group and therapy and the lesions observed leukemia shows diverse transcriptional profile[11]. Furthermore, the rearrangements mostly affect principal cellular pathways, such as cell cycle, apoptosis, cell differentiation and tumor suppression. Among all lesions, MLL type rearrangement presents different characteristics. While MLL is enough to stimulate a leukemic transformation, others may need additional hits. The mostly observed MLL type genetics lesions are (4;11), t(6;11), t(9;11), t(11;19) and clustered within a 9-kb BamHI genomic region and t(4;11) and t(9;11) lie within a 5-kb region in infant leukemia (Figure 1). According to the rearrangements diagnosis, overall survival rate and therapy change.

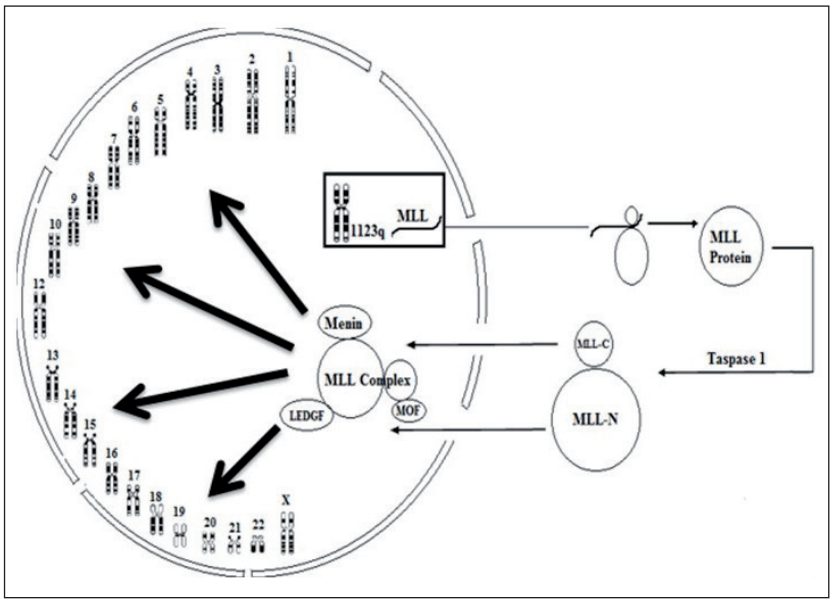

Fig. 1. MLL complex formation steps. The MLL problem starts in the nucleus, chromosomal rearrangement. After that MLL responsible protein is divided by Taspase-1 enzyme and reunites in the nucleus to affect chromatin structure. The whole MLL recombining the cleaved parts efficient form of the protein regulates the gene expression globally in the cell.

FUSION PROTEINS AND THEIR FUNCTIONS

The MLL gene can be separated in B and T cell origin, as well as myeloid leukemia and MLL protein, consists of N-terminal and C-terminal, while they are connected with at least four other proteins, around 100 different MLL fusion proteins have been recognized[12]. The MLL-protein complex regulates gene expression by modifying chromatin structure during early development, hematopoiesis and methylation of histone (H3K4). The complex is suggested to transform hematopoietic cells into leukemia cells successively. Some of the fusion proteins have a unique affinity with MLL protein and special functions. Here are some important proteins and their functions. MOF protein loosens up chromatin by histone charge neutralization[13]. WDR5 protein recognizes the histone H3 lysine 4 methyl-mark introduced by MLL and has been suggested that WDR5 ensures the progression of histone modification[14]. AT-hook is a DNA binding domain[15]. CxxC motif recognizes unmethylated CpG dinucleotides[16]. PHD, plant homeodomain[17]. SET, histone methyltransferase active site.

MLL PROTEIN AND THE COMPLEX FORMATION

MLL-N and MLL-C terminals consist of 2718 a.a and 1250 a.a respectively and are cleaved by Taspase 1 enzyme into two parts which they come together after being delivered to the nucleus[18]. There are 8 functional domains in MLL-N part which include Ala/Gyl/Ser rich, Poly/Gly, three AT hooks, Polypro, Zinc finger CXXC type, three zinc fingers PHD type, Bromodomain, FYR N-term. On the other hand, MLL-C part includes 5 functional domains TAD, FYRC-term, SET, 4post SET and S-adenosyl-L-methionine binding sites (Table 1). Recombining the cleaved parts efficient form of the protein regulates the gene expression globally in the cell (Figure 2). The complex regulates 3 main chromatin modifications; acetylation, methylation and nucleosome remodeling. Those epigenetic changes direct transcription factors recruit the MLL complex to start RNA synthesis.

MLL BREAKING POINT

While the breakpoint of *MLL* extends from exon8 to exon12, a high frequency of the breakpoints occurs within a 4 kb region between exon11 and adjacent to exon12 depending on the verification of the clinical data. The hot breakpoint is determined within intron11 that is mostly correlated with infant acute leukemia and therapy-related leukemia[19]. Oncogenic gene fusion is produced by broken of both DNA strands which are most frequently observed within an intron. The location neighboring to exon 12 is very fragile fragmentation and an important pioneer to rearrangement[20]. It is known that MLL exon12 is determined as highly sensitive to be broken than any of the adjoining introns or exons within MLL. DNase hypersensitivity site is frequently correlated with histone activity and transcription factor association[21]. This is abnormally expected to occur inside of a gene coding

TABLE 1. MLL protein complex components.

NCBI Name	Aliases	Human Chromosome Position	MLL interaction site
MEN1	Menin,MEAI, SCG2	11q13	N-terminus
PSIP1	p52,p75,PAIP, DFS70,LEDGF, PSIP2	9p22.3	N-terminus
PAF1		19q13.1	CXXC region
CTR9	SH2BP1, TSBP, p150,	11p15.3	CXXC region
BMI-1	RNF51	10p11.23	CXXC region
ELAC2	ELC2, HPC2	17p11.2	CXXC region
CTBP1	CtBP	4p16	CXXC region
HDAC1	HD1,RPD3, RPD3L1	1p34	CXXC region
PPIE	CYP-33, CYP33	1p32	PHD finger 3
ASB2		14q31-q32	PHD fingers
HCFC1	CFF,HCF-1,HCF1, VCAF	Xq28	adjacent to BD
HCFC2	HCF2	12q23.3	adjacent to BD
CREBBP	CBP,RSTS, KAT3A	16p13.3	MLL-C
KAT8	MOF, MYST1	16p11.2	MLL-C
WDR5	SWD3	9q34	SET domain
RBBP5	RBBP5,SWD1	1q32	SET domain
ASH2L	ASH2,ASH2L1,		
ASH2L2, Bre2	8p11q	SET domain	
DPY30	DPY-30, Saf19	2p22.3	SET domain

The table shows the all main gene names which are directly or indirectly related with MLL proteins. The MLL protein structure is completed with the gene types[61].

region. However, Scharf et al[22] demonstrated this in an exclusive work a mysterious promoter was identified within intron11, border of exon12. It has already been understood that topoisomerase II consensus sequence locates within exon12 which is the target of etoposide application. This proposes that etoposide stimulation of the cleavage is expressed at the distal site of the enzyme binding consensus sequence.

N AND C TERMINUS

N and C-terminus of the MLL subunit are preparing chromatin to efficient transcription and direct regeneration and alteration of hematopoietic stem cells and progenitor cells[23]. The two fragments have different functionally active domains. The functional domains of MLL-N and MLL-C are listed below with their names and functions.

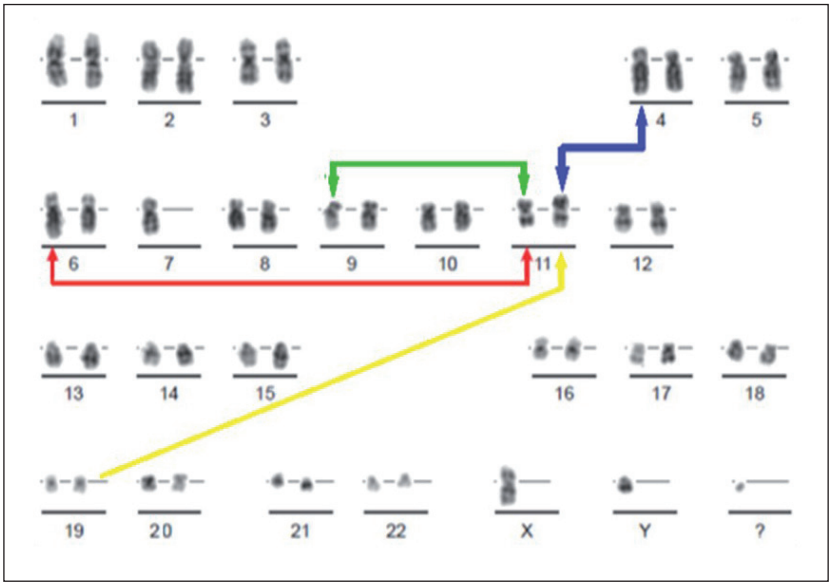

Fig. 2. Schematic representation of MLL type translocations. MLL type of leukemia has different chromosomal rearrangements. 4-11, 9-11, 6-11, 19-11 are the main types of MLL rearrangements. Each of the rearrangement has different genomic alterations and survival rate.

AT- HOOKS, CXXC, PHD

There are three AT-hooks and a CxxC domain that bind particularly to nonmethylated DNA and keep it away from *de novo* methylation, and a C-terminal 3–9, enhancer of zeste, trithorax (SET) domain with principal H3K4 methyltransferase. A-T hooks are specific DNA binding motif that exclusively identifies DNA with distortions, such as bends or kinks. CxxC domain recognizes the methylation status of DNA and binds purposely to unmethylated CpG dinucleotides and seems to be the main determinant of target gene selection and subnuclear localization[24]. Moreover, CxxC region has the potential to recruit suppressive factors such as; polycomb group proteins and histone deacetylases[25]. A four-group plant homeodomain fingers (PHD), which are located downstream of the CxxC, play a crucial role in localization and post-translation regulation of wild-type MLL. The third PHD finger finds trimethylated H3K4 and causes to MLL recruitment. Because of MLL cleavage and losing of H3K4 methyltransferase activity, the final product is the chimeric MLL fusion protein, which deregulates the activation of leukemic target genes, such as *HOXA9* and *MEIS*. The genes are observed in the huge majority of MLL-associated leukemia[26].

THE CONSERVED DOMAINS

SET is one of the highly conserved MLL domains which is located at the C-terminus of MLL and adds a methyl group to histone H3 at lysine 4[27]. COMPASS is a complex protein that is associated with SET1[28]. RBBP5 and ASH2L are necessary for powerful methyltransferase activity by stabilizing an active conformation of MLL permitting allosteric control[29]. SWI/SNF nucleosome remodeling complex consists of CBP and INI1 subunits which have been determined as interaction partners of MLL-C.

MENIN AND LEDGF

Menin and MLL generate a contact surface which is called LEDGF (lens epithelium derived growth factor). LEDGF interacts with chromatin via a PWWP domain. Menin and LEDGF are the product of the tumor suppressor gene, multiple endocrine neoplasias[30]. LEDGF is also concerned with HIV pathogenesis where it helps the integration of HIV proviruses into chromatin[31]. LEDGF-Menin binding motif and CxxC domain are extremely important for the overall function of MLL fusion[32].

MOLECULAR PATHWAYS IN ONCOGENIC MLL FUSIONS

MLL oncogenic activity is guided by the fusion proteins. To understand its mechanism, four distinct pathways have been proposed how the fusion partners could stimulate transcriptional activation. The molecular pathways consist of histone H3 lysine methylation, PolII phosphorylation, histone acetylation, histone H4 arginine methylation and dimerization in oncogenic MLL fusions proteins[4].

HISTONE PATHWAYS LEADING TO THE LEUKEMIA ACTIVITY

One of the most commonly found fusion partner is ENL and AF4 as members of the EAP complex that associates histone H3K79 methyltransferase activity. *DOT1L* catalyzes the activity with transcriptional elongation stimulation by pTEFb (positive transcription elongation factor b and a cyclinT) that phosphorylates the C-terminal repeat domain of RNA polymerase II. It is supposed that MLL fusion proteins abnormally redesign this complex for target chromatin. Active HDACs are combined with MLL in the MLL-CBP and MLL-p300 proteins. MLL-EEN reshapes the histone H4R3 arginine methyltransferase as a binding of the adaptor SAM68. The dimerization of MLL domains is supplied by the fusion partner that activates target genes by unknown mechanisms through coiled-coiled or other dimerization[4].

TYPES OF FUSION PROTEINS

Almost all *MLL* rearrangements are related by these six common partner proteins (*AF4, AF9, ENL, AF10, ELL*, *AF6*) consist of (> 85%) clinical report, descending order *AF4* (80%), *AF9* (16%), *ENL* (11%), *AF10* (7%) and *ELL* (4%)[33].While almost 50% of pediatric AML cases have t(9,11)(p22,q23) translocation, the rest 50% mainly consists of diverse translocations such as; t(11,19)(q23,p13.1), t(10,11)(p12,q23), t(6,11)(q27,q23) and t(1,11)(q21,q23) (Table 2). Some MLL partners, such as *MLLT1* [*ENL]*, *MLLT2 [AF4]* and *MLLT3 [AF9]* are sections of the super elongation complex. AFF1/AF4, MLLT3/AF9, MLLT1/ENL, MLLT1/AF1Q and ELL are the most commonly fused proteins. The epigenetic activation of gene expression during development is also mutated in subgroups of aggressive acute leukemias[34]. Although it is not clear, inhibition of the tumor suppressor protein p53 may increase MLL fusions susceptibility in a mutagenic form. ELL is homologous to AF4 and interacts with the other pro-

TABLE 2. MMLL fusion proteins and features.

Name	*Gene Alias*	*Features*	*Localization*
ENL[b]	MLLT1	Binds histone H3, assembles EAP[a] elongation/chromatin modification complex	Nuclear
AF9	MLLT3	ENL homolog also found in EAP	Nuclear
AF4	AAFF1, MLLT2	Founder of AF4 family, member of EAP	Nuclear
AF5q31	AFF4, MCEF	AF4 homolog found in EAP	Nuclear
LAF4	AFF3	AF4 homolog found in EAP	Nuclear
ELL		Elongation factor interacts with a protein related to AF4	Nuclear
AF10	MLLT10	Interacts with DOT1L histone methyltransferase	Nuclear
CBP	CREBBP	Histone acetyl-transferase in therapy-related MLL fusions	Nuclear
P300	EP300	CBP homolog in therapy, related MLL fusions	Nuclear
AF1p	EPSI5	Dimerization domain	Cytoplasm
GAS7		Dimerization domain	Cytoplasm
AF6	MLLT4	Dimerization domain	Cytoplasm
ABI1		Interacts with ENL when imported to the nucleus	Cytoplasm
EEN		Interacts with histone arginine methyltrasnferase PRMT1 when imported to the nucleus	Cytoplasm

The table apparently presents the MLL type gene aliases and their features and location in the cell. The table is adapted from R. Slany, 2009.

teins. that could make indirect contact with the EAP elongation machine. The ENL and AF9 association with AF4 family members is vital for the survival of MLL cells as small peptides breaking this communication[35].

PAFC PROTEIN

PAFc is a transcriptionally active protein that connects directly with RNA pol II and supports elongation by facilitating the deposition of some epigenetic marks, such as; transcription, H2BK120 ubiquitination, methylation of H3K4, H3K36 and H3K79[36]. Like Menin–LEDGF relations, MLL fusion proteins have also strong relation with PAFc for leukemogenesis. PAFc interaction properly targets to gene loci. The protein interaction with a domain of MLL is stood by the CxxC part of MLL and is always kept in all MLL fusion protein.

TRANSLOCATION TYPES

MLL translocations produce a chimeric protein which its amino–terminal portion of the protein fuses to the carboxy–terminal portion with more than 20 fusion partners. According to *MLL* translocation types, prognosis and overall survival present a different rate. It has been demonstrated that t(6;11)(q27;q23) and t(10;11)(p12;q23) are correlated with a poor prognosis, but the t(9;11)(p22;q23) types are correlated with a significantly longer survival rate[37].

TRANSLOCATION FREQUENCY

The location of MLL gene consists of diverse chromosomal activities and translocation frequency that has been detected in 104 distinct fusion genomic places[38]. The mostly observed translocations are given below with percentages.

- 43 (60.5%) reciprocal chromosomal translocations.
- 4 (5.7%) 11q23ter deletions.
- 8 (11.3%) 11q inversions.
- 13 (18.3%) MLL fusion partners and two DNA strand breaks and the insertion of 11q23 material into another chromosome.
- 3 distinct MLL fusion, very unusual, partners have been identified in 4.2% of all cases.

Before the term, mixed lineage leukemia, cytogeneticists had observed translocations, including t(4;11), and identified a special subset of ALL that was correlated with poor survival. Then 11q23 translocations are also typical for mixed-lineage leukemia. After diagnosis, 5-year survival rates come closer to 90%, but MLL therapy seems to have a barrier with hardly 40% of all infants surviving five years[10]. Mixed lineage leukemia relapses the second peak of frequency later in life, especially patients who have been treated previously with topoisomerase inhibitors like etoposide. 70% of infant ALL and 10% of all other ALL cases show 11q23 abnormalities[39]. Therapy-related leukemia includes about 10% of therapy stimulated AML and 3% of AML carrying 11q23 translocation.

DIFFERENCES AMONG AML, ALL AND MLL

While MLL t(4;11) translocations are found mostly in ALL, t(9;11)(p21;q23) translocations are mostly observed in AML, but rarely defined in ALL. On the other hand, t(11;19)(q23;p13) and MLL-ENL fusion are mostly observed in AML, B-cell precursor ALL and T-ALL[38]. The childhood leukemia generally shows an early relapse after chemotherapy including the MLL translocation in ALL.

MLL GENE EXPRESSION PROFILE

Amplification of the genes, a common characteristic in a wide range of tumors, is hardly ever determined in acute leukemia, approximately 1% of patients having it by guiding a cytogenetic result[40]. However, *MLL* gene amplification shows diverse gene expression profiles, such as underexpressed and overexpressed.

UNDER EXPRESSED IN MLL

Many underexpressed genes, *MME*, *CD24*, *CD22* and *DNTT,* have a purpose for early proper B-cell development in MLL. *TCF3*, *TCF4*, *POU2AF1, LIG4* and *SMARCA4* have correlated with B-precursor in AML/ALL comparison.

OVEREXPRESSION IN MLL

LGALS1, ANXA1, ANXA2, CD44 and *SPN* are quite overexpressed in MLL and *PROML1, FLT3* and *LMO2* are progenitor specific genes. On the other hand, *CCNA1, SERPINB1, CAPG* and *RNASE3* are myeloid-specific genes. *NKG2D* is a natural killer cell–associated gene[41]. HOXA9 and *PRG1* are reported as highly expressed in AML. In addition to that, overexpression of *HOXA9* has been correlated with a poor prognosis. *CD79B, CD24, CD44* and *SPN* expression indicate the early steps of lymphoid development. While *CD79B* and *CD24* expression represent highly, but *CD44 and SPN* expression decrease during maturation. *CD24, CD79B and CD44, SPN* expressions show antagonistic activity.

GENE EXPRESSION PROFILE OF DIFFERENT BLOOD CELLS IN AML, ALL AND MLL

AML, ALL and MLL gene expression have different profile especially in different blood cells and their formation process. While lymphoid-specific genes *CD24, MME*, *LIG4*, and *DNTT* are overexpressed in ALL samples, myeloid-specific genes *DF*, *CTSD* and *ANPEP* overexpressed in AML. On the other hand, *PROML1*, *LMO2*, *FLT3*, MLL are correlated with hematopoietic progenitors as overexpressed. According to gene expression analyses, almost 200 genes are significantly highly expressed in *MLL* as compared with the other two leukemia types. The gene expression profile comparison presents that MLL is a distinct disease. All MLL-rearranged leukemias show typical activation patterns of *HOXA* and *MEIS1* genes. The overexpression of HOXA9 and *MEIS1* can drive the myeloid phenotype in mice.

METHYLATION OF MLL

Epigenetics of histone protein

Epigenetics may change gene expression but not because of modifications in the DNA sequence. The epigenetic changes consist of histone modifications, DNA methylation and acetylation, small and non-coding RNAs[42]. MLL provides an important sample for research the correlated between epigenetic cell memory and human disease. DNA methyltransferase and histone deacetylase (HDAC), are kind of inhibitors and used for epigenetic based therapies that have strong and extensive effects on gene regulation in the cell. MLL is a necessary protein for normal development, while a highly mutated subset of them may influence the aggressive human leukemias. Therefore, the type of leukemia provides a helpful model for recognizing the connections between epigenetic cell memory and human disease. The nucleosome of chromatin, including H3/H4 tetramer and two H2A/H2B dimers, is the main target for epigenetic memory. On the other hand, post-translational modification (PTM) is another main source of protein diversity.

Histone methylation

Novel methylation of gene promoter and histone protein may show abnormal gene expression profiles. While high methylation of promoter sequence results from gene silencing, histone methylation has changed the conformation of chromatin structure and let transcription factors accession to the genes easily. Histone protein methylation and changes of chromatin conformation affect gene expression globally in *MLL*. The types of t(4;11), t(9;11) and t(11;19) translocations are the best-studied examples of promoter methylation in *MLL*[43]. The latest genomic research data reveal different epigenetic mechanisms that help for leukemia development. These are the existence of MLL-MLLT1, MLL-

MLLT3, MLL-MLLT10 or the reciprocal AFF1-MLL fusion protein and MLL-AFF1 fusion protein (ALL1-fused gene from chromosome 4, AF4).

DOT1L methylation

DOT1L methylates histone H3 at lysine 79 and initiates the chromatin modification during the transcriptional elongation[44]. AF10 binds *DOT1L* for recruitment which is essential for the transforming activity of MLL-AF10[45]. MLL fusion proteins also bind and increase H3K79 modification which is hypermethylated by activation of *HOXA9*[46].

Histone acetylation

Histone acetylation is another chromatin modification process that histone acetyltransferases CREB binding protein (CBP) and p300 are active in MLL. Histone deacetylase (*HDAC*) and DNA methyltransferase inhibitors are the main targets for epigenetic based therapies that they have a common and widespread effect on gene control in the cell.

Drugs for AML and MLL fusion

The treatments of leukemia are chemotherapy, radiotherapy, hormone treatments, or bone marrow transplant that show a distinct rate of cure depending on the type of leukemia and the age of the patient. Overall survival is almost four times better today than it was a half-century ago. The prognosis and decision of leukemia type are the difficult part of cancer. Two classes of chemotherapeutic agents, epipodophyllotoxin drugs; teniposide, etoposide and antracyclines are commonly known chemicals for 11q23 chromosome band abnormalities. The chemicals share a common mechanism of action that involves to bind and inhibit DNA topoisomerase II. Recent chemotherapy consists of a limited number of dense courses of cytarabine and anthracycline. However, the treatment of AML has the main problem which covers the high frequency of treatment-related deaths and long-term side effects[47]. Etoposide is an extremely suggestive treatment for MLL patients because of having DNA double-strand lesions in the etiology of *MLL* fusions.

HOXA gene family

HOXA genes encode active transcriptional factors that can change the gene expression. Improper expression of HOXA genes may produce malignant hematopoiesis. HOXA cluster gene expression abnormalities affect the proper work of the hematopoietic system during its development and stimulate the initiation of leukemogenesis. MLL types of leukemia have some *HOXA* gene family abnormalities, such as *HOXA4, HOXA7, HOXA9* and *HOXA10* genes. HOX proteins can block differentiation and promoting the survival of transformed cells for leukemogenesis[48]. It is well known that hematopoiesis activity can be regulated by reintroducing HOXA gene[49]. MLL protein is found in different tissues, such as liver, spleen, brain, heart, lungs, testes, colon, thymus, kidneys, tonsils and thyroid. However, gene mutations and rearrangements are mostly observed in hematopoietic cells. Some specific signals permit the activation of anti-apoptotic pathways which are caused by the presence of oncogenic MLL fusion proteins during fetal liver and hematopoiesis. The gene abnormalities are also observed in mice research and the mice die during embryonic day 12.5 and 16.5[50]. The hematopoiesis abnormality is the result of decreasing expression of *HOXA4, HOXA7, HOXA9*, and *HOXA1* in mice fetal liver and decreases the number of long and short-term reports of MLL-deficient mice demonstrated that MLL is needed for suitable segment structure in the axioskeletal system and also regulates hematopoiesis[51]. *HOXA9* is the most correlated gene for poor prognosis in AML and overexpression of the gene is enough to stimulate leukemia in mice. However, co-expression of *MEIS1* is speeding up disease starting. Some MLL involved fusion proteins can initiate leukemia independent of *HOXA9* which is covered by other HOXA cluster genes[52]. With the exception of *HOXA2* and *HOXA5*, almost all HOXA cluster genes overexpression direct to immortalization in the serial relating experiment[53]. Super elongation complex (SEC) known as transcription elongation factors is required for *HOX* gene expression in leukemic cells. It has already been realized that MLL fusion proteins can deregulate *HOX* genes, but there are exceptional reciprocal MLL fusion proteins[54]. *MEIS1* and *HOXA* gene family members expression increase in the leukemogenesis. *MEN1*, *LEDGF* and *MYB* proteins association at the N-terminal location of the MLL fusion are functionally important[55]. It was demonstrated *HOXA* gene under expression in the pediatric patients with translocation t(4;11), correlated with a bad prognosis[56].

Partial tandem repeats

Partial Tandem Repeats, PTD, are derived from other MLL gene translocations that produce a chimeric gene fusion. The PTD of the MLL gene preserves the MLL encoded protein domains. Although MLL-PTD is common in adult AML patients, not

in pediatric AML, has been identified at a low level in healthy humans. Around 7.5% of AML patients with a normal karyotype have a PTD of the *MLL* gene. The non-existence of PTD in *MLL* gene is correlated with poor prognosis in the form of AML and normal karyotype in adult patients.

CONCLUSIONS

MLL leukemia has different types because of chromosomal rearrangements. The arrangements result of different oncogenes and tumor suppressor genes activation[57-59]. The rearrangements are the main reason of MLL type of protein alterations[60]. Since MLL type of leukemia is one of the deadly types of cancer, even it can kill the babies in their mother's womb, analysis of genomic alterations and abnormalities of it is very important to understand its aggressiveness. Although one type of genomic alteration is enough to make a person cancer, MLL type shows more than one type of genomic alterations simultaneously. The chromosomal rearrangements are followed by abnormal histone modification via methylation and MLL-specific gene expression profile. The informative and clear explanation of the review will help to the researcher to cover the general information of MLL type of leukemia.

Conflict of Interest:

The author declares that they have no conflict of interest.

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