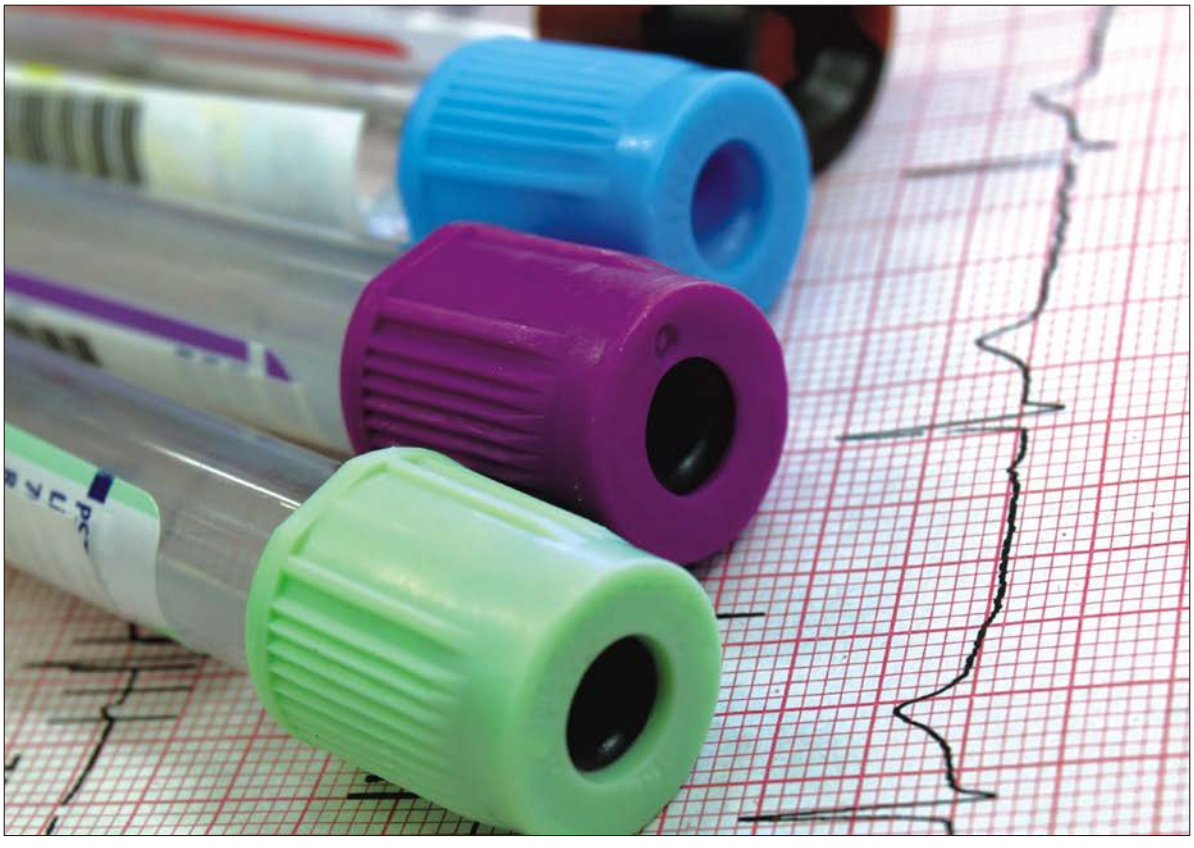

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Molecular Diagnostics and Therapy of Acute Myeloid Leukemia

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ABSTRACT

Acute Myeloid Leukemia (AML) is the most frequent cause of acute leukemia affecting adults, its incidence increases steadily with age and has been correlated with DNA methylation aberration and inactivation of tumor suppressor genes. Recent epigenomic studies showed that DNA methylation abnormalities have been observed in age related acute myeloid leukemia and indicated the importance of DNA methylation analysis for AML diagnosis. An insight of the connection between DNA methylation and AML might help in development of novel molecular diagnostic and genomic therapies. Epigenetic drugs of two families namely the DNA-demethylating agents and inhibitors of histone deacetylase have emerged as the most promising compounds in this area and several pharmaceutical compounds have received approval for the treatment of specific leukaemia and lymphoma subtypes. In addition possible combination between molecular therapeutic approaches including the activation of certain signal transduction pathway(s) like the interleukins family and chromatin-remodeling events is feasible by the application of epigenetic drugs. This approach might be one of the potential promising solutions for the AML treatment. The present study reviewed recent advances in the research related to genomic and epigenomic of acute myeloid leukemia.

Key words: DNA methylation, cancer, AML, tumor suppressor genes, chromatin remodeling

INTRODUCTION

Nowadays cancer is considered a major chronic disease leading to death; it is only second to heart disease and viewed as an age related disease (Ahmed *et al*., 2006; Dosay-Akbulut, 2006; Chatterjee, 2011; Gatoo *et al*., 2011; Singh *et al*., 2011). Leukemia is neoplastic proliferation of leukocyte precursor in the bone marrow. It could be classified to either lymphoid cells or myeloid cells by the type of white blood cell affected and acute or chronic according to the rate of cell growth. Acute leukemia develops rapidly and characterized by an overgrowth of immature blood cells, while chronic leukemia comprises an overgrowth of mature blood cells and develops slowly (Lowenberg *et al*., 1999; Bolufer *et al*., 2006). Furthermore, the two major groups of leukemia were classified into four subgroups, namely: Acute Lymphoblastic Leukemia (ALL), Acute Myeloid Leukemia (AML), Chronic Lymphoid Leukemia (CLL) and Chronic Myeloid Leukemia (CML). ALL is a severe blood disorder in which abnormal leukocytes are identified as immature forms of lymphocytes (Bassan *et al*., 2004; Bolufer *et al*., 2006). AML is a cancer of the

myeloid of blood cells and characterized by the rapid growth of abnormal white blood cells in the bone marrow, this specific nature of the disease leads to interference with the production of normal blood cells (Pinto *et al.*, 1998).

Implications of Cyclin-dependent kinases (CDKs), Cyclin-dependent kinase inhibitors (CKIs), DNA methylation and other molecular events in the development of leukemia have been investigated (Chan *et al.*, 1995; Hirai *et al.*, 1995; Ibrahim, 2010a, 2012). Taking into account the direct association of these factors in the development of cancer, the objective of this study was to review the perspective of recent advances in the research related to genomic and epigenomic of acute myeloid leukemia in leukemia cancer research and their impact on the development of new diagnostic and therapeutic approaches.

CLASSIFICATION AND DIAGNOSIS OF AML

AML development process in human is a multistage process where the chromosomal translocators in either the Hematopoietic Stem Cells (HSC) or in the progenitors induce the pre-leukemic state where successive mutational events lead to the AML development (Fig. 1).

AML patients show signs and symptoms of fatigue, hemorrhage, or infections and fever due to decreases in red cells, platelets, or white cells, respectively. AML main clinical findings are directly attributable to the leukemic infiltration of the bone marrow, with resultant anemia, granulocytopenia, or thrombocytopenia, with or without leukocytosis and leukemic infiltration of the various tissues, including liver (hepatomegaly), spleen (splenomegaly) and lymph nodes (lymphadenopathy) (Lowenberg *et al.*, 1999). The most commonly used method of AML classification is that developed by the French-American-British (FAB) group which divides AML into eight distinct subtypes (M0, M1, M2, M,3, M4, M5, M6 and M7) that differ with respect

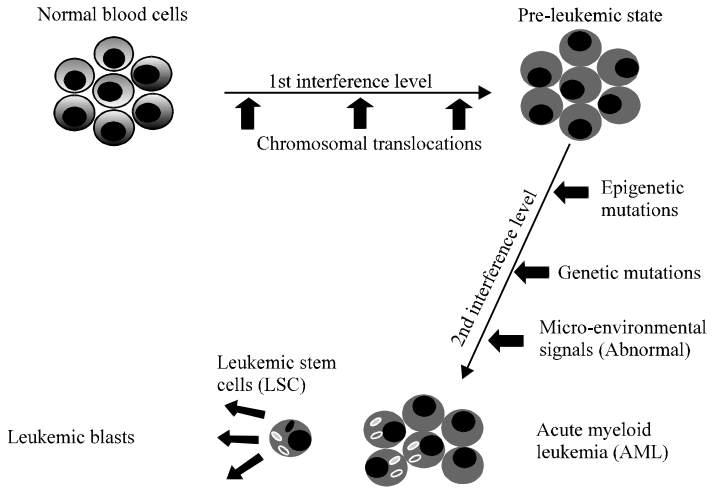

Fig. 1: Acute myeloid leukemia (AML) development process in human. This process is a multistage process where the chromosomal translocators in either the hematopoietic stem cells (HSC) or in the progenitors induce the pre-leukemic state where successive mutational events lead to the AML development. The AML phenotype is propagated and maintained in the leukemic stem cells (LSC) sub-population due to AML cells bearing the necessary mutations involved in the pathogenesis process

to the particular myeloid lineage involved and the degree of leukemic-cell differentiation. The more frequent chromosomal abnormalities shown in AML patients were t (8; 21), t (6; 9), inv (16), del (11q) and t (9; 11), respectively (Harrison, 2000).

Several methods have been used for diagnosis of AML; these methods include morphologic examination of blasts in bone marrow or blood and cytogenetic abnormalities (Betz and Hess, 2010). Cytogenetic analysis revealed that more than 50% AML patients have an abnormal karyotype (Grimwade and Hills, 2009). This type of analysis was also conducted on other types of cancers, e.g., malignant gliomas (Kandavelu and Vanisree, 2011). Recent study indicated the importance of molecular analysis based on various techniques, such as molecular analysis of protein (Hamad *et al.*, 2009) or Polymerase Chain Reaction (PCR) for leukemia diagnosis (Ibrahim *et al.*, 2009, 2010; Saleh *et al.*, 2010), these might have become an essential part of the diagnostic panel for acute leukemia. A new molecular method was used for detection of Arg72Pro that has been extensively genotyped for association with a wide variety of cancers. The method is based on the tetra-primer amplification refractory mutation system-polymerase chain reaction (ARMS-PCR), with a single PCR to discriminate both alleles (Lajin and Alachkar, 2011). In addition, it was suggested that cytomorphology, cytogenetics, fluorescence *in situ* hybridization (FISH) and immuno-phenotyping with multi-parameter flow cytometry (MFC) need to be applied for diagnosis (Moosavi *et al.*, 2008; Bacher *et al.*, 2009).

GENES INVOLVED IN SIGNAL TRANSDUCTION OF LEUKEMIA

Protein phosphorylation is an important mechanism in signal transduction pathways; specific enzymes perform this function, these are known as kinases which regulate the essential and vital routes linked to various cell growth activities including cell growth and proliferation (Broekman *et al.*, 2011). This mechanism is controlled by Cyclin-Dependent Kinases (CDKs) proteins which are well conserved serine/threonine kinases; they have a very low kinase activity and require the binding to cychns for their activation (Connell-Crowley *et al.*, 1993; Morgan, 1997). There is another group of proteins called cyclin-dependent kinase Inhibitors (CKIs), these are regulatory proteins which prevent the phosphorylation of targets by CDK/cyclin involved in cell cycle (Nigg, 1995; Elledge *et al.*, 1996; Bruggeman and van Lohuizen, 2006). Research in this field provided information about CDKs and CKIs role in controlling cell cycle and development of cancer. It have been shown in several forms of cancer, CKIs such as p16 and p27 are mutated (Serrano *et al.*, 1993; Kamb *et al.*, 1994; Nobori *et al.*, 1994). On the other hand, there are indications that they are degraded in several types of cancer; thus low levels of p27 are correlated with poor clinical prognosis (Porter *et al.*, 1997). Other studies have shown that there are two families of CKIs. The first family includes the INK4 proteins (inhibitors of CDK4), so named for their ability to specifically inhibit the catalytic subunits of CDK4 and CDK6. This family includes four proteins, p16INK4a, p15INK4b, p18INK4c and p19INK4d (Serrano *et al.*, 1993; Hannon and Beach, 1994; Guan *et al.*, 1994; Chan *et al.*, 1995; Hirai *et al.*, 1995). The four proteins (p15, p16, p18 and p19) which are associated with the development of AML are shown in Table 1.

DNA METHYLATION, CANCER AND AML

Epigenomics deals with molecular elements and mechanisms influencing gene expression (Heindel *et al.*, 2006; Guil and Esteller, 2009). Three types of epigenomic mechanisms have been identified in the human's genomic DNA; these are associated with gene expression and implicated

Table 1: The four proteins of cyclin-dependent kinase inhibitors (CKIs) associated with the development of Acute Myeloid Leukemia (AML), references are shown in the text

CKIs	Chromosome location	Tumor type	Alteration
p15	9p21	Acute lymphoblastic leukemia (ALL)	Genetic loss
		Acute myeloid leukemia (AML)	Genetic loss
p16	9p21	Acute lymphoblastic leukemia (ALL)	Genetic loss
		Acute myeloid leukemia (AML)	Genetic loss
		Chronic myelocytic leukemia (CML)	Genetic loss
p18	1p32	Acute myeloid leukemia (AML)	Upregulation
p19	19p13	Acute myeloid leukemia (AML)	Upregulation

in development, diseases and other biological phenomena (Ibrahim, 2010a, b, 2012). The three mechanisms are DNA methylation, histone modifications and RNA interference (Heindel *et al.*, 2006; Holliday, 2006; Suzuki and Yoshino, 2008; Taby and Issa, 2010). Genomic DNA methylation is the product of methylation of cytosine and formation of 5-methylcytosine (Strathdee and Brown, 2002). DNA methylation profile of human genome is called methylome (Lister and Ecker, 2009). Molecular analysis demonstrated that 3-4% of all cytosine molecules are methylated, yet approximately 0.75-1% 5-methylcytosine makes up of all nucleotide bases in the DNA of normal human tissue (Esteller and Herman, 2002; Esteller, 2008; Fraga *et al.*, 2002). It is worth mentioning an important characteristic of genomic DNA, that is the presence of CpG islands, which are stretches of CpG dinucleotides and are generally unmethylated but these regions are prone to DNA methylation (Bird, 1992).

Accumulating research works have provided increasing support for the association between of DNA methylation with various malignancies (Ibrahim, 2010a, b; Ibrahim and Makkiya, 2011; Schulz and Goering, 2011; Ibrahim, 2012). Nowadays it is accepted that changes in the normal DNA methylation patterns are the most common molecular lesions of genomic DNA of cancer cell. There are two types of DNA methylation aberrations; these are either alterations in the global hypomethylation which lead to oncogene activation and chromosomal rearrangement or hypermethylation of CG islands of the promoter regions of tumor suppressor genes (Nguyen *et al.*, 2001; Liu *et al.*, 2003). It is worth mentioning that hypermethylation of the promoter-associated CpG islands of suppressor genes will cause loss of gene expression, especially when this methylation affects tumor suppressor genes. Evidence for this came from the study of two such genes, VHL (the gene mutated in Von Hippel-Lindau disease) and CDKN2A (which encodes p16INK4A, a gene commonly mutated in many types of cancer) where, in a subset of tumors, dense promoter methylation was described, in association with loss of gene expression (which could be partially restored using methylation inhibitors), absence of coding region mutations and tumor-specific patterns of methylation (Kuerbitz *et al.*, 1999).

An interesting investigation was carried out on the breast cancer cell lines to investigate the relationship between promoter methylation (assessed by methylation-specific PCR, bisulfite sequencing and 5-aza-2'deoxycytidine treatment) and the DNA methyltransferase machinery (total DNMT activity and expression of DNMT1, DNMT3a and DNMT3b proteins) (Roll *et al.*, 2008). The study revealed two groups of cell lines that possessed distinct methylation signatures, hypermethylator cell lines and low-frequency methylator cell lines.

As indicated earlier, leukaemia has traditionally been viewed as a genetic disease; however recent investigation has shown that defects in the epigenomic DNA methylation also play an important role. In this respect a recent study illustrated that digestion of genomic DNA of normal

individuals with HpaII (which cleaves the sequence CCGG only if the internal cytosine residue is unmethylated) and MspI (which cleaves the same sequence regardless of methylation) showed lower degree of differences in RAPD-DNA patterns, indicating normal methylation patterns. However, different picture was shown when studying RAPD-DNA bands profile of CML genomic DNA following digestion with restriction HpaII and MspI, clear variations in bands patterns were observed when using selected RAPD primers to amplify digested genomic DNA by two enzymes (MspI and HpaII) for normal individuals and CML patients (Ibrahim, 2010a).

GENOMIC MUTATIONS OF TUMOR SUPPRESSOR GENES

Tumor suppressor genes can reduce the probability of transforming normal human cell into tumor cell (Yeo, 1999; Sherr, 2004; Ibrahim, 2010a). It is well known that mutations, chromosomal translocations and DNA methylation aberration can inactivate tumor suppressor genes thus leading to activation of oncogenes without altering the primary sequence of DNA (Robertson and Wolffe, 2000; Jones and Baylin, 2007; Esteller, 2008). Accordingly, some tumor suppressor genes have the ability to stop the cell from multiplying until the DNA damage is repaired; however, once tumor suppressor genes do not function correctly, the cells with DNA damage continue to divide and can accumulate further DNA damage that can eventually lead to the formation of a cancer cell (Macleod, 2000; Hussain *et al.*, 2001). Literatures provided information on the role of number of tumor-suppressor genes. Mutations in Rb suppressor gene are associated with retinoblastoma, a serious cancer of the retina that occurs in early childhood. In addition to retinoblastomas, mutations in the Rb gene have been detected in osteosarcomas, bladder carcinomas, small-cell lung carcinomas, prostate carcinomas, breast carcinomas, some types of leukemia and cervical carcinomas (Krug *et al.*, 2002). Mutations in the tumor suppressor gene p53 are found in a large proportion of human hematological malignancies and other cancers; increased amounts of cellular p53 protein after DNA damage have been associated with cell-cycle arrest and programmed cell death (apoptosis) and mutations or losses of p53 have been resulted in development of cancer (Velculescu and El-Deiry, 1996; Foulkes, 2007).

MOLECULAR AND DNA METHYLATION THERAPY

Several recent approaches have been reported for possible therapy of various types of cancers (Saeed *et al.*, 2005; Saxena and Moorthy, 2007; Mohan *et al.*, 2008; Hafidh *et al.*, 2009). One of these approaches is molecular utilization of interleukins in cancer therapy; the interleukins (ILs) represent another large family of cytokines, with at least 25 different constituent members (IL-1 to IL-25) having been characterized thus far. The immune stimulatory activity of IL-2 has proved beneficial in the treatment of some cancer types. Further studies have shown additional cancer types, most notably ovarian and bladder cancer, non-Hodgkin's lymphoma and acute myeloid leukemia, to be at least partially responsive to IL-2 treatment (Walsh, 2003a,b).

Moreover, there is an increasing trend toward utilization of an important fundamental distinction between epigenetics and genetics in new novel ways of cancer treatment; this difference is the potential reversible property of DNA methylation and histone alterations. Thus, it has been suggested the possibility of using this phenomena for cancer treatment. Reforming DNA methylation alterations in the methylome of cancer's patients is part of new approach of epigenomic therapy of cancer (Esteller, 2008; Jain *et al.*, 2009). The results in this field showed the possibility of amending the aberrated DNA methylation pattern of the affected CG regions of cancer's genomic DNAs by using specific drugs. Accordingly, the therapeutic approach is to adjust the aberrated DNA

methylation status of hypermethylated tumor-suppressor genes to normal state (Ibrahim, 2010a,b). One of the essential differences between human cancer genetics and epigenetics is that DNA methylation and histone modification changes are reversible under the right circumstances. Thus, epigenetic alterations are one of the weakest points in the defenses of the cancer cell, because those hypermethylated tumour-suppressor genes in their long "sleep" can be awoken and reactivated with the right drug regimens and exert their normal growth-inhibitory functions. Two families of epigenetic drugs, DNA-demethylating agents and inhibitors of histone deacetylase, have emerged as the most promising compounds in this area and several pharmaceutical compounds have received approval for the treatment of specific leukemia and lymphoma subtypes (Fandy *et al.*, 2007; Hellebrekers *et al.*, 2007; Abujamra *et al.*, 2010; Chen *et al.*, 2010; Mani and Herceg, 2010; Martinet and Bertrand, 2011). The successful story in these malignant disorders needs now to be translated to epithelial solid tumours.

CONCLUSIONS

Genomic and epigenomic molecular technologies can help in introducing new approaches for classification, diagnosis and therapies of cancers. AML like several other cancers are apparently epigenetic diseases of reverse nature making them target to specific medical drugs. Thus there is potential to develop new therapeutic tactic using combination of available molecular treatment approaches by interfering into the signal transduction pathway and DNA methylation, this might be the best treatment modality with future perspectives. Current direct or indirect molecular treatment of AML might increase the patient's bad prognosis. It is expected that further understanding of the functional molecular mechanisms of this disease is necessary for effective, successful design of therapeutic approaches leading to successful cure of the disease.

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