INTRODUCTION

Matrix metalloprotenases (MMPs) are a family of structurally and functionally related Zinc-dependent endopeptidases consisting of at least 20 enzymes which are capable to degrade all the protein components of the extracellular matrix (ECM). Most MMPs are secreted in catalytically patient forms. Their activity is regulated at the levels of gene expression ***(Lin et al., 2002)***.

The two matrix metalloproteinases MMP-2 gelatinase A and MMP-9 gelalivase B play key roles in tissue remodeling and tumor invasion by digestion of extracellular matrix barriers. MMP-9 measurements in blood have provided more encouraging results in cancer than MMP-2 assays ***(Lin et al., 2002).***

Matrix metalloproteinases (MMPs) and tissue inhibitors of metalloprotinases (TIMPs) regulate the turnover of the extracellular matrix and may module the biology of haematopoietic cells ***(Marquez-Curtiset al., 2001)***.

MMPs and TIMPs are key players in many physiological and pathological processes such as development, angiogenesis, connective tissue remodeling, wound healing and inflammation, as well as tumor invasion

and metastasis. The main role of MMPs in angiogenesis, tumor growth and metastasis is degradative of extracellular matrix (ECM) and release and/or activation of growth factors through their degragative activity. The degradative activity finally results in cancer progression ***(Klein et al., 2004)***.

MMP-9 was significantly increased in plasma of patients with breast cancer gastrointestinal tract cancer and hepatocellular carcinoma as compared with that of healthy subjects. In addition, clinical follow up of patients with metastatic cancer indicated that the survival time of patients with increased plasma levels of MMP9 was significantly shorter than that of patients with normal plasma levels ***(Akna et al., 1999)***.

Among TIMPs, TIMP-1 and TIMP-2 are secreted in the soluble form by a wide range of cell types including fibroblasts, monocytes, macrophages and endothelial cells.

TIMP-1 forms a 1:1 complex with the latent form of MMP9, whereas TIMP-2 binds with the highest affinity to the latent and active forms of MMP-2. TIMP-1 promotes the growth of K-562 erythroleukemia cell and may act on this cell line as an autocrine growth factor. The importance of this data is further supported by the fact that first MMP inhibitor drugs are in clinical trials ***(Jinga et al., 2006)***.

Leukemia is defined as the uncontrolled proliferation or expansion of hematopoietic cells that do not retain the capacity to differentiate normally to mature blood cells. In addition this abnormal blood cell development may result in a breakdown of cell to stroma interactions leading to the subsequent egress of immature blood elements from the bone marrow to the peripheral blood. Therefore, leukemia can serve as a paradigm for metastasis in general. However, the implications of MMPs or TIMPs in the bone marrow of patient with acute leukemia have not been explored. The assessment of the angiogenic factors are likely to be helpful in predicting patient outcome and selecting optimal therapeutic regimen ***(Aref et al., 2005).***

AIM OF THE WORK

The aim of the present study is to estimate marrow MMP-9 expression and to detect levels of TIMP-1 in patients with acute lymphoblastic leukemia and to correlate these results to other biological factors, such as immunophenotyping and cytogenetic analysis in an attempt to clarify their role as useful surrogate marker for monitoring disease status and propose it as a potential prognostic factor.

ACUTE LYMPHOBLASTIC LEUKEMIA

Introduction

Leukemias are diseases which develop as a consequence of abnormal uncontrolled proliferation of a single mutant hematopoetic progenitor cell that has the capability to expand by indefinite self renewal, giving rise to malignant poorly differentiated hematopoetic cells that progressively infiltrate the bone marrow ***(American Cancer Society, 2007)***.

Acute leukemia is broadly classified into myeloid and lymphoid cell types and subdivided according to stages of differentiation ***(Hoff brand et al., 2001).***

Acute lymphoblastic leukemia (ALL) is a malignant disorder that originates in a single B or T lymphocyte progenitor. Proliferation and accumulation of blast cells in the marrow result in suppression of hematopoiesis and thereafter, anemia, thrombocytopenia, and neutropenia. Lymphoblasts can accumulate in various extramedullary sites, especially the meninges, gonads, thymus, liver, spleen, and lymph nodes. ALL has many subtypes and can be classified by immunologic, cytogenetic, and molecular genetic methods. These methods can identify biologic subtypes requiring treatment approaches that differ in their

use of specific drugs or drug combinations, dosages of drug or duration of treatment required to achieve optimal results.

Epidemiology of ALL

ALL is the most common malignant disease affecting children, accounting for approximately 30% of childhood cancer ***(Peris Bonet et al., 1996).***

ALL incidence is highest at 3-4 years falling by 10 years of age and has a secondary rise after the age of 40 years ***(Grurney et al., 1995)***. Males are more commonly affected expect in infancy where there is a greater incidence in female ***(Grurney et al., 1995)***. There has not been major difference in incidence according to geographical areas or between urban, industrial and rural areas. ALL is commoner in white ***(Carroll and Bhojwani, 2003).***

Etiology of ALL

Genetic, environmental and viral factors have been suspected in the etiology of ALL, although most of ALL patients have not been exposed to those factors. Even when those associated conditions were found, the mechanism by which they caused the development of the disease was unknown. The most accepted theory is that those causative

factors most probably act through activation of oncogenes ***(Rabbitts, 1991).***

Genetic factors:

The most probable mechanism of development of ALL is the acquired mutation in hematopoietic stem cell. However, there is evidence for inherited predisposition to leukemia in children with genetic disorders and constitutional chromosomal abnormalities ***(Pui, 1995).*** The risk of developing ALL is 15-20 times more in children with Down's syndrome (trisomy21). Other syndromes like Bloom's syndrome (in which there is increased chromosomal fragility) and Klinfilter syndrome (XXY) have an association with ALL ***(Vanasse et al., 1999).***

Siblings of children with ALL have 2-4 folds greater risk to develop the disease. Also, when leukemia occurs in one identical twin, the other twin has a 20% chance of developing the disease due to intrauterine metastasis from one twin to the other via the shared placental circulation ***(Pui, 2006).***

Occupational and Environmental factors:

Radiation has been acknowledged as causative agent of ALL. The incidences were reported among young survivors of the atomic bomb and those exposed to

radioactive fallout from nuclear weapons testing ***(Preston et al., 1994)***.

Also, intrauterine (but not postnatal) exposure to diagnostic X-rays confers a slightly increased risk for the development of ALL, which correlates positively with the number of exposures. Therapy with alkylating agents has shown 14 folds increase in probability of development of leukemia in survivors of childhood cancers ***(Tuker et al., 1997).***

Pesticide exposure (occupational or home use), parental cigarette smoking and maternal alcohol consumption during pregnancy have been suggested as causes of childhood ALL ***(Sandler and Ross, 1997).***

Viral etiology:

The confirmed association is between ALL of L3 subtype and EBV infection which may be due to increase lymphoid proliferation that provides a setting in which a second oncogenic event, possibly myc oncogene activation can occur ***(De Vita et al., 2001).***

Secondary ALL:

Blast transformation in chronic myeloid leukemia and myelodysplastic syndrome is as 2ry ALL. It has also

been reported secondary to bone marrow transplantation ***(Deeg and Witherspoon, 1993).***

Pathogenesis:

Since the year 2001, there has been an exponential increase in the knowledge of the molecular events associated with the pathogenesis of human and experimental leukemias and lymphomas. Different chromosomal abnormalities have been identified in such diseases which might be structural or numerical ***(Baer, 2001).***

Genetic damage resulting in positive proliferative signals, loss of tumor suppressor gene function, inhibition of developmental programs, blocks in cell death pathways and avoidance of host defense mechanisms can all be documented to play a part in the pathogenesis of leukemia. The most obvious genetic changes are defined by the translocations that activate abnormal gene expression and frequently create chimeric structures in a broad range of leukemias and lymphomas. Such translocations provide a molecular definition for a specific type of leukemia and a focus for defining a portion of the potential growth control alterations ***(Charles et al., 2001).***

Alteration in the expression of genes or in the properties of the encoded proteins resulting from the rearrangement play an integral role in the process of malignant transformation. There are two general mechanisms by which chromosomal translocations result in altered gene function. The first is deregulation of gene expression. This mechanism is characteristic of the translocations in lymphoid leukemias that involve the Ig genes in B lineage tumors and TCR genes in T lineage tumors. These rearrangements result in inappropriate expression of the partner gene involved in translocation, with no alteration in its protein structure. The second mechanism is the expression of a novel fusion protein, resulting from the juxta position of coding sequences from two genes that are normally located on different chromosomes. Such chimeric proteins are "tumor specific", thus the detection of such a fusion gene or its protein products can be important in diagnosis or in the detection of residual disease or early relapse ***(Roman-Gomez et al., 2004).***

Mechanisms of Leukemogenesis at Different Levels:

1. ***Hematopoietic Growth Factors as Definitive Participants in Leukemogenesis****:*

A hyperproliferative state that can evolve into leukemic phenotype has been documented when cytokine genes are constitutively expressed. These hyperproliferative states are likely produced from hematopoietic elements ***(Hawley et al., 1998)***.

Much has been published correlating growth factor levels as a participant in the causation and evolution of human leukemia. Specific mechanisms have evolved to kinetically regulate the action of growth factors and their receptors. For every phosphorylation event occurring at the receptor level or downstream in a signaling pathway, there are balances with dephosphorylation mechanisms that limit the strength and duration of the signaling pathway. A particular good example of this type of mechanism is demonstrated by the family of suppressors of cytokine stimulation genes that are transcritpionally induced by cytokine action but function to bind and inactivate critical components in the signal cascade and limit the persistence of the signal ***(Abbas and Lichtman, 2003).***

2. <u>Receptor Alterations in Leukemogenesis:</u>

Receptor can be considered as any molecule capable of receiving a signal and participating in transmitting it into the cell. This is relatively easy to visualize for molecules such as transmembrane tyrosine kinase receptors like c-kit, CSF-1 receptor, or the insulin receptor, which bind a specific protein ligand in a cell-surface-bound or soluble form to activate growth and differentiation programs in immature hematopoietic precursors and other cell lineage. The intrinsic tyrosine kinase activity is activated by ligand engagement of the receptor molecules, which causes an allosteric change in shape and multimerization that is transmitted through the transmembrane portion to the intracellular domain, which leads to a program of tyrosine phosphorylation of the receptor, recruitment of other signaling molecules, and further signal transduction ***(Weiss and Schlessinger, 1998).***

Receptors of the large family of cytokines that regulate hematopoiesis can have more complicated subunit structures but lack an intrinsic enzyme activity ***(Baird et al., 1995).*** The intracellular domain of one or more subunit is coupled to kinases such as the JAK family, which can sense the binding of extracellular ligand, become activated, and pass on the signal to molecules to carry the signal into the cell ***(Abbas and Lichtman, 2003).***

Equally important are the molecular inter-actions that modify and regulate the efficiency of ligand presentation and hence signal strength to the primary receptor. In the transmembrane multi-subunit serine kinase family of receptors used by members of the transforming growth factor-β (TGF-β) family of ligands ***(Massague and Weis-Garcia, 1996)***. The signals that produced from this family of receptors can include strong negative effects on cell cycle proliferation and can serve as antagonists of factors having positive influences on the expansion of blood formed elements ***(Charles et al., 2001).***

Because the overall outcome of a cellular decision to divide, differentiate, or die must integrate different receptor activities, alterations in receptor function may be expected to happen prominently in human leukemogenesis. Mutations that vary the dosage of a receptor, its ability to activate in low or zero ligand concentration, or resist the down regulatory mechanisms that normally limit the duration of signaling for positive acting receptors should set the stage for secondary damage or progression to leukemia. Conversely, inactivation of negative regulatory receptors may change the balance of cell growth and promote leukemic changes. For example, the amplification and hyper-expression of growth factor receptors are well documented and strongly correlate with chances of progression of leukemia ***(Salmon et al., 1987).***

The second example is TGF-β superfamily. The members of this family are known to slow or block the hematopoietic cell proliferation for specific lineages. Reduction or loss of function of the TGF-β system on certain blood cells such as lymphocytes generally would be predicted to augment cell growth and participate in leukemogenesis ***(De Coteau et al., 1997).***

3. Intracellular Signaling Mechanisms:

When a blood cell at any stage of maturation encounters a signal through a cell surface receptor or combination of receptors, it must transmit that information into the cell for a decision process. Many such decisions involve transfer of information to the nucleus to drive gene transcription events associated with cell cycle progression or definitive differentiative changes ***(Charles et al., 2001).***

4. Transcription Factor Translocations:

The number of transcription factors identified at the sight chromosome translocations has continued to grow. Lymphoid and myeloid leukemias can be grouped according to frequency of specific translocations ***(Tenen et al., 1998).***

Translocations can alter the transcription factor target gene in two ways. In numerous lymphoid leukemias, one

partner in the translocation is the immunoglobulin or T-cell receptor locus, which creates a strong transcriptional signal adjacent to the target gene without directly affecting its structure ***(Roman-Gomez et al., 2004).*** The resulting leukemias develop as a consequence of increased dosage of the transcription factor target because of high-level over expression of the normal gene product ***(Hatano et al., 1991).***

A more frequent mechanism for transcription factor deregulation by chromosome translocations in leukemia is through the creation of fusion genes, analogous to the tyrosine kinase fusions involving Ab1, pDGFR, and JAK. Many of these genes play essential role in hematopoietic development or differentiation, and the fusion proteins interfere with this function. The transcription factor gene fusions can function as oncogenes by distinct mechanisms; these genes are categorized as genes that primarily play a role in hematopoietic development, hematopoietic differentiation, homeotic patterning, or apoptosis ***(Charles et al., 2001; Roman-Gomez et al., 2004).***

5- Abnormalities in genes involved in cell cycle progression:

In the cell cycle field there are several pathways that serve as checkpoints to regulate cell cycle entry progression at appropriate times. One such pathway regulates the G_1/S-phase checkpoint and involves cyclin-