Expression of Phosphorylated STAT5 in Acute Myeloid Leukemia

Thesis

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ABSTRACT

Background: AML is characterized by maturation arrest of a malignant clone of myeloid cells. Signal transducer and activator of transcription 5 (STAT5) is a transcription factor that regulates many aspects of cell growth, survival and differentiation. Constitutive activation of STAT5 has been identified in a number of hematopoietic malignancies including AML.

Aim: The aim of this study was to assess the protein expression of phosphorylated-STAT5 in leukemic blasts of newly diagnosed AML patients to correlate its expression with the clinical outcome in these patients.

Subjects and Methods: Thirty patients suffering from de novo AML were included in the study. Assessment of phosphorylated-STAT5 protein in AML blasts of bone marrow aspirate/peripheral blood smear films was performed by immunocytochemistry using rabbit anti-phospho-STAT5A/B (Y694/Y699) and fluorescence-labelled anti-rabbit IgG antibodies.

Results: Expression of total p-STAT5 was detected in 23/30 (76.7%) of cases. Expression of cytoplasmic and nuclear p-STAT5 was detected in 24/30 (80%) and in 7/30 (23.3%) of cases, respectively. Median blast count percentage in peripheral blood and bone marrow at presentation was significantly higher in the total p-STAT5 positive AML patients than the p-STAT5 negative AML patients (median 30% *vs.* 10%, P=0.028 and median 60% *vs.* 45%, P=0.048; respectively). No statistically significant difference was found between the response to induction therapy and p-STAT5 expression.

Conclusion: The present study confirmed the expression of total phosphorylated-STAT5 in about two-thirds of newly diagnosed AML cases. Cytoplasmic p-STAT5 was detected in a larger set of AML patients than nuclear p-STAT5. No statistically significant impact of p-STAT5 expression was encountered on the clinical outcome in these patients.

Key words:
AML, phosphorylated STAT5, nuclear p-STAT5, cytoplasmic p-STAT5, FLT3.

Contents

List of Abbreviations

ABL	Abelson gene
AIDS	Acquired immunodeficiency syndrome
ALL	Acute lymphoblastic leukemia
AML	Acute myeloid leukemia
AMML	Acute myelomonocytic leukemia
APL	Acute Promyelocytic Leukemia (M3)
ATRA	All trans- retinoic acid
BAALC	Brain and acute leukemia cytoplasmic gene
BCR	Breakpoint cluster region
BMA	Bone marrow aspirate
BMSC	bone marrow stromal cell
BMT	Bone marrow Transplantation
BUN	Blood urea nitrogen
CBC	Complete blood count
CBFB	Core Binding Factor Beta gene
CD	Cluster of differentiation
CEBPA	CCAAT/enhancer-binding protein α
CLL	Chronic lymphocytic leukemia
CXCR4	CXC chemokine receptor 4
DIC	Disseminated Intravascular Coagulation
DN	Dominant-negative
DNA	Deoxyribonucleic acid
EDETA	Ethylene diamine tetra acetic acid
EPO	Erythropoietin
ETO	Eight-Twentyone gene
EVI1	Ecotropic virus integration-1
FAB	French American British
FISH	Fluorescence Insitu Hybridization
FLT3	Fetal liver Tyrosine Kinase 3
G-CSF	Granulcoytoe colony stimulating factor
GH	Growth Hormone

GM	Granulocyte monocyte
GvHD	Graft-versus-Host Disease
GvL	Graft versus Leukemia
HB	Haemoglibin
HLA-DR	Human leucocyte antigen class II
HNSCCs	Human head and neck squamous cell carcinomas
HSC	Hematopoietic stem cell
HTLV-I	Human T-cell lymphotrophic virus type I
IFN	Interferon
IHC	Immunohistochemical staining
IL	InterLeukin
ITD	internal tandem duplication
JAKs	Janus family tyrosine kinases
LCL	Cherry lymphoblastoid cells
LDH	Lactate dehydrogenase
M3v	M3 variant
M4Eo	M4 associated with Eosinophilia
MAP	Mitogen-activated protein
MAPK	Mitogen-activated protein kinase
MDR-1	Multi Drug Resistance-1
MDS	Myelodysplastic syndrome
MGG	May Grunwald-Giemsa
MLL	Mixed lineage Leukemia gene
MPD	Myeloproliferative Disorders
MPO	Myeloperoxidase
MYH11	Smooth Muscle Myosine Heavy Chain 11gene
NPM1	Nucleophosmin1
ODNs	Oligodeoxynucleotides
PAS	Periodic Acid Schiff
PCR	Polymerase Chain Reaction
PI3K	Phosphatidylinositol 3 kinase
PML	Promyelocytic leukemia
PRL	Prolactin Hormone

P value	Probability value
RARα	Retinoic Acid Receptor α gene
RB1	Retinoblastoma1
RBM15	RNA-binding protein 15
RT	Reverse Transcription
RUNX1	Runt-related transcription factor 1
SBB	Sudan Black B
SD	Standard Deviation
STAT	Signal transducer and activator of transcription
STAT3	Signal transducer and activation of transcription 3
STAT5	Signal transducer and activation of transcription 5
STK-1	Stem cell tyrosine kinase
TdT	Terminal deoxynucleotide transferase
TLC	Total leucocytic count
TPO	Thrombopoietin
WBC	White Blood Cells
WHO	World Health Organization

List of Tables

List of Tables (Cont.)

List of Figures

Introduction

The signal transducers and activators of transcription (STAT) gene family consist of seven proteins. STAT mediated signal transduction pathways influence neoplastic processes on a broad basis by perturbing control over survival, differentiation, proliferation, and apoptosis ***(Krebs and Hilton, 2001).***

STAT5 is one of seven members of the STAT family of transcription factors. Aberrant activation of STAT5 has been demonstrated in diverse groups of neoplasms, including AML. Activation of STAT is caused by tyrosine phosphorylation followed by the consecutive translocation of p-STAT into the nucleus. In normal hematopoiesis, STAT5 is activated by receptor ligation of Fms-like tyrosine kinase 3 (FLT3) to its ligand as well as by several other cytokines as IL3, GM-CSF and IL5. STAT5 is considered the preferred second messenger of FLT3-mediated signaling ***(Meier et al., 2009)***.

FLT3 and its downstream STAT-related pathways play a fundamental role in AML. Expression of p-STAT5 is significantly correlated to FLT3-internal tandem duplications (FLT3-ITD) in myeloid blasts. Strong adverse prognostic impact of FLT3-ITD in AML was reported in literature, with an association with an adverse clinical outcome with poor overall survival and a higher risk of relapse, particularly in AML with normal karyotypes. Therefore, assessment of FLT3 mutations as well as addressing their functional significance by measurement of p-STAT5 might be important ***(Kottaridis et al., 2001)***.

Analysis of p-STAT has been attempted by different methodologies such as electrophoretic mobility shift assays ***(Xia et al., 1998)***, western blot analysis ***(Spiekermann et al., 2002),*** flowcytometry

(Pallis et al., 2003), immunofluorescence ***(Bodo et al., 2009)*** and immunohistochemistry ***(Obermann et al., 2010).*** Interestingly, the percentage of cases with p-STAT expression varied between 7% and 95%. Some discrepancies can probably be attributed to the applied methodologies as well as the sensitivity and specificity of the antibodies. Assessment of STAT5 gene expression by quantitative PCR would not be helpful as well since the phosphorylated (active) protein amount and its microtopographic distribution is of relevance .This raises the question of whether immunocytochemistry of peripheral blood or bone marrow smears, which has the potential for delivering reproducible evaluation of cellular protein levels, would be a suitable methodology for the measurement of phosphorylated-STAT5 in AML**.**

Aim of the Work

The aim of this study was to assess the protein expression of phosphorylated-STAT5 in leukemic blasts of newly diagnosed AML patients by immunocytochemistry using rabbit anti-phospho-STAT5A/B (Y694/Y699) and fluorescence-labelled anti-rabbit IgG antibodies to correlate its expression with the clinical outcome in these patients.

Acute Myeloid Leukemia

Acute leukemias are clonal malignant disorders resulting from genetic alterations in hematopoietic stem cells that limit the ability of stem cells to differentiate into red cells, granulocytes, and platelets, and lead to the proliferation of abnormal leukemic cells or "blasts." Acute myeloid leukemias (AML), also referred to as acute nonlymphocytic leukemias, are heterogeneous disorders. Most AML subtypes are distinguished from other related blood disorders by the presence of more than 20% blasts in the bone marrow. It is characterized by the rapid growth of abnormal white blood cells that accumulate in the bone marrow and interfere with the production of normal blood cells ***(Smith et al., 2004).***

Epidemiology

Approximately 11,000 new cases of AML are diagnosed annually in the United States, with an overall annual incidence of 3.4 new cases per 100,000 people ***(Jemal et al., 2008).*** The incidence of AML is higher in males than in females and in whites than in blacks. The incidence of AML increases with age; the median age at diagnosis is 63 years. AML accounts for about 90% of all acute leukemias in adults, but is rare in children ***(Jemal et al., 2002).*** Congenital leukemia is usually AML rather than ALL (acute lymphoblastic leukemia) and is often monocytic, with high incidence of extramedullary disease particularly involving the skin and the central nervous system ***(Greer et al., 2004).***

Etiology

Although several factors have been implicated in the causation of AML, most patients who present with de novo AML have no identifiable risk factor.

Risk factors for AML ***(Lichtman and Liesveld, 2001):***

1- Environmental factors
   - Radiation
   - Benzene
   - Alkylating agents and cytotoxic drugs

2-Acquired diseases

   A- Clonal hematopoietic diseases
      - Chronic myelogenous leukemia
      - Idiopathic myelofibrosis
      - Primary thrombocythemia
      - Polycythemia vera
      - Acquired sideroblastic anemia
      - Paroxysmal nocturnal hemoglobinuria

   B- Other hematopoietic diseases
      - Aplastic anemia

3-Inherited conditions

- Down's syndrome
- Bloom's syndrome
- Ataxia telangiectasia
- Dyskeratosis congenita
- Combined immunodeficiency syndrome
- Neurofibromatosis and Schwachman-Diamond syndrome