Chapter- 1

General Introduction
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1.1. LEUKEMIA
During hematopoiesis or formation of cellular components of blood, erythrocytes, granulocytes, lymphocytes and monocytes are formed from the hematopoietic stem cells, the first two in myeloid tissue and the latter in lymphatic tissue. Abnormal proliferation of any of these cells or their precursors during the various stages of differentiation produces the disease Leukemia, which is part of the broader group of hematological neoplasms (Figure 1.1).

Figure 1.1. Hematopoiesis. All cells shown arise from hematopoietic stem cell [http://www.health.sa.gov.au/cancare].

Leukemia is categorized clinically and pathologically into acute and chronic form. Acute leukemia is characterized by the rapid growth of immature blood cells. Acute forms of leukemia can occur in children and young adults. Immediate treatment is required in acute leukemias due to the rapid progression and accumulation of the malignant cells, which then spill over into the bloodstream and spread to other organs of the body. If left untreated, the patients will die within months or even weeks. Chronic leukemia is distinguished by the excessive buildup of relatively mature, but still abnormal, blood cells. Typically taking months to years to progress, the cells are produced at a much higher rate than normal cells, resulting in many abnormal white blood cells in the blood. Chronic leukemia mostly occurs in older people [Judge Mand Whitteker JA 1992].

The diseases are further classified according to the type of abnormal cell found most in the blood. When leukemia affects lymphoid cells, it is called lymphocytic leukemia. When myeloid cells are affected, the disease is called myeloid leukemia.
Combining these two classifications provides a total of four main categories:

Acute Myeloid Leukemia (also known as acute myelogenous leukemia or AML) occurs more commonly in adults than in children. This type of leukemia was previously called acute nonlymphocytic leukemia (ANLL).

Acute lymphocytic leukemia (also known as Acute Lymphoblastic Leukemia or ALL) is the most common type of leukemia in young children. This disease also affects adults, especially those ages 65 and older.

Chronic lymphocytic leukemia (CLL) most often affects adults over the age of 55. It sometimes occurs in younger adults, but it almost never affects children.

Chronic myeloid leukemia or chronic myelogenous leukemia (CML) occurs mainly in adults. A very small number of children also develop this disease.

1.2. ACUTE MYELOID LEUKEMIA

Acute myeloid leukemia (AML) is a cancer of the myeloid line of white blood cells, characterized by the rapid proliferation of abnormal cells which accumulate in the bone marrow and interfere with the production of normal blood cells. It is the most common acute leukemia affecting adults, and its occurrence increases with age [Whittaker JA and Judge M 1992].

1.2.1. Signs and symptoms of leukemia

Symptoms of leukemia vary depending on the exact type of the disease and where it has spread in the body. In chronic leukemia, the symptoms may not appear for a long time. In acute leukemia, the symptoms usually develop quickly and rapidly become more severe.

Normal bone marrow cells are displaced by the abnormal bone marrow cells with increase in number of immature white blood cells, resulting in lack of blood platelets, which are important in the blood clotting process. This means people with leukemia may become bruised, bleed excessively, or develop pinprick bleeds (petechiae).

White blood cells, which are involved in fighting pathogens, may be suppressed or dysfunctional, putting the patient at the risk of developing infections.

Finally, the red blood cell deficiency leads to anemia, which may cause dyspnea. All symptoms may also be attributable to other diseases.

Some other related symptoms:

- Fatigue
- Fever, chills, and other flu-like symptoms
- Loss of appetite and/or weight loss,
- Shortness of breath
- Excessive sweating, particularly at night
- Swollen or bleeding gums
• Neurological symptoms (headache)
• Enlarged liver and spleen
are the different symptoms of leukemia.

12.2. Risk factor for leukemia
The exact cause of leukemia has not been identified. The different leukemias probably have different causes, and very little is certain about what causes them. A number of risk factors have been identified:

a) Exposure to high levels of ionizing radiation (X-ray, gamma ray etc),
Survivors of the atomic bombings of Hiroshima and Nagasaki had an increased rate of AML [Bizzozero OJ et al. 1966].
b) Occupational chemical exposure to benzene and other aromatic organic solvents.
c) Exposure to anti-cancer chemotherapy, in particular alkylating agents.
d) "Pre-leukemic" blood disorders such as Myelodysplastic syndrome (MDS)
e) Smoking habit
f) Down syndrome & other specific genetic diseases [Evans DI et al. 1972].
g) Human T-cell leukemia virus-I
h) Li-Fraumeni syndrome.

However, many patients with the disease do not have any of the known risk factors.

1.2.3. Diagnosis of AML
The first clue to a diagnosis of AML is usually an abnormal result on a complete blood count. Whereas a presumptive diagnosis of AML can be made via examination of the peripheral blood smear when there are circulating leukemic blasts, a definitive diagnosis typically requires an adequate bone marrow aspiration and biopsy. To identify the type of abnormal blood cells, a bone marrow examination is regularly performed; however, if there are a large number of leukemic cells circulating in the peripheral blood, a bone marrow biopsy may not be essential. Marrow or blood is examined by light microscopy and flow cytometry to diagnose the existence of leukemia, to differentiate AML from other types of leukemia (e.g. ALL), and to classify the subtype of disease (see below). A sample of marrow or blood is usually also tested for chromosomal aberrations by routine cytogenetics or fluorescent in situ hybridization (FISH).

The diagnosis and classification of AML can be demanding, and should be performed by a hematologist expert. According to the extensively used WHO criteria, the diagnosis of AML is established by demonstrating involvement of more than 20% of the blood and/or bone marrow by leukemic myeloblasts [Harris NL et al. 1999]. Acute promyelocytic leukemia (APL) a subtype of AML has the highest curability
and requires a unique form of treatment, it is important to quickly establish or exclude the diagnosis of this subtype of leukemia.

1.2.4. Classification

French-American-British classification

The French-American-British (FAB) classification system divided AML into 8 subtypes M0 through to M7, based on the type of cell from which the leukemia has developed and its degree of maturity. This is done by examining the appearance of the malignant cells under light microscopy and/or by using cytogenetics to characterize any underlying chromosomal aberrations. The subtypes have varying prognoses and responses to therapy. The eight FAB subtypes are [Bennett JM et al. 1976]

- M0 (undifferentiated AML)
- M1 (myeloblastic, without maturation)
- M2 (myeloblastic, with maturation)
- M3 (promyelocytic), or acute promyelocytic leukemia (APL)
- M4 (myelomonocytic)
- M4eo (myelomonocytic together with bone marrow eosinophilia)
- M5 monoblastic leukemia (M5a) or monocytic leukemia (M5b)
- M6 (erythrocytic), or erythroleukemia
- M7 (megakaryoblastic)

Although the WHO classification may be more useful, the FAB system is still widely used as of mid-2006.

WHO classification: i) AML with characteristic genetic abnormalities, ii) AML with multilineage dysplasia, iii) AML and MDS, therapy-related, iv) AML not otherwise categorized, v) Acute leukemias of ambiguous lineage. [Vardiman J et al. 2002].

1.2.5. Treatment

Treatment of AML consists mainly of chemotherapy, and is separated into two phases: induction and post-remission (or consolidation) therapy. The objective of induction therapy is to attain a complete remission by decreasing the amount of leukemic cells to an undetectable level; the aim of consolidation therapy is to remove any residual undetectable disease and achieve a cure.

Relapsed AML

Regardless of aggressive therapy, however, only 20%-30% of patients enjoy long-term disease-free survival. For relapsed AML patients, the only proven potentially curative therapy is a stem cell transplant [Appelbaum FR 2001, 2002]. Since treatment options for relapsed AML are so limited, another option which may be offered is palliative care.
For relapsed acute promyeloeytic leukemia (APL), arsenic trioxide has been tested in trials and approved by the Food and Drug Administration. Like ATRA, arsenic trioxide does not work with other subtypes of AML [Soignet SL et al. 2001].

1.3. CHRONIC MYELOID LEUKEMIA

Chronic myeloid leukemia (CML) is a form of chronic leukemia characterized by increased and unregulated clonal proliferation of myeloid cells in the bone marrow. CML is a myeloproliferative disease associated with a characteristic chromosomal translocation called the Philadelphia chromosome.

CML occurs in all age groups, but most commonly in the middle-aged and elderly. Its annual incidence is 1 to 2 per 100,000 people, and slightly more men than women are affected. CML represents about 15-20% of all cases of adult leukemia in Western populations [Faderl S et al. 1999]. The only well-described risk factor for CML is exposure to ionizing radiation; for example, increased rates of CML were seen in people exposed to the atomic bombings of Hiroshima and Nagasaki [Moloney WC 1987]. In India there is no such data available.

1.3.1. Signs and symptoms

Patients are often asymptomatic at diagnosis, presenting incidentally with a high white blood cell count on a routine laboratory test. However other leukemoid reactions can have a similar manifestation on a blood smear. Symptoms of CML may include: malaise, low-grade fever, increased susceptibility to infections, anemia, and thrombocytopenia with easy bruising (although an increased platelet count may also occur in CML). Splenomegaly may also be seen.

1.3.2. Diagnosis

CML is frequently suspected on the basis on the total blood count, which shows increased granulocytes of all types, usually including immature myeloid cells. Basophils and eosinophils are generally increased; this characteristic may help differentiate CML from other leukemias. A bone marrow biopsy is frequently done as part of the assessment for CML, but bone marrow morphology alone is not sufficient to diagnose CML. Eventually, CML is diagnosed by detecting the Philadelphia chromosome by routine cytogenetics, by fluorescent in situ hybridization, or by RT-PCR for the BCR-ABL fusion gene. Controversy exists over so-called Ph-negative CML, or cases of suspected CML in which the Philadelphia chromosome cannot be detected. Many such patients in fact have complex chromosomal abnormalities which mask the t(9;22) translocation, or have evidence of the translocation by FISH or RT-PCR regardless of normal routine karyotyping [Savage DG et al. 1997].
Chapter 1 General Introduction

1.3.3. Phases of CML
CML is divided into three phases based on clinical characteristics and laboratory findings. In the absence of intervention, CML typically begins in the chronic phase, and over the course of several years progresses to an accelerated phase and finally to a blast crisis. Blast crisis is the incurable phase of CML and clinically acts like an acute leukemia.

1.3.3.1. Chronic phase
About 85% of patients with CML are in the chronic phase at the time of diagnosis. In this phase, patients are usually asymptomatic or have only mild symptoms of fatigue or abdominal fullness. The time-span of chronic phase is variable and depends on how early the disease was diagnosed and the therapies used. Eventually, in the absence of curative treatment, the disease progresses to an accelerated phase.

1.3.3.2. Accelerated phase
Criteria for diagnosing transition into the accelerated phase are quite variable; the most extensively used criteria are those proposed by investigators at M.D. Anderson Cancer Center [Kantarjian H et al. 1988], by Sokal J et al. 1988 and the World Health Organization [Vardiman J et al. 2002]. The WHO criteria are perhaps most widely used, and include:
- 10-19% myeloblasts in the blood or bone marrow, Platelet count <10^{11}/l, unrelated to therapy, Platelet count >10^{12}/l, unresponsive to therapy; >20% basophils in the blood or bone marrow
- Cytogenetic evolution with new chromosomal aberrations in addition to the Philadelphia chromosome
- Increasing splenomegaly or white blood cell count, unresponsive to therapy
The patient is considered to be in the accelerated phase if any of the above are present. The accelerated phase is significant because it signals that the disease is progressing and transformation to blast crisis is pending.

1.3.3.3. Blast crisis
Blast crisis is the last phase in the evolution of CML, and behaves similar to an acute leukemia, with rapid progression and short survival. Blast crisis is diagnosed if any of the following are present in a patient with CML:
- >20% myeloblasts or lymphoblasts in the blood or bone marrow; Large clusters of blasts in the bone marrow on biopsy; Development of a chloroma (solid focus of leukemia outside the bone marrow).

1.3.4. Treatment
1.3.4.1. Chronic phase
In the past, it has been treated with chemotherapy, interferon and bone marrow transplantation, although targeted therapies introduced at the beginning of the 21st century have drastically changed the management of CML. Chronic phase CML is
treated with imatinib mesylate (marketed as Gleevec® or Glivec®; previously known as STI-571).

1.3.4.2. Blast crisis

Blast crisis carries all the symptoms and characteristics of either acute myeloid leukemia or acute lymphoblastic leukemia, and has a very high mortality rate. This stage can most effectively be treated by a bone marrow transplant after high-dose chemotherapy.

Most cancers occur through alterations leading to gain of function in oncogenes and loss of function in tumor suppressor genes. These can be achieved by gene deletions, mutations and epigenetic modifications of the genome. Eukaryotic cells proliferate only when they are instructed to do so and otherwise stay in the resting phase. Journey of the cell through the cell cycle is tightly regulated by multiple genes and a section of these are the focus of this study.

1.4. THE CELL CYCLE

Eukaryotic cells multiply in a cyclical process, which is composed of four phases (Figure 1.2). The fundamental task of the cell cycle is to ensure that DNA is faithfully replicated once during S phase and identical chromosomal copies are distributed equally to two daughter cells during M phase [Sherr CJ 1996]. In between S and M phase there are two gap periods (G1 and G2), during which cells prepare themselves for S and M phases. When cells are not dividing, they exit the cell cycle to stay in a quiescent state (G0). The propagation through different steps in the cell cycle is firmly regulated by variable actions of protein complexes composed of cyclins and their respective kinases (cyclin-dependent kinases; CDKs) specific for particular phases of the cell cycle [Johnson DG and Walker CL 1999, Nurse P 2000] (Figure 1.2). The activity of cyclin/CDK complexes is regulated by numerous mechanisms, particularly by fluctuating protein synthesis and degradation of cyclins, inhibition of CDKs phosphorylations, and by binding of factors of the two types of cyclin-kinase inhibitor (CKI) families [Arellano M and Moreno S 1997]. INK4 (inhibitor of cdk4/6) family consists of four members, p16^{INK4A}, p15^{INK4B}, p18^{INK4C}, and p19^{INK4D}, which specifically inhibit cyclin D-cyclin dependent kinases to prevent phosphorylation of RB family protein [Sherr CJ and McCormick F 2002]. Members of the CIP/KIP-family of inhibitors, p21^{CIP1}, p27^{KIP1}, and p57^{KIP2}, preferentially inhibit action of CDK2, but bind also CDK4-containing complexes [Johnson DG and Walker CL 1999, Vidal A and Koff A 2000].
The initial "start" of cell proliferation is progression from G1 to S phase. The proliferation of mammalian cells is generally determined by extracellular signals that take on a program of gene expression and protein regulation required for cell division [Sherr CJ 1996]. During mid- to late G1 phase, a cell monitors for growth-promoting and -inhibiting signals. If the cellular environment is favorable for propagation, the cell makes a decision to enter S phase by bypassing the restriction point (R) (Figure 1.2). In nonstressed circumstances, this decision to replicate DNA and divide is irreversible until the next G1 phase [Bartek J and Lukas J 2001]. Retinoblastoma (Rb) protein and its homologs p107 and p130 control G1/S transition [Weinberg RA 1995, Bartek J and Lukas J 2001]. The hypophosphorylated RB protein sequesters the transcription factor E2F at G1. During cell cycle progression cyclin-CDK complexes in late G1 phosphorylate pRB which then releases E2F for transcription activation of genes whose products are necessary for G1/S transition [Weinberg RA 1995]. CDK inhibitors, on the other hand oppose cyclin-CDK complexes, when cell's functional integrity and readiness to progress across the cell cycle is lacking. Transcription factor Myc is another important factor for G1/S transition which induces several factors promoting S phase entry [Pelengaris S et al. 2002]. Throughout the cell cycle, a cell monitors successful accomplishment of the earlier phase before entering the next one. These key transitions are often referred to as the cell cycle checkpoints or restriction points [Hartwell LH and Weinert TA 1989].

![Figure 1.2. The cell cycle. Cells multiply by dividing in the cell cycle driven by activities of different cyclin-CDK complexes (see text for details).](image_url)

**1.4.1. Cancer cell cycle**
Transformation of cultured primary cell into tumorigenic variants is a multistep procedure, whereby each genetic change gives a proliferative benefit [Hahn WC and Weinberg RA 2002]. The development of cancer in living animals is drastically more complex, in view of the fact that specific interactions between tumor cells and host
tissues are crucial for angiogenesis, tissue invasion and metastasis. However, despite
the existence of many forms of cancer and global changes in gene expression profile
observed in cancer cells versus normal cells, a comparatively small number of
essential alterations are sheared by the majority and possibly all, cancers [Hanahan D
and Weinberg RA 2000]. Such mutations can disrupt normal growth control in
response to environmental cues, or can take apart cell cycle checkpoints that
otherwise limit cell division or that induces cell suicide in response to DNA damage
or oncogene activation. RB and p53 are central to these processes [Sherr CJ et al.
2002].

Alterations in two types of genes contribute the majority to development of cancer.
Tumor suppressor genes generally suppress development of cancer; a function which
is recessively lost to make probable tumorigenesis. Proto-oncogenes promote
tumorigenesis by a dominant gain of growth promoting activity. These frequently
have opposing functions in the same, vital cellular pathways. For example, the Rb
pathway, important for controlling normal cell proliferation and targeted in most -- if
not all - human cancers, harbors a number of oncogenes and tumor suppressor genes
(Figure 1.3) [Ho A and Dowdy SF 2002]. The other major pathway targeted in human
cancers is the tumor suppressor p53 pathway which functions in the damage
checkpoints.

1.4.2. The Retinoblastoma Protein pRB in cancer
The first identified and characterized tumor suppressor gene in humans was
retinoblastoma gene. Genetic investigations of patients with retinoblastoma eye tumor
which appear in childhood showed a defect in a gene sequence known as the
retinoblastoma (RB) gene. In the hereditary form of this childhood cancer, a defect is
inherited in RB gene via germ line. Second allele of the RB gene inactivated by
mutation or deletion leads to complete failure of the function of the gene and goes to
tumor formation [Krauss G 2003]. Somatic mutations of pRB gene are found in more
frequently occurring tumors, including lung carcinomas, osteosarcomas and bladder
carcinomas. However, pRB gene is rarely mutated in leukemia.

The phosphorylation status of pRB (and its relatives) is controlled by a network of
interactions between D-type cyclins, CDK4/6, cyclin E/CDK2, and the CDK
inhibitors, mainly of the INK4 family.

Proliferation and apoptosis control by pRB occurs through active repression of E2F-
dependent promoters. pRB in the under phosphorylated form has both anti-
proliferative and anti-apoptotic effect. It inhibits transcription of E2F-controlled
genes, among which are genes necessary for S- phase propagation and thus for cell
proliferation. E2F controlled genes also contain pro-apoptotic gene for instance the
gene for Apafl and for p73. E2F also keeps levels of p53 low, an effect mediated by
activation of ARF through ARF-MDM2-p53 network (Figure 1.3). If the controlled
function of pRB is lost by pRB mutations/deletion, E2F will inappropriately activate the pro-apoptotic genes and growth arrest and/or apoptosis will result. However, when together with loss of pRB function key players regulating apoptosis and/or cell cycle arrest (e.g. ARF, p53, Apaf1) are mutated or overexpressed (MDM2), cells will be going to hyperproliferation.

Figure 1.3. RB – p53 pathways

1.4.3. Cyclin dependent kinase inhibitors (CDKI)
Cyclin dependent kinases (CDKs) activity in the cell cycle are negatively control by group of specific inhibitor proteins known as cyclin dependent kinase inhibitors (CDKI) [Lee MH and Yang HY 2001]. The CDKIs are divided into two groups based on sequence homology:

CIP/KIP family: p21\(^{CIP1}\) (also known as CIP1, Waf1), p27\(^{KIP1}\) (KIP1) and p57\(^{KIP2}\).
INK4 family: p16\(^{INK4A}\), p15\(^{INK4A}\), p18\(^{INK4C}\), p19\(^{INK4D}\).

There is specificity in the inhibition of the various cyclin-CDK complexes.
The CDKIs are crucial control elements that regulate the G1/S transition and the transition of cells from a quiescent to a dividing state and vice versa. Primary targets of CDKIs are the CDKs, either free or complexed with cyclins.

1.4.3.1. CIP family
The p21\(^{CIP1}\) inhibitor binds mainly to the complexes of CDK2 with cyclin A and E, leading to their inactivation and cell cycle arrest. It is regulated at the transcriptional level by the p53 during DNA damage response. Increase in level of p21 mRNA are observed upon treatment of cells with DNA damaging agents, and this transcriptional regulation is part of the DNA damage checkpoint during G1/S phase. p21 is also associated with the replication accessory protein PCNA (proliferating cell nuclear antigen) [Luo Y \textit{et al.} 1995]. PCNA is required for DNA synthesis and function in
clamping DNA polymerase 8 to the DNA, thereby increasing the processivity of DNA synthesis. It is believed that this is another mechanism by which p21 can inhibit DNA synthesis and S-phase progression. The main targets of both p27 and p57 are the cyclin A-CDK2 and cyclin E-CDK2 complexes like p21. This group of proteins also interacts with cyclin D-CDK4/6, but with lesser efficiency than with INK4 family.

1.4.3.2. INK4 family
The INK4 family members bind predominantly CDK4/6 complexes, preventing association of D-type cyclins. Regulation of INK4 is cell type specific and shows complex pattern. The observation that the human chromosome 9p21 region is frequent site of deletion and rearrangement in many cancers including leukemia [Diaz MO et al. 1988], implied the existence of a tumor suppressor gene which is involved in tumor formation. Later, the gene p16 (also known as MTS1, INK4A, CDKN4I) was identified in this region and was found to posses many features suggesting that it is relevant to heterozygosity at this site [Kamb A et al. 1994, Nobori T et al. 1994]. Another gene termed p15
INK4B (or MTS2), with high homology to p16 is located in the same chromosomal region [Kamb A et al. 1994]. Particularly, the level of p15 is subject to induction at the mRNA level by the antimitogenic cytokine TGFβ [Hannon GJ and Beach D 1994]. Subsequently, two further specific polypeptide inhibitors of CDK4/6 protein, termed p18
INK4c (located on chromosome 1p32) and p19
INK4d (located on chromosome 19p13), inducing G1 arrest have been discovered [Guan KL et al. 1994, Okuda T et al. 1995]. Alteration of p18 and p19 are rarely found in leukemia [Drexler HG 1998]. The INK4 family, especially p15 and p16 alter frequently in leukemia by different mechanisms e.g deletion, mutation, and hypermethylation.

1.4.4. The MDM2-p53 Network and cancer
A functional inactivation of the MDM2-p53 network [Alarcon-Vargas D and Ronai Z 2002; Michael D and Oren M 2002] (Figure 1.4) is observed in almost all cancers. Inactivating mutations have been found to increase cell division activity and to diminished apoptosis by affecting both the proliferative and the anti proliferative function of the network. The p53 network inactivation can occur at numerous positions. Mutation of p53 itself is observed in the majority of cancers. However, p53 mutation is rarely associated with leukemias [Boyapati A et al. 2004, Peller S et al. 2003, Stirewalt DL et al. 2001]. These mutations are mostly nonsense mutations and affect predominantly the DNA-binding domain region of p53, impairing its transactivation function.
Interestingly, mutated p53 proteins are often more stable than wild type p53 and accumulate in tumor cells. The preferred mutation of the DNA binding domain during tumorigenesis suggested for its role in tumor suppression.

Loss of the p53 response can also occur via inactivation of other components of the MDM2-p53 network (Figure 1.4). MDM2 overexpression and amplification of the MDM2 gene is observed in many tumors, leading to enhanced degradation of p53. Overexpression of MDM2 mRNA in acute myeloid leukemia (AML) has been well documented, although no evidence of amplification of MDM2 gene was found in leukemias [Bueso-Ramos CE et al. 1993]. Another way to inactivate the p53-MDM2 module uses the p14^{ARF} protein. A decreased level of ARF, which has the characteristics of tumor suppressor, is frequently observed in tumor cells. This often occurs without mutation of p14^{ARF} gene itself. Rather, aberrant CpG methylation of promoter sequences at the p14^{ARF} gene locus appears to be responsible in these cases for silencing the ARF gene.

The pathways that mediate genotoxic, non-genotoxic and oncogenic stresses are the target of a huge number of selection pressures, and these pressures will come together on the p53-MDM2 module, acting either separately or in combination.

1.4.5. Regulation of p53 stability by degradation through the proteasome

Throughout normal metabolism in eukaryotic cells, the 26S proteasome degrades nearly all proteins outside the lysosomal pathway [Kierszenbaum AL 2000]. Under normal cellular growth conditions, p53 is expressed constantly, although its levels are kept low by uninterrupted protein degradation [Prives C and Hall PA 1999] with a
half-life of less than 20-30 minute. Upon stress, p53 is accumulating in cells and this occurs by blocking its degradation, which leads to enhanced protein half-life, rather than by upregulating gene expression or mRNA stability. p53 is degraded through the 26S proteasome; a process which is driven by the ubiquitination of p53 to several C-terminal lysine residues [Rodriguez MS et al. 2000]. The major E3 ubiquitin ligase for p53 is MDM2, the most vital regulator of p53 [Momand J et al. 2000, Alarcon-Vargas D and Ronai Z 2002]. MDM2 inhibits the transcriptional activity of p53 by binding to the TA domain of p53 [Juven-Gershon T and Oren M 1999, Momand J et al. 2000] and ubiquitinating p53 and itself, leading to degradation of the proteins [Honda R et al. 1999, Haupt Y et al. 1997, Kubbutat MH et al. 1997]. Although it is thought that MDM2 shuttles p53 from nucleus to cytoplasm [Roth J et al. 1998], nuclear export is not necessary for p53 degradation [Yu ZK et al. 2000, Lohrum MA et al. 2001, Xirodimas DP et al. 2001], and p53 can be degraded by both nuclear and cytoplasmic proteasomes [Shirangi TR et al. 2002]. Following stress, p53 accumulation occurs during weakening of p53-MDM2 interaction, which can be achieved by two mechanisms: inhibition of MDM2 by interacting proteins, or changes in posttranslational modifications of p53 and/or MDM2 (Figure 1.5). The best characterized MDM2-interacting protein capable of inhibiting p53 degradation is the alternative reading frame protein (p14ARF). In response to E2F1 or MYC, it accumulates and antagonizes MDM2, this leads to p53 mediated apoptosis [Sherr CJ and Weber JD 2000] (Figure 1.5; see below). MDM2 is induced by p53, making an autoregulatory loop to downregulate p53 at the end of a stress response [Juven-Gershon D and Oren M 1999, Momand J et al. 2000]. One more E3 ligase for p53 has been identified: Pirh2 can ubiquitinate p53 in vitro and promote p53 degradation in vivo independently of MDM2 [Leng RP et al. 2003]. Interestingly, Pirh2 is a p53 responsive gene, probably forming a feedback regulatory loop [Leng RP et al. 2003].

DNA damage

Figure 1.5. p53 protein regulation. See text for details.
1.4.6. MDM2
The mouse double minute-2 (MDM2) was discovered as an amplified and overexpressed gene in spontaneously transformed derivative of mouse BALB/c cell line 3T3DM [Fakharzadeh SS et al. 1991]. MDM2 (also referred to as HDM2 in human cells) is an oncogene due to its inhibitory effects on p53 [Deb SP 2003]. HDM2 gene is located on chromosome 12q13-14, a region often amplified in human cancers. It has been shown that MDM2 can release growth arrest in cell line with phosphorylation resistant RB indicating that it can interact with RB too [Momand J et al. 2000, Daujat S et al. 2001]. Like an alternative to p53 mutation, overexpression of MDM2 protein is a tumor-associated mechanism to inactivate p53, and amplification of HDM2 occurs in 7% of human cancers [Momand J et al. 2000]. As described p53 is autoregulated by MDM2. The main MDM2 transcript in human cells codes 491 amino acids with a 90-kDa molecular weight protein and a RING finger motif on its C terminus, responsible for the E3 ubiquitin ligase activity [Momand J et al. 2000]. A number of regulatory phosphorylation sites on MDM2 have been recognized both in its N terminus, which binds p53, and in its central acidic domain mediating p53 ubiquitination [Hay TJ and Meek DW 2000]. MDM2 is phosphorylated by Akt kinase, which promotes nuclear translocation of MDM2 and its ability to ubiquitinate p53 [Mayo LD and Donner DB 2001, Zhou BP et al. 2001, Ashcroft M et al. 2002, Lin HK et al. 2002]. Other MDM2 modifications have also been reported, which may influence MDM2 ability to promote p53 degradation. Following ionizing radiation exposure, dephosphorylation of several residues in the central domain of MDM2 decreases its capability to promote p53 degradation [Blattner et al. 2002]. Recently, an MDM2 homologue has been cloned. MDMX, is also able to inhibit p53-mediated transactivation [Stad R et al. 2000] of genes. Degradation of p53 is not promoted by MDMX, but it can inhibit MDM2 doing so, as a result stabilizing both p53 and MDM2 [Jackson MW and Berberich SJ 2000, Stad R et al. 2001]. In some cancers MDMX is overexpressed, and its gene MDM4 amplified, associating with high levels of p53 [Ramos YW et al. 2001]. In mice loss of the MDM4 gene is embryonic lethal and can be rescued with loss of p53. This indicates that regulation of p53 by MDM2 and MDMX work in non-overlapping pathways [Parant J et al. 2001].

1.4.7. INK4A/ARF
Studies into cancer cells indicate that proteins encoded by the p16INK4A gene locus function as tumor suppressors and are of great importance for cancer development [Sherr CJ 2001]. Two proteins encodes at the gene locus for p16INK4A, namely the p16INK4A a cyclin dependent inhibitor and the ARF protein (ARF = alternative reading
frame: pl9ARF in mice and pl4ARF in human) [Quelle DE et al. 1995], as a result linking the p53 and Rb pathways important for suppression of tumorigenesis. Each gene uses a different promoter and is transcribed in a different reading frame, thus resulting in totally different amino acid sequences despite utilizing the same exon 2 and 3 [Sidransky D 1996]. Both proteins act as inhibitors of the G1 to S transition, even though they function in two different pathways: p16 acts as an inhibitor of cyclin D-CDK4/6 complexes and p14 stabilizes p53 by inhibition of MDM2.

P14\textsuperscript{ARF} is a small, basic protein that interacts with the C terminus of MDM2, abrogating the capability of MDM2 to promote p53 degradation [Kamijo T et al. 1998, Pomerantz J et al. 1998, Stott FJ et al. 1998, Zhang Y et al. 1998, Honda R and Yasuda H 1999]. ARF is capable of translocating MDM2 to the nucleoli [Weber JD et al. 1999], but this is not required for inhibition of MDM2 activity [Llanos et al. 2001]. ARF is also able to interact with MDMX and sequester it to the nucleoli, ensuing in an increase in the transcriptional activity of p53 [Jackson MW et al. 2001]. ARF acts in response to abnormal cell proliferation resulting from oncogenic stimulus and viral transformation, and it can be induced by at least Myc [Zindy F et al. 1998], Ras [Palmero I et al. 1998], E2F1 [Bates S et al. 1998], and adenoviral E1A [de Stanchina E et al. 1998]. Induction of ARF causes growth arrest and represses transformation during induction of p53 [Quelle DE et al. 1995, Kamijo T et al. 1997, Pomerantz J et al. 1998]. However, ARF-null cells immortalized in cell culture often maintain functional p53, signifying that suppression of cellular transformation by p53 is arbitrated by ARF [Kamijo T et al. 1997]. ARF is also able to induce antiproliferative genes and to inhibit ribosomal RNA processing independently of p53 [Kuo ML et al. 2003, Sugimoto M et al. 2003]. ARF is not essential for the DNA damage response of p53 [Kamijo T et al. 1997]. The p53 negatively regulates ARF expression, as a result returning the capability of MDM2 to downregulate p53 [Stott FJ et al. 1998].

Linggi B et al. 2002 demonstrated that p14 is a direct transcriptional target of the AML1-ETO fusion protein that results from the (8;21) translocation associated with acute myeloid leukemia. Repression of p14 may explain why p53 is very rarely mutated in (8;21)-containing leukemias and suggests that p14 is an important tumor suppressor in a large number of human leukemias.

1.4.8. Bel-2

Family members of Bel-2 are the vital regulators that control mitochondria mediated apoptosis [Parone PA and Martinou JC 2002]. The Bel-2 is the founding member of this family which was first identified as an oncprotein coded by a gene affected by translocation of chromosomes 14 and 18 in B cell lymphomas [Cleary ML et al. 1986, Tsujimoto Y et al. 1986]. However, many other oncproteins the Bel-2 is not implicated in the cell cycle regulation, and hence does not fit into the classical
oncogene picture. Now, greater than 20 members are known of the Bcl-2 family, which can have a positive or a negative effect on the initiation on the apoptotic program. Every family member has at least a copy of so-called BH motif (BH, Bcl-2 homolog), of which there are four types (BH1-BH4). On the basis of structural and functional criteria, the Bcl-2 family has been divided into three groups [Cory S and Adams JM 2002]. Group I comprise anti-apoptotic protein characterized by four short, conserved Bcl-2 homology (BH) domains, known as BH1-BH4. Overexpression of group I member can prevent initiation of apoptotic program in various cell types including blood precursor cells. Group II includes the proapoptotic proteins Bax and Bak which are similar in structure to the group I proteins but lack the N-terminal BH domains. Their activity is necessary for the induction of mitochondria mediated apoptosis. Group III consists of pro-apoptotic polypeptides including Bad, Bid, Nora etc. that contain a single BH3 domain. Bcl-2 family is transcriptional targets of p53. Of note are the genes for the proapoptotic protein Bax, which is activated by p53, and the gene for the antiapoptotic protein Bcl-2, which is repressed by p53 [Krauss G 2003].

High expression levels of the Bcl-2 gene and other family members were seen in many hematologic malignancies, including AML [Reed JC 1997]. A number of studies have been examined in correlation to treatment outcome in AML [Campos L et al. 1993, Maung ZT et al. 1994, Filipits M et al. 2000, Naumovski L et al. 1998, Karakas T et al. 1998]. Higher Bax/Bcl-2 ratio was significantly correlated with a higher CR rate, longer time to relapse, and a longer OS and DFS [Del Poeta G et al. 2003]. High levels of Bcl-2 protein correlated with an adverse outcome when associated with favorable and intermediate cytogenetics, but low levels predicted an adverse outcome within the group with unfavorable cytogenetics [Kornblau SM et al. 1999].

1.5. THE p53 FAMILY

Although most solid tumors contain inactivating mutations of the p53 tumor suppressor, in hematological malignancies those alterations of the p53 gene are not frequent (less than 20%) [Boyapati A et al. 2004, Peller S et al. 2003]. How the tumors arise in the presence of a tumor suppressor like p53 remains to be elucidated. Following fifteen years of being alone, p53 was found to have two family members, p73 and p63 [Kaghad M et al. 1997, Yang A et al. 1998]. The family members are differentially involved in the regulation of cell cycle and DNA damage induced apoptosis [Benard J et al. 2003]. Additionally, p63 and p73 have been shown to interfere with mechanisms of cell differentiation and development [Moll UM et al. 2004].
1.5.1. p73

The p73 protein was discovered in 1997 by Kaghad et al. [Kaghad M et al. 1997]. It is encoded by the p73 gene of fourteen exons, located on chromosome 1, in 1p36 locus, shows strong homology with p53, especially in terms of overall domains: a trans activation domain (TA), a proline rich domain (PR), a DNA binding domain (DBD), an oligomerization domain (OD), a C-terminal sterile α-motif (SAM) domain involved in protein-protein interactions, and a post-SAM basic domain, which has an inhibitory effect on the transactivation properties of the proteins. p73 and p53 share 30% amino acid identity in their TA domain, 79% identity in their DBD and 33% similarity to the OD [Kaghad M et al. 1997]. Thus it is not surprising that both proteins bind to the same DNA sequences and transactivates the same promoters [Melino G et al. 2002, 2003].

Figure 1.6. Similarity in structure between p53 and p73 proteins and organization of different p73 isoforms. Due to alternative NH2- and COOH-terminal splicing of transcripts, P73 gives rise to a diversity of isoforms. (*) Formation of ΔN -isoforms requires activation of the alternative P2 promoter in exon/intron 3'. Formation of ΔN' requires initiation of transcription from the P1 promoter and includes all exons (1–14) with an additional insertion of exon 3'. In addition, during the translation, owing to different translational sites the final proteins ΔN and ΔN' become identical. TA, transactivation domain; DBD, DNA-binding domain; OD, oligomerization domain; SAM, sterile alpha motif [Adapted from Pluta A et al. 2006].
The p73 gene gives rise to multiple protein isoforms, which are generated both by alternative splicing and by the use of alternative promoters (Figure 1.6) [Melino G et al. 2003, Melino G et al. 2002, Ueda Y et al. 1999, Levrero M et al. 2000, Stiewe T et al. 2002, Scaruffi P et al. 2000]. The isoforms produced by two promoters are called TAp73 and ANp73, the former retains a TA domain and latter has a shorter amino terminus lacking the TA domain. Also, N-terminally truncated, lack of transactivation p73 protein (ΔTAp73) has been described in human cancer cells [Stiewe T et al. 2002]. These oncogenic proteins are produced by aberrant splicing (p73ΔNex2, p73ΔNex2/3, ΔNp73), whereas ΔNp73 is generated via the use of an alternative promoter within the exon/intron 3’. In addition, alternative splicing produces at least nine transcripts with different carboxy terminals (α, β, γ, δ, e, η, η1, and 0) [Ueda Y et al. 1999, Scaruffi P et al. 2000, Ishimoto O et al. 2002, De Laurenzi V et al. 1998, De Laurenzi VD et al. 1999], which are functionally distinct.

Full length TAp73 can bind to numerous p53 binding sites, transactivate certain p53 target genes, including MDM2, p21cip1, Bax, Gadd45, and stimulate cell cycle arrest or apoptosis [Yang A et al. 2002, Benard J et al. 2003]. p73 may inhibit transcriptional activity of p53 by competing for the same binding sites on DNA, but also co-operate in target gene activation. For example, in mouse embryo fibroblasts (MEFs) p73 are required for p53-induced apoptosis through their capability to stabilize p53 binding to the apoptotic promoters [Flores ER et al. 2002]. Mutant p53 interacts with p73 and impairs their sequence-specific DNA binding, thereby inhibiting their transcriptional activity [Strano S et al. 2002]. The ΔNp73 forms have an important regulatory role, as they exert dominant negative effect on both p53 and TAp73 transcriptional activities. This inhibitory function is exerted either at the oligomerization level (for TAp73) or by competing for binding to the same DNA target sequence (as p53) [Yang A et al. 2002]. p53 and TAp73 can induces ΔNp73 isoform both on mRNA and protein levels, which create a dominant negative feedback loop to terminate p53-mediated stress response and function of TAp73 [Kartasheva NN et al. 2002, Ishimoto O et al. 2002]. This may be supported by the ability of ΔNp73 to inhibit Rb, and as a result promote cell proliferation [Stiewe T et al. 2003].

Both MDM2 and MDMX can bind p73 forms containing the TA Domain, inhibit their transcriptional activities, but not induce their degradation [Irwin MS and Kaelin WG 2001]. MDM2 can also induce alterations in the subcellular localization of p73, MDMX at least in the localization of p73 [Wang X et al. 2001]. p73 is stabilized by MDM2 and MDMX [Irwin MS and Kaelin WG 2001]. Instead, viral proteins do not bind and inactivate p73 as they do for p53 [Irwin MS and Kaelin WG 2001].

The p73 gene was also considered as a candidate of the imprinted tumor suppressor gene because monoallelic expression of p73, possibly owing to the genomic
imprinting of a parentally transmitted allele, has been described in neuroblastoma, and also in several cancer cell line as well as normal tissue [Kaghan M et al. 1997]. However, biallelic expression of this gene was also observed in some normal tissues, and in both bladder cancer and normal bladder [Yokomizo A et al. 1999]. Thus, p73 is not monoallelically expressed in all human tissues, and it could be tissue dependent [Yokomizo A et al. 1999].

Although members of the p53 family of proteins are structurally and functionally related, p63 and p73 seem to have at least to some extent individual roles distinct from p53. As p53 is crucial for cellular stress responses and tumor suppression, p73 seem mostly to have cell type-specific developmental roles [Irwin MS and Kaelin WG 2001]. Even though p73 lies in 1p36, a region with common LOH in human cancers, it seems that p73 is not a tumor suppressor [Benard J et al. 2003]. However, despite extensive studies of p73 in a variety of primary cancers, only few tumors with p73 mutation have been found. p73-null mice do not show increased rate of spontaneous tumorigenesis [Yang A et al. 2002]. On the other hand, in a variety of cancer types, the expression of p73 is greater than in normal tissues [Moll UM et al. 2001, Stiewe T et al. 2002]. Tumor related upregulation of p73 and genetic data from human cancers and p73-deficient mice refuse a classical Knudson-type tumor suppressor role [Knudson AG et al. 2001]. As an alternative, p73 may have roles in pheromonal, neurological and inflammatory pathways. Sympathetic neurons are protected by p73 from p53-induced apoptosis during development, as these cells demonstrate reduced survival in p73-null mice [Pozniak CD et al. 2000]. The clinical impact of p73 has been established to date only for neuroblastoma, where ΔNp73 expression correlates with unfavorable prognosis [Casciano I et al. 2002]. Oncogenes, such as E2F1, c-Myc, and E1A, can activate p73 to induce apoptosis independently of p53 [Irwin MS and Kaelin WG 2001]. A role for p73 is indicated in E2F1-induced apoptosis in thymocytes, and p73 could be involved in radiation-induced murine T-cell lymphomas [Irwin MS and Kaelin WG 2001, Benard J et al. 2003]. p73 is DNA damage-responsive, as it is phosphorylated by c-ABL and stabilized upon ionizing radiation exposure [Irwin MS and Kaelin WG 2001]. DNA damage dependent acetylation of p73 by p300 selectively enhances the activation of the apoptotic target genes [Costanzo A et al. 2002].

1.5.2. p63
This protein is strikingly conserved, and has an N-terminal transactivation domain (TA), a DNA binding domain, and an oligomerization domain [Yang A et al. 1998]. The p63 shows to be the evolutionary ancestor, and p63 and p73 are more similar to each other than p53. p63 has a C-terminal sterile α-motif (SAM) domain involved in protein-protein interactions, and a post-SAM basic domain, which has an inhibitory
effect on the transactivation properties of the proteins. p63 has two promoters, 30-40 kb apart and with unique regulatory sites, driving transcription of sequences coding for proteins either with or without the N-terminal TA (designated as ΔNp63). In addition, alternative splicing creates variation in the C termini, resulting in at least six major transcripts [Yang A et al. 2002]. Despite a structural similarity with p53, its plays a unique developmental role in epidermal morphogenesis and differentiation [Moll UM et al. 2004]. p63 and its isoforms are differentially expressed in normal tissues and neoplastic cells [Di Como CJ et al. 2002, Nylander K et al. 2002]. Both MDM2 and MDMX can bind p63 to the TA domain, inhibit its transcriptional activities, but not induce degradation [Irwin MS and Kaelin WG 2001, Kadakia M et al. 2001, Calabro V et al. 2002]. MDM2 can also induce alterations in the subcellular localization of p63 [Kadakia M et al. 2001].

1.6. ROLE OF EPIGENETICS IN GENE EXPRESSION
Epigenetic events play an important role in the development and prognosis of cancer. 'Epigenetics' refers to heritable changes in gene expression that do not result from alterations of nucleotide sequence in the gene. Mutations taking place in oncogenes frequently result in a gain of function, whereas mutations or deletions coupled with tumor suppressor genes cause an inactivation or loss of negative regulators. Tumor suppressor gene function losses can also occur through epigenetic changes such as DNA methylation. The promoter region of genes, where transcription is initiated are inactivated and silenced by DNA methylation [Bird A 2002, Jones PA and Baylin SB 2002]. In cancer, epigenetic silencing through methylation leading to aberrant silencing of tumor suppressor function occurs at least as frequently as mutations or deletion [Jones PA and Baylin SB 2002, Herman JG and Baylin SB 2003]. Posttranslational modification of histone proteins, the building blocks that package DNA into repeated nucleosomes, affects both chromatin folding and association with non-histone regulatory proteins [Jenuwein T and Allis CD 2001]. Increase of histone acetylation is associated with gene activation whereas histone deacetylation represses gene transcription. These events occur during all stages of tumorigenesis and represent an alternative to deletion and mutations for inactivating tumor suppressor genes [Feinberg A et al. 2006]. Although epigenetic aberrations are the most frequent alterations in cancer, very little is known regarding the cause and effects of these changes on tumor initiation and progression. Perturbation of the ideal epigenetic equilibrium found in normal cells leads to its transformation. The epigenetic aberrations observed can be summarized as falling into two categories: tumor suppressor genes transcriptionally silenced of by CpG island promoter hypermethylation and global genomic hypomethylation.
1.6.1. DNA methylation at CpG islands

DNA methylation arises by covalent addition of a methyl group at the 5' carbon of the cytosine ring, resulting in 5-methylcytosine [Bird A 2002]. In mammalian DNA, 5-methylcytosine is found in 4% of genomic DNA, mostly at cytosine-guanosine dinucleotide (CpGs). Such CpG sites occur at lower than anticipated frequencies throughout the human genome but are found in more often at small stretches of DNA called CpG islands. These islands are usually found in or near promoter regions of genes, where transcription is initiated [Herman JG and Baylin SB 2003]. In comparison to the bulk genomic DNA, where most CpG sites are heavily methylated, CpG islands remain unmethylated in germ line tissue and promoters of normal somatic cells, allowing gene expression to occur [Bird A 2002].

DNA methylation helps to maintain transcriptional inactivation in nonexpressed or noncoding areas of the genome. Such as, pericentromeric heterochromatin, which is condensed and transcriptionally silent, is heavily methylated. By contrast, these sites are usually unmethylated in promoter regions of euchromatin, despite the transcriptional state of the gene. Exception of this rule, however, can be found in mammalian cells where these regions are methylated to maintain transcriptional silence. Thus, CpG islands are methylated in promoters of genes located in the inactivated X chromosome of females, as are certain imprinted genes where only the paternal or maternal allele is expressed [Bird A 2002, Hermann JG and Baylin SB 2003]. Hypermethylation can also induce predictable alterations in gene expression. Methylation of MGMT and hMLH1 DNA repair genes can lead to their inactivation, resulting in increased frequency of mutations and microsatellite instability, respectively [Herman JG et al. 1998, Esteller M et al. 2000]. Methylation can also encourage spontaneous deamination, increase DNA binding of carcinogens, and enhance the rate of mutations and DNA adduct formation and subsequent gene inactivation [Baylin SB 2005].

1.6.2. DNA methylation regulation

DNA methylation is controlled at several different levels in tumor and normal cells. A family of enzymes called DNA methyltransferases (DNMTs) carries out covalent addition of methyl groups [Baylin SB 2005]. Chromatin structure in the promoter region of genes also affects DNA methylation and transcriptional activity. These are in turn regulated by several factors such as nucleosome spacing and histone acetylases, which affect access to transcriptional factors [Baylin SB 2005].

1.6.3. DNA methyltransferases family

The addition of methyl groups to cytosine residue in DNA are catalyzed by DNMTs. DNMTs found in mammalian cells include DNMT1, DNMT3a, and DNMT3b [Bestor
TH 2000, Okano M et al. 1999, Okano M et al. 1998]. In mouse development, DNMT1 seems to be responsible for maintenance of established patterns of DNA methylation, while DNMTs 3a and 3b appear to mediate establishment of new, or de novo, DNA methylation patterns [Bestor TH 2000, Okano M et al. 1999, Okano M et al. 1998]. Incidentally cancer cells may be different in which DNMT1 alone is not responsible for maintaining abnormal gene hypermethylation and both DNMTs 1 and 3a may cooperate for this purpose [Rhee I et al. 2000, Rhee I et al. 2002].

1.6.4. Chromatin Structure and DNA methylation
Although methylation controls gene activity, it alone is not enough to repress transcription. The local chromatin structure also adds in determining whether genes are transcribed or repressed. Since its discovery, DNA methylation has been coupled with a transcriptionally silent state of chromatin; but the mechanisms by which DNA methylation is translated into transcriptionally inactive chromatin have only recently started to be disclosed.

Previously, a number of hypotheses have been proposed to give explanation the way by which DNA methylation is interpreted by nuclear factors. Hypotheses are:

1. DNA methylation hampers the binding of sequence specific transcription factors to their binding sites that have CpG [Tate PH and Bird AP 1993]. In this context, a protein with an affinity for unmethylated CpG has also been identified that is coupled with actively transcribed regions of the genome [Lee JH and Skalnik DG 2002]. In this case, methylation of CpGs would result in release of protein.

2. The second model proposed that methylation may have direct consequences for nucleosome positioning, for example, by leading to the assembly of specialized nucleosomal structures on methylated DNA that silence transcription more efficiently than conventional chromatin [Kass SU et al. 1997].

3. The third is that methylation guides to the recruitment of specific factors that selectively recognize methylated DNA and either obstruct binding of other nuclear factors or have a straight effect on repressing transcription [Lewis JD et al. 1992]. However, there are examples that support all three possibilities, the active recruitment of methyl-CpG binding activities shows to be the most extensive mechanism of methylation-dependent repression.

In 1997, the laboratories of Dr. Adrian Bird and Dr. Alan Wolffe reported that MeCP2 represses the transcription of methylated DNA through the recruitment of a histone deacetylase-containing complex [Jones PL et al. 1998, Nan X et al. 1998]. This result established for the first time a mechanistic link between DNA methylation and transcriptional repression by the alteration of chromatin. Further reports have established the mechanism in which the remaining methyl CpG binding proteins (MBPs) connect DNA methylation and gene inactivation [Wade PA et al. 1999, Ng HH et al. 1999, Ng HH et al. 2000]. Ng HH et al. 1999 reported that MBP2 is indeed
a component of the previously recognized MeCP1 complex, which shows histone
deacetylase activity. Alternatively, Wolffe’s laboratory identified MBP3 as a
component of the M-2/NURD complex, which exhibits both histone deacetylase and
ATPase dependent nucleosome remodeling activities [Wade PA et al. 1999].

To realize the implications of the relations between DNA methylation and histones, it
is essential to define the significance of these posttranslational histone modifications
to the determination of diverse chromatin states. Most histone modifications occur in
their protruding N-terminal tails. This specificity in the pattern of modifications under
particular circumstances guided to the proposal of the histone code hypothesis, in
which histone modifications perform sequentially or in combination to form a code
that may be read by nuclear factors [Strahl BD and Allis CD 2000]. There are a
number of modifications that are compatible with gene silencing. In general, histone
deacetylation leads to gene silencing. Furthermore, methylation of lysine 9 of histone
H3 has been coupled with gene silencing.

Figure 1.7. Effects of DNA methylation and chromatin structure on gene transcription in
normal and tumor cells. Ovals with black asterisks, nucleosomes with histones having
deacetylation of key histone amino acids such as lysine 9 of histone H3 and methylation of
this same residue (both marks of inactive transcription); ovals with light asterisks,
nucleosomes with acetylation of lysine 9 of histone H3 and methylation of lysine 4 of H3 (both
marks of active transcription); white circles, CpG sites; black circles, hypermethylated sites;
arrow with X, transcriptional repression; curved arrows, transcriptional activity. CA,
transcriptional coactivators; CR, transcriptional corepressor; DNMTs, DNA
methyltransferases; HAT, histone acetyltransferase; HDAC, histone deacetylase; MBPs,
methylcytosinebinding proteins; TF, primary transcription factors. [Adapted from Herman JG
and Baylin SB 2003].

Subsequent to finding of the coupling between DNA methylation and histone
deacetylation by MBPs, additional associations have been found. On one hand,
DNMTs are also known to recruit histone deacetylases [Fuks F et al. 2001, Robertson
KD et al. 2000]; on the other hand, both DNMTs and MBPs have been reported to recruit histone methyltransferases that modify lysine 9 of histone H3 [Fuks F et al. 2003, Fujita N et al. 2003].

Thus, several connections are established between hypermethylation of the CpG islands of tumor-suppressor genes in cancer and their transcriptional inactivation. In case of MBP proteins, involvement with hypermethylated promoters and their association in silencing their corresponding genes has now been established in a number of cases [Magdinier F et al. 2001, Nguyen CT et al. 2001, Ballestar E et al. 2003]. Indeed, MBP proteins seem to be a common feature of the methylated promoter of these genes and also display a notable specificity in vitro [Fraga MF et al. 2003] and in vivo [Ballestar E et al. 2003]. Thus, a MBP-specific profile for hypermethylated CpG islands is starting to be exposed (Figure 1.7).

1.6.5. Methylation of genes in Cancer

The widely accepted 'two hit' hypothesis of carcinogenesis proposed by Knudson [Knudson AG 2001], loss of function of both alleles in a given gene (tumor suppressor) is necessary for malignant transformation. The first hit usually is in the form of a mutation in a crucial gene, followed by loss of wild-type allele by deletion or loss of heterozygosity. In familial cancers this first hit arises through germ line mutation, while in non-inherited sporadic cancers somatic mutations are more frequently observed. Subsequent loss of the other allele through deletion, mutation or loss of heterozygosity can abolish the remaining functional gene. Regulatory gene inactivation in this manner can lead to the evolution of cancer.

Same results can be achieved during epigenetic gene inactivation by promoter hypermethylation. CpG islands located in the promoter region of tumor suppressor gene, normally unmethylated at these regions like all the other genes, undergo a dense hypermethylation in cancer cells leading to turn off of critical genes that could otherwise suppress tumorigenesis (Figure 1.8). Not every gene is methylated in every tumor type, but strong specificity is apparent with respect to the tissue of origin [Costello JF et al. 2000, Esteller M 2005]. Yet, it is not known why some genes became hypermethylated in certain tumor, while others with similar properties remain methylation-free. It has been hypothesized, as researchers have done before with genetic mutations, that a particular gene is preferentially methylated with respect to others in certain tumor types because inactivation confers selective advantage, in the Darwinian sense, on the former [Esteller M 2005].

The tumor suppressor genes, bona fide and "look-alike," that undergo abnormal CpG island methylation in human cancer affect all the cellular pathways and have relevant consequences [Esteller M 2002]. A brief list of the most significant genes inactivated by DNA hypermethylation is represented in Table 1.1 [Esteller M 2005, Herman JG and Baylin SB 2003].
Figure 1.8. DNA methylation in normal and cancer cells. White circles, CpG sites; black circles, hypermethylated sites; 1, 2, 3, exons of the depicted gene; X, transcriptional repression. DNMT, DNA methyltransferases [Adapted from Herman JG and Baylin SB 2003].

Table 1.1: Pathways interrupted by promoter hypermethylation of gene and associated gene inactivation in cancer.

<table>
<thead>
<tr>
<th>Pathway</th>
<th>Genes</th>
</tr>
</thead>
<tbody>
<tr>
<td>Altered cell-cycle control</td>
<td>p16, p15, p14, p73, Rb</td>
</tr>
<tr>
<td>Apoptosis</td>
<td>caspase 8, DAP kinase, TMS-1</td>
</tr>
<tr>
<td>Repair of DNA damage</td>
<td>MLH1, BRCA1, GST-Pi, O6-MGMT</td>
</tr>
<tr>
<td>Tumor architecture or tumor-cell invasion</td>
<td>E-cadherin, H-cadherin, FAT, VHL, APC, LKB1, TMP-3, THBS1</td>
</tr>
<tr>
<td>Growth factor response</td>
<td>RARβ1, SOCS1, SOCS3, ER, PR, AR, PRLR</td>
</tr>
<tr>
<td>Others</td>
<td>RASSF1A, THBS-1, NORE1A, HIC-1, SFRP1, COX1, GATA-4, GATA-5, SRBC, SYK, RIZ1, TPEF/HPP1</td>
</tr>
</tbody>
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1.6.6. Reversal of gene silencing to prevent or treatment of cancer

It is clear from the types of genes that are epigenetically inactivated in tumor that reactivation of their expression could have a deep antitumor effect. Since the mid-1980s, scientists have been able to reactivate hypermethylated genes in vitro using demethylating agents as 5-azacytidine and its deoxy derivative 5-aza-2'-deoxycytidine. However, particularly good results have been observed in continued studies of the preleukemic myelodysplastic syndrome [Silverman LR et al. 2002, Kornblith AB et al. 2002].
1.7. MOLECULAR GENETICS OF MYEOLID LEUKEMIA

To understand the genetic basis of human leukemia, there have been significant strides over the past many years. Most of leukemias are sporadic, but recurrent chromosomal translocations have made identification of disease alleles easier. In majority of cases, cloning of the chromosomal translocation breakpoint produces fusion genes that are casually implicated in disease pathogenesis of human leukemias. However, more than 300 recurring chromosomal translocations have been identified, of which about 100 have been cloned. So, it is clear that many more chromosomal translocations are there than present leukemic phenotypes. However, the similarity of structure and function of expressed fusion genes in some translocations has given insights into improvement of therapeutic agents that might have wide applicability [Kelly LM and Gilliland DG 2002].

1.7.1. MOLECULAR PATHOGENESIS OF ACUTE MYELOID LEUKEMIA

The molecular pathogenesis of acute myeloid leukemia (AML) is complex, but the multiple genetic defects that converge on a set of biological properties of leukemic cells can be understood.

Class I mutations
Confer proliferation and/or survival advantage but do not affect differentiation

BCR-ABL, TEU/PDGFBH
N-RAS and K-RAS mutants
FLT3 activating mutations

Class II mutations
Serve primarily to impair hematopoietic differentiation and subsequent apoptosis

AML/ETO, CBFB/SMMHC, and PML/RARα fusions,
Point mutations in AML1/C/EBPα

CML-like

AML

MDS-like

**Figure 1.9.** Multistep pathogenesis of AML. This model hypothesizes that AML is the consequence of collaboration between at least two broad classes of mutations. Class I mutations, exemplified by constitutively activated tyrosine kinases or their downstream effectors, confer a proliferative and/or survival advantage to hematopoietic cells. When expressed alone, these mutant genes confer a CML-like disease characterized by leukocytosis with normal maturation and function of cells. Class II mutations result in loss of function of transcription factors that are important for normal hematopoietic differentiation. Class II mutations would also be predicted to impair subsequent apoptosis in cells that do not undergo terminal differentiation. When expressed alone, these mutations may confer a phenotype most like MDS. Despite the timing or order of acquisition of mutations, individuals who accrue both Class I and Class II mutations have a clinical phenotype of AML [Adapted from Kelly LM and Gilliland DG 2002]
From the available evidence it appears that AML is the consequence of collaboration between at least two broad classes of mutations:

Class I mutations that give a survival and/or proliferative advantage to hematopoietic cells. Class II mutations that primarily impair hematopoietic differentiation and may provide a selective advantage for these cells as well by impairing subsequent apoptosis. (Figure 1.9)

Chromosomal translocations which target transcription factors and transcriptional coactivators of hematopoietic system.

In AML chromosomal translocation target the transcription factors, which include core binding factor (CBF), retinoic acid receptor alpha (RARα), and members of the HOX family of transcription factors. Chromosomal translocations also target the proteins which are transcriptional coactivators, such as Creb-binding protein (CBP), p300, and MLL etc. All these transcription factors and coactivators have a crucial role in normal hematopoietic development. Chromosomal translocations lead to loss of function of transcription factors and impaired hematopoietic differentiation. The transcription factor CBF is a heterodimeric protein with a DNA binding component, AML1 (also the HUGO designation RUNX1, CBFA2, and PEBP2A), and AML1 transcriptional activity increases by CBFB subunit which does not bind with DNA. It transactivates expression of wide range of genes which are essential for normal hematopoiesis [Speck NA et al. 1999]. It has been reported that mice deficient of either AML1 or CBFB are lacking hematopoiesis [Castilla LH et al. 1999, Okuda T et al. 1996, Wang Q et al. 1996]. So, loss of function of CBF during chromosomal translocations would be anticipated to impair hematopoietic development.

Involvement of CBF is found during the three most frequent chromosomal translocations t(8;21), inv(16) and t(12;21) that result in expression of the AML1-ETO [Erickson P et al. 1992, Kozu T et al. 1993], CBFβ-SMMHC [Liu P et al. 1993], and TEL-AML1 [Golub TR et al. 1995, Romana SP et al. 1995] chimeric proteins respectively. All act as dominant negative inhibitor of CBF-mediated transcription [Frank R et al. 1995, Hiebert SW et al. 1996, Meyers S et al. 1995]. In mice model it has been demonstrated that expression of the fusion genes dominantly interferes with the function of the normal allele, as a result CBF completely loses its function [Yergeau DA et al. 1997, Kelly LM and Gilliland DG 2002]. The transcriptional repression of CBF target genes by CBF related fusion proteins is mediated in part by aberrant recruitment of the nuclear corepressor/histone deacetylase complex [Kelly LM and Gilliland DG 2002], one more recurring topic in the pathogenesis of AML.

The t(8;21) translocation juxtaposes the AML1 gene on 21q22 with the ETO (also called MTG8) gene on 8q22 [Erickson P et al. 1992, Mitterbauer M et al. 1998, Miyoshi H et al. 1991], resulting in the production of a chimeric AML1-ETO mRNA and protein [Downing JR et al. 1993, Nucifora G et al. 1993, Kozu T et al. 1993].
The AML1 (CBFa2) protein is a member of the family of transcription factors with homology to the pair-rule Drosophila gene Runt. AML1 heterodimerizes with another protein CBFβ and generates a transcription factor. The AML1/CBFβ transcription factor bind directly to the enhancer core motif that is in the transcriptional regulatory regions of a number of genes that are critical for myeloid cell growth, differentiation and function [Meyers S et al. 1995]. The reciprocal translocation t(8;21)(q22;q22) is one of the most frequent chromosomal abnormalities in AML [Bitter MA et al. 1987, Nucifora G et al. 1995, Rowley JD 1990] with an overall frequency of 10% in adults [Berger R et al. 1987, Campana D et al. 1995] and it is strongly (about 40%) associated with the M2 FAB subtype [Peterson LF and Zhang DE 2004]. The leukemic cells of AML-M2 patients with t(8;21) show typical morphological features and frequently express the immunophenotypic marker CD19 and, less often, CD56 [Hurwitz CA et al. 1992, Kita K et al. 1992, Porwit-MacDonald A et al. 1996, Seymour JF et al. 1994]. However, t(8;21) may also be seen in AML cases with M1 and M4 subtypes. Translocation (8;21)-positive leukemias have good clinical remission rates as well as long-term survival [Mitterbauer M et al. 1998, Schiffer CA et al. 1989]. This nonrandom translocation is often coupled with additional chromosomal aberration [Sakurai M et al. 1974, Schoch C et al. 1996] and t(8;21) is a cytogenetics risk factor for extramedullary leukemia, which seems to be a bad prognosis in these patients [Byrd JC et al. 1997]. The fusion gene is consistently detected by reverse transcriptase-polymerase chain reaction (RT-PCR) in patients with a documented t(8;21) in cytogenetic analysis.

Chromosomal aberration inv(16) is strongly associated with AML-M4 or M4Eo subtypes and among patients with AML-M4, 23 percent had an inv(16) [Larson RA et al. 1986]. The inversion break point at 16q22 occurs close to the end of the coding region of the CBFβ gene [Liu P et al. 1993], which encodes one subunit of the heterodimeric AML1/CBFβ transcription factor mentioned above [Meyers S et al. 1995]. A smooth muscle myosin heavy chain gene (MYH11) is interrupted by the breakpoint on 16p. A fusion protein containing the 5' region of CBFβ (or CBFβ) (165 of 182 amino acids), together with the domain that heterodimerizes with AML1, fused to the 3' portion of MYH11 is produced [Liu P et al. 1993, Shurtleff SA et al. 1995]. This portion of MYH11 contains a repeated alpha helical structure involved in myosin filament interactions and may therefore be essential in dimerization of the fusion protein in M4 leukemic cells. CBFβ/MYH11 fusion protein emerges to act by disrupting the function of the AML1/CBFβ transcription factor, resulting in repression of transcription [Lutterbach B et al. 1999].

Acute promyelocytic leukemia (APL or AML-M3) is always coupled with fusion gene involving RARα, and is associated with the most common chromosomal translocation t(15;17) which produces PML/RARα fusion protein. As a result of
expression of this fusion protein differentiation at the promyelocyte stage is blocked due to the recruitment of nuclear corepressor complex. All-trans-retinoic acid (ATRA) binding to PML-RARα results in release the nuclear corepressor complex and is reported by several groups. ATRA is widely used for APL treatment.

However, the exact mechanism of action of fusions involving HOX family members is not identified. HOX genes are expressed early in hematopoietic development, and their expression is downregulated during differentiation.

Apart from the chromosomal translocations, point mutations in hematopoietic transcription factors also play a crucial role in development of AML. For example, familial platelet defect (FPD) disorder with tendency to develop AML demonstrated loss of function by mutations in AML1 [Michaud J et al. 2002, Song W-J et al. 1999]. Approximately 3%-5% of sporadic AML have demonstrated loss of function by point mutations in hematopoietic transcription factors [Kelly LM and Gilliland DG 2002].

Several facts support the hypothesis that loss of function of hematopoietic transcription factor is not sufficient for development of AML. Firstly, requirement of second mutation to develop AML from FPD/AML syndrome which is coupled with germ line mutation in AML1 [Song W-J et al. 1999]. Secondly, introduction of the aml-1eto transgene in mouse germ line do not produce cancer [Higuchi M et al. 2002].

1.7.1.1. Mutations in AML that provide proliferative advantage to hematopoietic cells

1.7.1.1.1. N-RAS and K-RAS Mutations

Activating mutations at codons 12, 13 or 61 of N-RAS and K-RAS are associated with AML and MDS [Kelly LM and Gilliland DG 2002]. The incidence of these mutations varies among the studies [Beaupre DM et al. 1999]. A cooperative group trial demonstrated 18% occurrence of N- and K-RAS mutations and conferred a poor prognostic factor [Neubauer A et al. 1994].

1.7.1.1.2. The roles of FLT3 in acute myeloid leukemia

In 1991, FLT3 (Fms-like tyrosine kinase3) also known as FLK-2 (fetal liver kinase-2) and STK-1 (human stem cell kinase-1), was cloned independently by two groups [Matthews W et al. 1991, Rosenet O et al. 1991]. FLT3 has strong sequence similarities with receptor tyrosine kinase (RTK) receptor family of other members of the class III (RTKIII), such as FMS, platelet-derived growth factor receptor (PDGFR) and KIT. All of them are characterized by an extracellular domain comprised of 5 immunoglobulin like (Ig-like) domains and by cytoplasmic domain with a split tyrosine kinase motif [Rosnet O et al. 1993, Agnes F et al. 1994] (Figure 1.10). FLT3 encodes a 993 amino acid protein [Rosnet O et al. 1993] and is expressed in immature hematopoietic cells, placenta, gonads and brain [deLapeyriere O et al. 1995, Maroc N
FLT3 is expressed at high levels in 70% to 100% of AML patients and in a high percentage of ALL cases [Birg F et al. 1992, 1994]. Thus, the data indicate that FLT3 expression may play a role in the survival or proliferation of leukemic blasts [Gilliland DG and Griffin JD 2002].

Figure 1.10. Sketch of FLT3 structure. Shown in schematic style, the 5 immunoglobulin-like folds that make up the ligand-binding extracellular domain, single transmembrane domain, and cytoplasmic domain made up of a kinase domain interrupted by a kinase insert. The juxtamembrane domain where internal tandem duplications (ITDs) take place and aspartic acid 835 where most kinase domain mutations occur are indicated by arrows [Adapted from Small D 2006].

1.7.1.1.3. FLT3 mutation in human leukemias
FLT3 mutations are one of the most common somatic alterations in AML, occurring in about 1/3 of patients. FLT3 mutations consist of two major types: internal tandem duplication (ITD) or length mutation (LM) mutations of 3 to 400 bp (in frame or contains foreign sequence/additions of extra nucleotides) that map to the juxtamembrane region and point mutations that most frequently involve aspartic acid 835 of kinase domain (KD) but have also been found less frequently in several other sites [Small D 2006].

1.7.1.1.4. FLT3 Internal Tandem Duplications (ITDs)
In 1996, Nakao and colleagues first reported the presence of ITDs in the juxtamembrane (JM) domain of FLT3 in AML and suggested that these mutations might play an important role in pathogenesis of AML [Nakao M et al. 1996]. Subsequent confirmatory findings demonstrate that the overall frequency is 986/4299 (23%) in adult AML patients of total cases reported in the literature so far [Small D 2006, Kelly LM and Gilliland DG 2002]. The frequency of FLT3-ITD is approximately 10% to15% in pediatric AML patients and is more frequent in older adults with AML [Kelly LM and Gilliland DG 2002, Xu F et al. 1999, 2000, Stirewalt DL et al. 2001]. FLT3-ITDs have been identified in all FAB subtypes of AML, with the highest reported incidence in the M3 subtype and less frequently in the M2 subtype [Kelly LM and Gilliland DG 2002]. It’s occurred in about 15% of all secondary AMLs [Horiike S et al. 1997], and may be associated with disease progression or relapse of AML [Horiike S et al. 1997, Nakano Y et al. 1999]. FLT3-ITDs have not been detected in limited number of patients with CML, CML blast crisis, JMML, non-Hodgkin lymphoma, adult T-cell ALL, CLL or multiple myeloma [Gilliland DG and Griffin JD 2002, Kelly LM and Gilliland DG 2002, Yokota S et al. 2000].
1997]. In addition, FLT3-ITDs have not been detected in normal hematopoietic cells, including cord blood and bone marrow in which there are high levels of expression of FLT3 [Ishii E et al. 1999]. Juxtamembrane domain (JD) has autoinhibitory function that retains the kinase inactive conformation. So, there is a high frequency of FLT3-ITD in AML that increases with age, and FLT3-ITDs are less common in other hematologic malignancies [Kelly LM and Gilliland DG 2002].

1.7.1.1.5. FLT3 “Activation Loop or Kinase Domain” Mutations in Human Leukemias

In 2001, Yamamoto and colleagues reported that a cohort of AML patients contains mutations in a domain referred to as the activation loop of FLT3, in particular, substitution mutations at aspartic acid residue 835. Activation loop domain has autoinhibitory function that maintains the kinase in an inactive conformation. The activation loop or kinase (KD) is a common part of tyrosine kinases and when the kinase is in the “inactive” state, it functions to obstruct access of adenosine triphosphate (ATP) and substrate to the kinase domain. Following activation, which would normally be through ligand binding in the case of RTKIII family members, a specific tyrosine residue within the loop is normally phosphorylated, causing the loop to implement an “activated” configuration allowing access to the kinase [Gilliland DG and Griffin JD 2002]. D835 substitutions have been reported in 7% to 12% in AML patients and rarely detected in MDS and ALL. D835Y was the most common substitution, but other substitutions included D835V, D835H, D835H, D835E and D835N [Yamamoto Y et al. 2001, Abu-Duhier FM et al. 2001].

Altogether these data indicate approximately 30% have acquired mutations in FLT3 composed of either FLT3-ITD mutation or FLT3-KD mutations. Thus, FLT3 is the single most commonly mutated gene in AML [Gilliland DG and Griffin JD 2002]. Particularly, the mutations in the FLT3 JD and KD might be predicted to result in loss of autoinhibitory function, with constitutive activation of the FLT3 kinase and downstream targets, including STAT5, RAS/MAPK, and PI3K/AKT [Kelly LM and Gilliland DG 2002].

In murine hematopoietic progenitors the expression of FLT3-ITD is not sufficient to cause AML [Kelly LM and Gilliland DG 2002]. So, second mutations may be required for development of AML linked with FLT3-ITD. The hypothesis that FLT3 mutations work together with second mutations to cause AML is supported by the observations that FLT3-ITDs occur consequentally with other gene aberration [Kelly LM and Gilliland DG 2002].

1.7.1.1.6. FLT3 as a target for AML therapy

FLT3 is the single, most regularly mutated gene in AML and associated a poor prognosis in the majority of patients [Abu-Duhler FM et al. 2001, Frohling S et al.
As a consequence, there has been an intensive effort to develop FLT3-tyrosine kinase selective inhibitors (FLT3-TKI) as therapeutic agents. Tyrosine kinase ABL inhibitor STI571 or imatinib methylase or Gleevec has been validated in CML patients for BCR-ABL fusion gene as the above approach. A large number of FLT3 inhibitors have been discovered and investigated, in large part through collaborations between academia and industry [Levis M and Small D 2005]. A number of FLT3 inhibitors were studied in cell lines or mice model [Levis M et al. 2001, Naoe T et al. 2001, Tse KF et al. 2001]. Several FLT3 inhibitors have reached clinical trials in relapsed or refractory AML patients, some or all of whom had FLT3 mutations [Smith BD et al. 2004, Stone RM et al. 2005, Fiedler W et al. 2005]. In trials that have treated both FLT3 mutant and wild-type FLT3 AML patients, there is more frequent response to FLT3-TKI in the mutant FLT3 patients. In most patients, the clinical response is short-lived with most patients’ peripheral blast returning within weeks to months [Small D 2006]. Preclinical studies established synergistic killing of leukemic cells in vitro when FLT3-TKI are combined with conventional chemotherapy in a sequence specific manner [Levis M et al. 2004]. A number of current trials of FLT3-TKI are using this approach of combination treatment in either newly diagnosed adult or relapse AML studies. CEP-701 (Lestaurtinib) combined with chemotherapy is in a phase III trial in relapse FLT3 mutant AML patients in many countries [Levis M et al. 2005, Stone R et al. 2005].

1.7.2. MOLECULAR GENETICS OF CHRONIC MYELOID LEUKEMIA
Almost certainly the most extensively studied human cancer is Chronic Myeloid Leukemia (CML). In 1960, Nowell and Hungerford discovered Philadelphia (Ph) chromosome, as first consistent chromosomal abnormality associated with a specific type of leukemia which was a breakthrough in cancer biology [Nowell P and Hungerford D 1960]. The Ph chromosome is the result of a t(9;22) reciprocal chromosomal translocation [Rowley JD 1973] and was shown to involve the ABL proto-oncogene on chromosome 9 and a break point cluster region (BCR) gene on chromosome 22 [Bartram CR et al. 1983, Groffen J et al. 1984]. Over 95% of patients with clinically ‘acceptable’ CML have a BCR-ABL gene in their leukemia cells [Shepherd P et al. 1995, Melo JV 1996], consequently the t(9;22) can generally be considered as the hallmark of CML. Though, it is not restricted to CML because it is detected in 10% to 20% of adult ALL [Westbrook CA et al. 1992] and in 2% to 5% of childhood ALL [Russo C et al. 1991, Suryanarayan K et al. 1991], as well as in occasional cases of AML [Kurzrock R et al. 1987, Najfeld V et al. 1989, Preudhomme C et al. 1992], lymphoma [Mitani K et al. 1990, Fujii H et al. 1990] and
1.7.2.1. Molecular structure of the BCR-ABL translocation

The breakpoint within the ABL gene at 9q34 can occur anywhere within a >300kb area at the 5' end of the gene, either upstream of the first alternative exon lb i.e. between exons lb and la, or downstream of exon la (Figure 1.12). Regardless of the exact location of the breakpoint, splicing of the primary hybrid transcript generates an mRNA molecule in which BCR sequences are fused to ABL exon a2. On the contrary to ABL, breakpoints within BCR localize to 1 of 3 so-called breakpoint cluster regions (bcr). In the majority of CML patients and in approximately one third of patients with Ph-positive ALL, the break occurs within a 5.8 kb area spanning BCR exons 12-16 (also referred as b1-b5), defined as the major breakpoint cluster region (M-bcr). Due to alternative splicing, fusion transcripts with either b2a2 or b3a2 junctions can be formed. A 210 kd fusion protein (P210BCRABL) is derived from this mRNA. In the remaining patients with ALL and rarely patients with CML, characterized clinically by prominent monocytosis [Melo JV et al. 1994, Ravandi F et al. 1999], the breakpoint are more upstream in the 54.4 kb area between the alternative BCR exon e2' and e2, named the minor breakpoint cluster region (m-bcr). The resultant e1a2 mRNA is translated into 190 kd protein (P190BCR-ABL). Recently, a third breakpoint cluster region (µ-bcr) was identified downstream of exon 19, giving rise to a 230 kd chimeric protein (p230BCR-ABL) associated with the rare Ph-positive chronic neutrophilic leukemia [Pane F et al. 1996], though not in all cases [Wilson G et al. 1997, Mondal BC et al. 2006]. If sensitive techniques such as nested RT-PCR are used, the e1a2 transcript is detectable in many patients with typical P210BCR-ABL CML [van Rhee F et al. 1996]. The low level of expression of these P190-type transcripts compared to P210 indicates that they are likely to be the result of alternative splicing of the primary mRNA. Occasional cases with other junctions, such as b2a3 [Liu LG et al. 2003], b3a3, e1a3, e6a2 [Melo JV 1997], e8a2 [Branford S et al. 2000] or e2a2 [Leibundgut EO et al. 1999], have been documented in CML and ALL patients.
The function of various parts of BCR and ABL in the oncogenic chimeric protein provides important information in the different type of transcript found in nature. Interestingly, ABL exon 1, even if retained in the genomic fusion, is never part of chimeric mRNA. So, it has to be spliced out during processing of the primary mRNA; the mechanism underlying this apparent peculiarity is unidentified. Derived from the observation that the ABL part in the chimeric protein is nearly invariable while the BCR portion varies significantly, one may assume that ABL is likely to carry the transforming code whereas the diverse sizes of the BCR sequence may dictate the phenotype of the disease [Deininger MW et al. 2000]. In support of this idea, uncommon cases of ALL express a TEL-ABL fusion gene, [Golub TR et al. 1996, Papadopoulos P et al. 1995] representing that the BCR moiety can in principle be substituted by other sequences and still cause leukemia. Although all 3 major BCR-ABL fusion proteins induce a CML-like disease in mice, they differ in their ability to induce lymphoid leukemia [Li S et al. 1999], and in contrast to P190 and P210, transformation to growth factor independence by P230BCR-ABL is incomplete [Quackenbush RC et al. 2000, Inokuchi K et al. 2003], which is consistent with the relatively benign clinical course of P230-positive chronic neutrophilic leukemia [Pane F et al. 1996].
1.7.2.2. The physiologic function of the ABL and BCR

The ABL gene is the human homologue of the v-ABL oncogene carried by the Abelson murine leukemia virus (A-MuLV) [Abelson HT et al. 1970], and it encodes the nonreceptor tyrosine kinase [Laneuville P 1995]. Human ABL is a ubiquitously expressed 145 kd protein with 2 isoforms arising from alternative splicing of the first exon [Laneuville P 1995]. A number of structural domains can be distinct within the protein (Figure 1.13). Three SRC homology domains (SH1-SH3) are located near the NH₂ terminus. The SH1 domain carries the tyrosine kinase function, while the SH2 and SH3 domains allow for interaction with other proteins [Cohen GB et al. 1995]. Proline-rich sequences in the middle of the molecule can, in turn, interact with SH3 domains of other proteins, such as Crk [Feller SM et al. 1994]. Toward the 3' end, nuclear localization signals [Van Etten RA et al. 1989] and the DNA-binding [Kipreos ET et al. 1992] and actin-binding motifs [McWhirter JR et al. 1993] are found.

![Figure 1.13. Structure of the ABL protein.](image)

Some quite diverse functions have been attributed to ABL, and the emerging picture is complex. As a result, the normal ABL protein is implicated in the regulation of the cell cycle [Kipreos ET and Wang JY 1990, Sawyers CL et al. 1994], in the cellular response to genotoxic stress [Yuan ZM et al. 1999], and in the transmission of information about the cellular environment through integrin signaling [Lewis JM et al. 1998, Van Etten RA 1999]. Overall, it integrates signals from different extracellular and intracellular sources that control decisions in regard to cell cycle and apoptosis. It has to be stressed, however, that lots of the data are based exclusively on in vitro studies in fibroblasts, not hematopoietic cells, and are still controversial. Unfortunately, the creation of ABL knockout mice failed to resolve the majority of the outstanding issues [Tybulewicz VL et al. 1991, Schwartzberg PL et al. 1991]. Like ABL, BCR is a 160 kd protein expressed ubiquitously [Laneuville P 1995].
Some structural motifs can be delineated (Figure 1.14). The first 5' exons encode a serine-threonine kinase. The single substrates of this kinase identified so far are Bap-1, a member of the 14-3-3 family of protein [Reuther GW et al. 1994], and probably BCR itself [Laneuville P 1995]. A coiled coil domain at the N-terminus of BCR permits dimer formation in vivo [McWhirter JR et al. 1993]. The middle portion of the protein contains a region with dbl-like and pleckstrin-homology (PH) domains that appreciate the exchange of GTP for GDP on Rho guanine exchange factors (GEF) [Denhardt DT et al. 1996], which in turn may stimulate transcription factors such as NF-κB [Montaner S et al. 1998]. The carboxy terminus has GTPase activity for Rac [Diekmann D et al. 1991], a small GTPase of RAS super-family that controls actin polymerization and the activity of an NADPH oxidase in phagocytic cells [Diekmann D et al. 1995], BCR can be phosphorylated on several tyrosine residues, especially tyrosine 177, which binds Grb-2, an essential adapter molecule implicated in the activation of the Ras pathway [Ma G et al. 1997]. The fact that BCR knockout mice are viable and the reality that an increased oxidative burst in neutrophils is so far the only documented defect [Voncken JW et al. 1995] possibly reflect the redundancy of signaling pathways. If there is a role for BCR in the pathogenesis of Ph-positive leukemias, it is not clearly apparent because the incidence and biology of P190BCR-ABL-induced leukemia are the same in BCR− mice as they are in wild-type mice [Voncken JW et al. 1998].

1.7.2.3. Molecular targets for therapy in CML
Efforts at designing therapeutic tools for CML based on molecular and cellular biology of the disease have concentrated primarily on three major areas— the stimulation of the immune system's capability to recognize and destroy leukemic cells, specific gene expression inhibited at the translation level by anti-sense strategies, the modulation of protein function by precise signal transduction inhibitors.
The issue of immunologic stimulation, be it in the form of adoptive immunotherapy by donor lymphocyte infusion [Dazzi F et al. 1999] or of BCR-ABL junction peptide vaccination [Pinilla-Ibarz J et al. 2000], is another opportunity being extensively explored for treatment of CML. The antisense oligonucleotide [O'Brien SG and Smetsers TF 1997, Gewirtz AM et al. 1998] and ribozyme [James HA et al. 1998] approaches received a lot of interest in the last decade but have a general failed to achieve their theoretical promises.

Perhaps the most exciting of the molecularly designed therapeutic strategies was brought about by the arrival of signal transduction inhibitors (STI), which prevent or block a protein from exerting its responsibility in the oncogenic pathway. Because the major transforming property of BCR-ABL protein is generated through its constitutive tyrosine kinase activity, direct inhibition of such activity appears to be the most logical means of silencing the oncoprotein. To this consequence, a number of tyrosine kinase inhibitors have been evaluated form different sources for their potential to modify the phenotype of CML cells. Subsequently, synthetic compounds were developed through a rational design of chemical structures able to compete with the adenosine triphosphate (ATP) or the protein substrate for the binding in the catalytic center of the kinase [Levitzki A et al. 1995] (Figure 1.15). The most promising of these compounds is the 2-phenylaminopyrimidine STI571, which specifically inhibits Abl tyrosine kinase at micromolar concentrations [Buchdunger E et al. 1995]. In addition, it selectively suppresses the growth of CML primary cells and cell lines in vitro [Druker BJ et al. 1996, Deininger MW et al. 1997] and in mice [Druker CJ et al. 1996, le Coutre P et al. 1999]. Its remarkable specificity and efficacy led to consideration of the drug for therapeutic use. At present STI571 (also known as imatinib mesylate, Gleevec) is internationally accepted for treatment of CML patients.

Figure 1.15. Mechanism of tyrosine kinase inhibitors action. The drug competes with ATP for its specific binding site in the kinase domain. So, whereas the physiologic binding of ATP to its pocket allows BCR-ABL to phosphorylate selected tyrosine residues on its substrates (left diagram), a synthetic ATP mimic for example STI571 fits this pocket equally well but does not give the essential phosphate group to be transferred to the substrate (right diagram). The downstream series of reactions is then arrested because, with its tyrosines in the unphosphorylated form, this protein does not assume the required conformation to ensure association with its effector [Deininger MW et al. 2000].
1.7.2.4. Signaling pathways and biologic properties of BCR-ABL positive cells

Three major mechanisms have been involved in the malignant transformation by BCR-ABL, mainly adhesion to stroma cells and extracellular matrix [Gordon MY et al. 1987], activation of mitogenic signaling [Puil L 1994] and apoptosis [Bedi A et al. 1994]. A fourth probable mechanism is the newly described proteasome-mediated degradation of Abl inhibitory proteins [Dai Z et al. 1998].

1.7.2.4.1. Altered adhesion properties

CML progenitor cells show decreased adhesion to bone marrow stroma cells and extracellular matrix [Gordon MY et al. 1987, Puil L et al. 1994]. In this situation, cell proliferation is negatively regulated by adhesion to stroma, and CML cells escape this regulation by virtue of their perturbed adhesion properties. Recent data suggest a vital role for β-integrins in the interaction between stroma and progenitor cells. An adhesion-inhibitory variant of β1 integrin expressed in CML cells, is not found in normal progenitors [Zhao RC et al. 1997].

1.7.2.4.2. Mitogenic signaling pathways activation

Several signaling pathways activated in BCR-ABL positive cells, namely Ras and the MAP kinase, Jak-Stat, PI3 kinase and Myc pathways (Figure 1.16).

*Ras and the MAP kinase pathways.* Several links between Bcr-Abl and Ras have been defined. Autophosphorylation of tyrosine 177 provides a docking site for the adapter molecule Grb-2 [Pendergast AM et al. 1993]. Grb-2, after binding to the Sos protein, stabilizes Ras in its active GTP bound form. Two other adapter molecules, She and Crkl, can also activate Ras. Both are substrates of Bcr-Abl [Oda T et al. 1994, Pelicci G et al. 1995] and bind Bcr-Abl through their SH2 (She) or SH3 (Crkl) domains. Circumstantial evidence that Ras activation is important for the pathogenesis of Ph positive leukemias comes from the observation that activating mutations are uncommon, even in the blastic phase of the disease [Watzinger F et al. 1994], unlike in most other tumors.

*Jak-Stat pathway.* Constitutive phosphorylation of Stat transcription factors (Stat1 and Stat5) have been reported in several BCR-ABL-positive cell lines [Ilaria RL et al. 1996] and in primary CML cells [Chai SK et al. 1997], and Stat5 activation appears to contribute to malignant transformation [de Groot RP et al. 1999]. Although Stat5 has pleiotropic physiologic functions [Nosaka T et al. 1999], its effect in BCR-ABL-transformed cells appears to be primarily anti-apoptotic and involves transcriptional activation of Bcl-xL [Horita M et al. 2000, Sillaber C et al. 2000]. In contrast to the activation of the Jak-Stat pathway by physiologic stimuli, Bcr-Abl may directly activate Stat1 and Stat5 without prior phosphorylation of Jak proteins. There seems to be specificity for Stat6 activation by P190$^{BCR-ABL}$ proteins as opposed to P210$^{BCR-ABL}$ [Ilaria RL et al. 1996].
**PI3 kinase pathway.** It has been documented that PI3 kinase activity is required for the proliferation of BCR-ABL-positive cells [Skorski T et al. 1995]. Bcr-Abl forms multimeric complexes with PI3 kinase, Cbl, and the adapter molecules Crk and Crkl [Sattler M et al. 1996], in which PI3 kinase is activated. The next relevant substrate in this cascade appears to be the serine-threonine kinase Akt [Skorski T et al. 1997]. This kinase had previously been implicated in anti-apoptotic signaling [Franke TF et al. 1997]. A recent report placed Akt in the downstream cascade of the IL-3 receptor and identified the pro-apoptotic protein Bad as a key substrate of Akt [del Peso L et al. 1997]. Phosphorylated Bad is inactive because it is no longer able to bind anti-apoptotic proteins such as Bcl-xl and it is trapped by cytoplasmic 14-3-3 proteins. Altogether this indicates that Bcr-Abl might be able to mimic the physiologic IL-3 survival signal in a PI3 kinase dependent manner. Thus, Bcr-Abl appears to have a profound effect on phosphoinositol metabolism, which might shift the balance to a pattern similar to physiologic growth factor stimulation.

**Myc pathway.** Activation of Myc by Bcr-Abl is dependent on the SH2 domain [Sawyers CL et al. 1992]. The pathway linking Myc to the SH2 domain of Bcr-Abl is still unknown. However, result obtained in v-abl-transformed cells suggest that the signal is transduced through Ras/Raf, cyclin dependent kinases (cdks), and E2F transcription factors that ultimately activate the MYC promoter [Zou X et al. 1997].

**1.7.2.4.3. Inhibition of apoptosis**
Expression of Bcr-Abl in factor dependent murine [Daley GQ et al. 1988] and human [Sirard C et al. 1994] cell lines prevents apoptosis after growth-factor withdrawal, an effect that is critically dependent on tyrosine kinase activity and that correlates with
the activation of Ras [Puil L et al. 1994, Bedi A et al. 1994]. Moreover, several studies showed that BCR-ABL-positive cell lines are resistant to apoptosis induced by DNA damage [Bedi A et al. 1994, Bedi A et al. 1995]. The underlying biologic mechanisms are still not well understood. Bcr-Abl may block the release of cytochrome C from the mitochondria and thus the activation of caspases [Dubrez L et al. 1998, Amarante Mendes GP et al. 1998]. This effect upstream of caspase activation might be mediated by the Bcl-2 family of proteins. Bcr-Abl has been shown to up-regulate Bcl-2 in a Ras-[Sanchez Garcia I et al. 1997] or a PI3 kinase-dependent [Skorski T et al. 1997] manner in Baf/3 and 32D cells, respectively. Moreover, as mentioned previously, Bcl-xl is transcriptionally activated by Stat5 in BCR-ABL-positive cells [Horita M et al. 2000, Sillaber C et al. 2000].

1.7.2.4.4. Degradation of inhibitory proteins
The recent discovery that Bcr-Abl induces the proteasome-mediated degradation of Abi-1 and Abi-2 [Dai Z et al. 1998], 2 proteins with inhibitory function, may be the first indication of yet another way by which Bcr-Abl induces cellular transformation.

1.8. GENES WHICH ARE INVOLVED IN BIOACTIVATION OF PROCARCINOGEN AND DETOXIFICATION OF CARCINOGEN
Carcinogenesis is the result of heritable changes in somatic cell DNA that alter the growth characteristics of cells. Xenobiotic substances capable of producing such changes are present in the environment and are ingested with food additives, drugs, and pollutants. To cope from these, all higher animals including humans have evolved a series of genes coding enzymes involved in oxidation/activation (Phase-I) and conjugation/detoxification (Phase-II) of these compounds. Important among these are two main groups of drug metabolizing enzymes include phase-I enzymes Cytochrome P-450 family and the phase-II enzymes Glutathione S- transferases (GSTs) family. In Phase-I, the major reaction involved is hydroxylation, catalyzed by members of a class of enzymes referred as monooxygenases or cytochrome P-450 family. In phase II, the hydroxylated or other compounds produced in phase I are converted by specific enzymes to various polar metabolites by conjugation with glucuronic acid, sulphate, acetate, glutathione, or certain amino acids, or by methylation. The overall purpose of the two phases of metabolism of xenobiotics is to increase their water solubility (polarity) and facilitate their excretion from the body [Murray RK 1990]. These enzymes have overlapping substrate profiles and are characterized by polymorphic
Environmental exposures and genetic susceptibility play a role in the etiology of leukemias [Infante-Rivard C et al. 1999]. DNA damage in the hematopoietic precursor cell is the essential prerequisite for the development of leukemia and endogenous and exogenous substances cause these damages. Polymorphisms in the genes metabolizing these agents are known and suspected to be one of the risk factors of cancer. Association of these polymorphic variations with hematopoietic malignancies has also been tested, with conflicting outcomes.

Gliutathione S-transferases (GSTs) are a family of phase II detoxification enzymes that catalyze the conjugation of glutathione (GSH) to a broad spectrum of xenobiotics. This detoxification capability acts a role in cellular protection from environmental and oxidative stress, however, is also implicated in cellular resistance to drugs. Overexpression of GST isozymes have been found in chemotherapeutic resistant in cell lines. This overexpression guides to an accelerated detoxification of drug substrates and hence an acquired resistance.

1.8.1. GST classification and human polymorphisms

Human GSTs are divided into three major families: mitochondrial, membrane-bound microsomal and cytosolic. Microsomal GSTs are designated as ‘membrane associated proteins in eicosanoid and glutathione metabolism’ (MAPEGs). MAPEGs are structurally different from cytosolic GSTs although are functionally similar in the capability to catalyse the conjugation of GSH to electrophilic compounds [Hayes JD et al. 2005]. The mammalian cytosolic family of GSTs are catalytically active in a homo- or heterodimeric state though it exists as monomers [Mannervik B and Danielson UH 1988]. The cytosolic family is further divided into seven classes: Alpha, Mu, Omega, Pi, Sigma, Theta and Zeta. Classification is based on sharing greater than 60% identity within a class and focuses mainly on the more highly conserved N-terminal domain that contains a catalytically active tyrosine, cysteine or serine residue. The catalytic residue interacts with the thiol group of GSH (G-site), and crystal structure has confirmed a substrate-binding site (H-site) that facilitates catalysis and is in proximity to the G-site [Dirr H et al. 1994, Armstrong RN 1997].

1.8.1.1. Alpha family

The GSTα isoform is mainly expressed in the liver and is encoded by a gene cluster localized on chromosome 6p12. This cluster contains 5 genes encoding proteins belonging to GSTA1-A5. Human tissues widely express transcripts for GSTA1, A2 and A4, whereas expression of GSTA3 is rare and GSTA5 is yet to be detected in...
human tissues. Epidemiological results show that aberrant expression of GSTA has been linked to an increased risk in colorectal cancer, ovarian cancer and clear cell renal cell carcinoma [Coles B et al. 2001, Tetlow N et al. 2004, Chuang ST et al. 2005, McIlwain CC et al. 2006].

1.8.1.2. Pi family
The GST Pi class is encoded by a single gene spanning about 3kb and located on chromosome 11 [Cowell IG et al. 1988]. In this class four active, functionally different polymorphisms (GSTP1*A–D) have been identified [Ali-Osman F et al. 1997]. The GSTP1 genotype has been associated with differences in chemotherapeutic response and cancer susceptibility and is overexpressed in a wide variety of tumors including ovarian, NSCLC, breast, colon, pancreas and lymphoma [Tew KD 1994].

1.8.1.3. Zeta family
The Zeta class (GSTZ1) is a single 10.9 kb gene located on chromosome 14 that encodes for a 29 kDa protein [Blackburn AC et al. 1998]. This gene family has been recently identified on the basis of sequence alignment and phylogenetic studies [Board PG et al. 1997]. A recombinant human enzyme catalyzed the oxygenation of dichloroacetic acid to glyoxylic acid. Clinical data for GSTZ1 are insufficient to deduce a role for GSTZ1 in disease [McIlwain CC et al. 2006].

1.8.1.4. Omega family
The Omega class of GSTs included two members (GSTO1 and GSTO2) and a pseudogene (GSTO3p). Based on two defining features, X-ray crystallography and substrate specificity, the Omega class is both structurally and functionally distinct from other eukaryotic GSTs [Townsend D and Tew K 2003]. GSTO1 is a single gene located on chromosome 10 that codes for proteins expressed abundantly in the liver, glial, macrophages and endocrine cells [Board PG et al. 2000, Yin Z et al. 2000]. So far, four polymorphisms have been known, GSTO1*A–D [Board PG et al. 2000]. Among the Australian, African and Chinese populations, GSTO1*A was the most common haplotype with a frequency ranging from 0.6 to 0.9; whereas GSTO1*B*A was the least frequent, with a frequency of 0.01–0.05 [Whitbread AK et al. 2003]. GSTO1*A demonstrated a GSH-dependent reduction of dehydroascorbate, a role characteristic of glutaredoxins rather than GSTs [Board PG et al. 2000]. This allele was first described as the human monomethylarsenic acid reductase, (MMA[V]), and is the rate-limiting enzyme of inorganic arsenic metabolism [Zakharyan RA et al. 2001]. Thioltransferase activity differs among the GSTO1*A–C polymorphisms and may add to an individual’s ability to metabolize arsenic [Tanaka-Kagawa T et al. 2003]. GSTO2, while separated from GSTO1 by 7.5 kb on chromosome 10, shares
64% amino-acid identity [Whitbread AK et al. 2003]. GST02, like GSTO1, is ubiquitously expressed and shares GSH-dependent dehydroascorbate reductase activity.

1.8.1.5. Mu family

The mu genes are ordered, 5' GSTM4-GSTM2- GSTM1-GSTM5-GSTM3 3', in a 20kb cluster [Xu S et al. 1998]. Three GSTM1 alleles, GSTM 1*0, GSTM 1*A, GSTM 1*B, are described. GSTM 1*0 is deleted. GSTM 1*A and GSTM 1*B differ by one base in exon 7 and encode monomers that form active homo- and heterodimeric enzymes. McLellan RA et al. 1997 have described two Saudi Arabians with ultrarapid GSTM1 activity as a result of having two copies of the gene. It was inferred that this rapid detoxification phenotype could have an increased defensive effect against carcinogens [McLellan RA et al. 1997]. GST M1 has been implicated in detoxification of polycyclic aromatic hydrocarbons like benzo (α) pyrene, benzene, styrene etc.

Loss of GSTM1 enzyme function is ascribed to a homozygous deletion of this gene resulting in the GSTM1*0 allele [Smith CM et al. 1994]. It has been suggested that the mutation is a result of an unequal crossing over of the M1 and M2 loci [Xu S et al. 1998], which are in close physical proximity and share 99% nucleotide sequence identity [Pearson WR et al. 1993]. The frequency of GTSM1*0 individuals is approximately 67% in Australians, 50% in Caucasians and 22% in Nigerians [Smith G et al. 1995]. GSTM1 null genotype frequency varies among the different part of India. Frequency of GST M1 null genotype in the population of West Bengal has been reported to vary between 20-48% [Roy B et al. 2001]. GSTM1 null phenotype is associated with an increased risk of the lung, colon and bladder cancer and has also been associated with response rates to some chemotherapy [Smith CM et al. 1994; Lohmueller KE et al. 2003]. For example, GSTM1*0 patients with acute myeloid leukemia (AML) have been shown to have a better response to adriamycin and cyclophosphamide treatment, although precise reasons for this correlation are not clear [Ambrosone CB et al. 2001].

1.8.1.6. Theta family

The Theta class of GSTs consists of two different subfamilies: GSTT1 and GSTT2. Genes encoding both proteins are colocalized on chromosome 22 and are separated by 50 kb [Pemble S et al. 1994; Tan KL et al. 1995]. Substrates for GSTT1 are of small reactive hydrocarbons like epoxides, 1,3-butadiene and their derivatives, dichloromethane [Landi S 2000]. Polymorphisms exist within both genes including a null phenotype (GSTT1*0) that exhibits decreased catalytic activity and has been
Chapter I

General Introduction

associated with an increased risk of cancers of the head, neck and oral cavity [Strange RC and Fryer AA 1999, Landi S 2000].

The frequency of the null genotype has been assessed in some ethnic groups worldwide. The frequency of null genotype is highest among Chinese (64.4%) followed by Koreans (60.2%). Among Caucasians the frequency was measured to be 15%, Turkish 19.7%, Malaysians 38%, Egyptians 14.7%, Among Africans-Americans the frequency was measured to range form 20 to 24%, whereas the occurrence was low among Mexican-Americans (9.7%). In Brazil, the frequency of null genotype was 18.5% and 19% among the Caucasian and African descendants, respectively, but was 11% among the Amazonian Indians [Landi S 2000]. GSTT1 null genotypes frequency varies in different part of India. Frequency of GST T1 null genotype in the population of West Bengal has been reported to vary between 3-13% [Roy B et al. 2001].

1.8.1.2. GST null genotype and cancer prognosis

Deletion of the GSTM1 and GSTT1 genes results in a ‘null’ genotype characterized by a common deficit in enzymatic activity. Individuals homozygous for these deletions are thought to be at increased risk for malignancies as a consequence of a decreased ability to detoxify probable carcinogens. Wherever this phenotype has been studied as a predictive factor for cancer prognosis or response to therapy, the results have been contingent upon tumor type. Patients with GSTM1*0 or GSTT1*0 showed a better survival rate after chemotherapeutic treatment for invasive ovarian cancer compared to other patients [Howells RE et al. 2001]. However, results were contrary for ovarian cancer patients who were double null for GSTM1 and GSTT1. When compared to patients with the wild-type GSTM1 or GSTT1 allele, the null genotype patients exhibited a reduced response to chemotherapy resulting in both a poorer prognosis as well as a decrease in remission rates [Howells RE et al. 1998]. Several studies have been documented on association between polymorphisms and incidence of leukemias (AML, ALL, CLL, MDS) [Rollinson S et al. 2000, Chen CL et al. 1997, Stanulla M et al. 2000, Yuille M et al. 2002, Chen H et al. 1996].
Aims of the study
AIMS OF THE STUDY

Aims of the present study were to shed light on the current knowledge of molecular genetics and epigenetics in leukemia. For this I have studied alteration of some cancer related genes in myeloid leukemia namely acute myeloid leukemia (AML) and chronic myeloid leukemia (CML) arising in eastern India.

More specifically, the objectives of this thesis research were to:

- Characterize the gene expression pattern at the mRNA level of cell cycle regulatory genes in de novo AML patient samples.
- Characterize the CpG island methylation status near to the gene promoter of nine cancer related genes in primary AML patients.
- Detect and characterize the chromosomal translocations, gene duplication and mutation which are frequently found in primary AML and CML.
- Characterize the status of glutathione S-transferase gene polymorphisms in CML patients with respect to normal population.

These data have been correlated with the clinical data and course of overall survival.