Chapter 1

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1.1. HEMATOPOIESIS

Hematopoiesis is a precisely regulated process that generates terminally differentiated cells in the peripheral blood from immature progenitors in the bone marrow. Mature blood cells consist of a variety of components, such as erythrocytes, granulocytes, lymphocytes and platelets. The number of these cells is strictly maintained in spite of the short life of these cells. For such tight control, hematopoietic progenitors are required to properly proliferate, differentiate into mature cells, or undergo apoptosis according to circumstances. Different lines of evidence have indicated that hematopoiesis can be achieved by a finely tuned cooperation of hematopoietic genes in the progenitor cells. The orderly production of blood cells is a highly regulated process involving successive stages of commitment and differentiation. This involves complex interactions of cells and cellular products of both, the hematopoietic system and their stromal microenvironment. All hematopoietic functions are ultimately regulated at the level of HSCs, which are present in bone marrow and have self-renewing capability. In response to specific growth factors, interleukins and hormones, these cells undergo two sequential differentiating processes. The first is commitment, by which stem cells lose their self-renewal capability and differentiate to progenitors with a more limiting differentiation potential (which give rise to only one or sometimes two cell lineages). The second is maturation, which allows the terminal differentiation of cells committed to a specific lineage. Both, the commitment and maturation of stem cells arise from the temporal and spatial expression of lineage-specific genes. Gene transcription requires the assembly of RNA polymerase II with a multi protein pre-initiation complex at specific DNA sequences including the TATA box in a promoter. Interactions of general transcription factors with basal promoter elements are generally essential for basal transcription but not sufficient to modulate its rate. Activation or repression of gene transcription takes place when several transcription factors bind to their cognate sequences on the lineage-specific promoters and stimulate or inhibit transcription through protein-protein interactions with the basal transcription machinery (GOODRICH et al. 1996). The action of lineage-specific transcription factors determines the selective expression of various lineage-specific genes. These transcription factors are either expressed
constitutively or induced at a certain stage of differentiation. Rather than being controlled by any single master regulator, lineage-specific gene expression appears to depend on the combination of regulatory proteins that bind in overlapping functional domains.

Many transcription factors are required for blood cell development, and several, such as Scl, Rbtn, E2A and CBFβ, also play key roles in the deregulation of hematopoiesis, which is manifested as leukemia. Figure 1.1 depicts the conventional view of hematopoiesis in which multipotent stem cells are self-regenerating and also produce precursor cells with increasing restriction of their lineage and proliferative potential. Within each lineage population, cell numbers rise with increasing maturity.

![Figure 1.1: Schematic diagram of stages in hematopoietic differentiation and CBFβ expression (adults and embryo).](image)

Cells in which CBFβ-GFP is expressed are represented in **green**, and cells that do not seem to express CBFβ-GFP are shown in black. The common myeloid progenitor (CMP), common lymphoid progenitor (CLP), and megakaryocyte-erythroid precursor (MEP) populations were not isolated and analyzed directly. These populations in which CBFβ expression is presumed are shown in blue. (KUNDU and LIU 2003)

Acute Myeloid Leukemia (AML) is a malignant neoplasm of hematopoietic cells characterized by an abnormal proliferation of myeloid precursor cells, decreased rate of self-destruction and an arrest in cellular differentiation. All cells are continually faced with decision to divide, differentiate, or undergo programmed cell death. The normal cell regulation is balanced and delicately orchestrated by genes promoting and suppressing cell growth. AML is associated with a sequence of genetic changes that cause deregulation in the cell cycle division, cell differentiation, or programmed cell death. AML is a heterogeneous disease,
which is generally characterized by the presence or absence of specific cytogenetic abnormalities. One of the key transcription factors which play an important role in AML is RUNX1. With regard to the anatomy of RUNX1, the Core Binding Factor (CBF) is a transcriptional regulator complex, which is composed of two sub-units; α subunit (RUNX) and β subunit (CBFβ) (Kamachi et al. 1990). The transcription factor RUNX1/AML1 is one of the main regulators of genesis of blood (haematopoiesis) in vertebrates.

1.2. EVOLUTION OF RUNX1

In higher primates including humans, RUNX1 which is 260-kb long gene coding for the consequent transcription factor is located on chromosome 21. RUNX2 (PEBP2aA/CBFA1/AML3), which has 6p21 chromosomal location, plays a substantial role in osteogenesis during embryonic development and RUNX3 (PEBP2αC/CBFA3/AML2) which positions itself at 1p36 in the chromosomal arrangement of human genome, is majorly expressed in hematopoietic cells (Baek et al. 1995; Levanon et al. 1994; Speck et al. 1999).

RUNX1 has 9 exons and 12 possible isoforms. RUNX2 has 8 exons and 12 possible isoforms. RUNX3, the smallest of the three, has 6 exons and a very few isoforms (Figure 1.2). Rennert and coworker in 2003 described the phylogenecity of RUNX gene based on its sequence homology as shown in Figure 1.3.

![Figure 1.2: The mammalian RUNX genes structure and mode of function](ZFARONI et al. 2002). The three mammalian RUNX genes have similar genomic organization with two promoters (P1 and P2) and a very large first intron. The two promoters give rise to two biologically distinct 5′ untranslated regions (UTRs) (yellow and orange). In humans and mice, each gene resides on different chromosomes (human 21, 6 and 1, and mouse 16, 17 and 4, respectively). The highly conserved runt domain is encoded by the three exons is marked in green. (Baek et al. 1994; Zaffaroni et al. 2002)
Figure 1.3: Phylogenetic illustration of RUNX genes showing the gene number and promoter usage in different animals. The three lower non-designated branches in the phylogenetic tree represent the animal groups (from bottom up) sponges, cnidarians (e.g. jellyfish) and acoelomates (e.g. flatworms). The most primitive animals contain one gene regulated by the P2 promoter (RENNERT et al. 2003).

Phylogenetic studies have established that the number of isoforms of RUNX genes have increased along with evolution (summarized in Figure 1.4). Bilaterians ancestrally contain a single RUNX gene, suggesting that the multiple RUNX1 genes in vertebrates and insects arose by independent duplication events within those respective lineages. At least two introns are present in the primordial bilaterian RUNX1 gene. Alternative promoter usage arose prior to the duplication events gave rise to three members of RUNX1 genes in vertebrates (RENNERT et al. 2003). It is exigent to understand the biological functions of RUNX1 because of the presence of large number of isoforms generated in the cell by different processes like exon skipping, presence of more than one promoter, multiple poly (A) tails and post translational modifications etc. RUNX gene is transcribed from two alternative promoters, which are generally referred to as the distal (P1) and the proximal (P2) promoters, and are approximately 160kb apart (GHÖZİ et al. 1996; LEVANON et al. 2001b). RUNX1 has four domains 1) N-terminal domain, 2) Runt domain, 3) C-terminal auto-inhibitory domain, and 4) transcriptional activation domain. (Figure 1.5)
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Figure 1.4: Phylogenetic tree on the basis of number of isoforms of RUNX1.

Figure 1.5: Functional anatomy of RUNX1. DNA binding & heterodimerization with CBFβ are mediated by the Runt domain (green). The Runt domain also associates with other DNA-binding proteins. The negative regulatory regions for DNA binding are located adjacent to the Runt domain (gray). Shown are the locations of other sequences for transactivation (red), transcriptional repression (blue), and nuclear matrix attachment. ERK phosphorylation sites, and binding regions for coactivators sp300/CBP, ALY, YAP, corepressors mSin3A, Ear-2, and TLE. Arrows indicate breakpoints in t(8;21), t(3;21), and t(12;21). Almost all sequences downstream to the Runt domain are lost in t(8;21) and t(3;21), while they are retained in t(12;21). ERK, extracellular signal-regulated kinase; ALY, ally of RUNX1 & LEF-1; YAP, Yes associated protein. (KUROKAWA and HIRAI 2003)
1.3. REGULATION OF RUNX1

In various experimental setups, these aforementioned two promoters (P1 and P2) were found to be active in cells of a variety of lineages. This is despite the fact that RUNX1 is preferentially expressed in haematopoietic cells. There are two regulatory elements in the RUNX1 gene that guide its tissue-specific expression. One of these two regulatory elements, located in intron 1, is a haematopoiesis-specific enhancer. The second regulatory element, located in intron 5, contributes to the formation of an active chromatin hub, which integrates the above-mentioned enhancer and the distal promoter, P1 and proximal promoter, P2 (see Figure 1.6).

![Figure 1.6: Scheme depicting the common structure of mammalian P1-5'UTR and P2 5' UTR. (a). It contains four exons; the middle two are alternatively spliced and designated as exon/introns. RR marks the two perfectly conserved RUNX-binding sites, underlined in the conserved 18bp sequence 5' CAACCACAGAACCACAAG-3'. (b). Mammalian P2-5' UTR. It spans one exon, which terminates with an in-exon splice site (AG) and is preceded by the intronic branch point signal CTRAY. The P2-5'UTRs contain IRES and is surrounded by an exceptionally large CpG island marked by the gray cloud (LEVANON and GRONER 2004).](image)

The transcripts formed under the regulation of proximal, P2 promoter are particularly prevalent in fetal liver (FL) hematopoietic stem cells (HSC) (FUJITA et al. 2001; SAMOKHVALOV et al. 2006; TELFER and ROTHENBERG 2001), while those regulated by distal promoter P1 prevalent in HSC-fated cells (SAMOKHVALOV et al. 2007) and are critically required for FL hematopoiesis (POZNER et al. 2007) (Figure 1.7). Because of the shift in the promoter regulation by P1 and P2 promoters, the gene leads to the formation of a protein with slightly different N-terminus. Tefler and Rothenberg reported in 2001 that the first 19 amino acids of N-terminal portion of the protein synthesized by the regulation of P1 promoter, were replaced by five amino
acids of the protein synthesized by genes regulated by P2 promoter (TELFER and ROTHENBERG 2001). The short length of the protein synthesized from the gene regulated by P2 promoter may be because of the fact that the 5’ UTR region being lengthy (~1.6 kb), contains many ATGs, which lead to the initiation of protein synthesis from various points resulting in the synthesis of short isoform of the protein. Inversely, 5’ UTR of RUNX1 synthesized by the regulation of P1 promoter is short and thus leads to the formation of efficient long RUNX1 protein (RENNERT et al. 2003). The alternative splicing mechanism produced three major isoforms of RUNX1 i.e. AML1c (regulated by P1 promoter), AML1b (regulated by P2 promoter) and AML1a (regulated by P2 promoter) (LA FIURA et al. 2008). Any of the Runx protein forms a part of large macromolecular assembly in which various proteins interact with each other to regulate not only expression of its own family member but also of their target genes.

![Expression of AML1](image)

**Figure 1.7:** Expression of AML in both endothelial and hematopoietic cells from E8.5 (initial sites of development) to adult.

RUNX1 might (as a speculative extrapolation) also play a role in other systems as it is expressed in many other embryonic tissues (NORTH et al. 1999) and in epithelial cells (LEVANON et al. 2001a; LIAN et al. 2003). **Figure 1.8** gives a detailed knowledge about the expression of RUNX1, RUNX2 and RUNX3 during the formation of bone.

RUNX genes may sometimes be expressed in the same biological tissues and act synergistically and/or at different compartments. Finally, because all
three RUNX family members bind to the same DNA sequence, some redundancy does occur, although often the expression patterns in the same tissue may vary in intensity (COHEN 2009). In addition to this plethora of expression, it is overexpressed in endometrioid carcinoma (PLANAGUMA et al. 2004) and down-regulated in gastric cancer (SAKAKURA et al. 2005). It is, therefore, very important and imperative (and yet incomplete) to study the function of RUNX1.

1.4. RUNX1- A PROTEIN WITH DUAL FUNCTION

The Runx1 protein can act as a regulator of definitive hematopoiesis by acting as a scaffold protein in enhanceosomal or repressosomal complexes. As is evident from Figure 1.9, in case of the enhanceosomal complexes it can act as a transcriptional activator while it behaves as a repressor in the repressosomal complexes.

There are a number of hematopoietic gene-specific promoters which are activated by Runx1. This includes promoters for IL-3, GM-CSF, granzyme B, neutrophil elastase and subunits of TCR and BCR, which substantiate the fact
that it indeed facilitates the assembly of transcriptional activation complex. RUNX1 associates with transcriptional cofactors including p300 and CREB-binding protein (CBP), which facilitates transcription by acetylating histones and making the promoters more accessible (KITABAYASHI et al. 1998).

![Figure 1.9: Proposed mechanism of RUNX1 ability to activate and repress transcription](image)

Runx1 can also function as a transcriptional repressor, depending on the target gene and the cellular milieu, by recruiting co-repressors, such as transducin-like enhancer (TLE) by interacting with the carboxy-terminal VWRPY penta-peptide motif, the nuclear receptor Ear-2 and mSin3A (IMAI et al. 1998; JAVED et al. 2000; LEVANON et al. 1998; WANG et al. 1998) (Figure 1.10). The p21/WAF1/CIP1 promoter was identified as a putative target for RUNX1, in search for promoter sequences that contain AML1 binding sites. It was later observed that Grochro interaction is not required for Runx1-dependent repression of p21, instead two domains in C-terminal of RUNX1 are the basic requirement. It acts as transcriptional repressor for p21/WAF1/CIP1 (cdk inhibitor important for cell cycle checkpoint control and terminal differentiation) in NIH3T3 cells (LUTTERBACH et al. 2000) and CD4 in thymocytes (TANIUCHI et al. 2002).
On the genetic point of view, there are some sequences that directs Runx transcription factors to nuclear matrix-associated sites and in turn, supports transcription in a cell cycle-dependent manner; via showing the specificity of its binding at intranuclear site where the regulatory machinery for its expression is present (STEIN et al. 2003; TANG et al. 1998). Runx1 protein binds to a specific nucleotide sequence, TGT/Cgt, which is present in the promoter region of several hematopoietic genes.

1.5. RUNX1 AND ITS TARGET GENES

We are of the opinion that RUNX1 target genes must be expressed in the same tissues or cells as RUNX1. Therefore, the expression patterns of a number of differentially expressed genes, chosen due to potential functions in leukemia development, were compared to that of RUNX1. The expression of number of genes in human tissues, many hematopoietic cell lines and normal human bone cells were assessed using cDNA panels (MICHAUD et al. 2000). Many of these genes show a high expression in a number of hematopoietic cell lines and all the others show common expression with RUNX1 in various tissues such as liver and peripheral blood leukocytes (PBLs). This lends support to our supposition.

1.5.1. Targeted Genes of RUNX1

Researchers have also identified new potential RUNX1 target genes by analyzing the regulatory regions and the expression pattern of differentially
expressed genes present in the overlaps between different platforms. Many RUNX1 target genes have already been described in the literature, mainly from in-vitro studies and in mouse cells (MICHAUD et al. 2003; OKADA et al. 1998). Four of the published target genes, CSF1R, MYB, MPO and TIMP1, were differentially expressed in the RUNX1 knockout embryos. In addition, target genes that were described more recently, including CCND3 (BERNARDIN-FRIED et al. 2004) and IGFBP3 (IWATSUKI et al. 2005), were identified following overexpression of the CBF complex. Interesting candidates were among the 16 genes differentially expressed in almost every dataset, such as Annexin I (ANXA1), which was shown to reduce inflammation, by inhibiting neutrophil recruitment (PERRETTI et al. 1993) and has an anti-proliferative effect by inducing aberrant cytoskeleton formation (ALDRIDGE and BRYANT 2003). This gene is likely to play an important role downstream of RUNX1.

1.5.2. RUNX3

Runt-related transcription factor-3 (RUNX3) is a member of the runt domain family of transcription factors, also known as polyomavirus enhancer-binding protein 2 (PEBP2)/core binding factors (CBF). RUNX3 is a known regulator of major developmental pathways, and has recently been reported as a candidate for tumor suppressor. RUNX3 is located on chromosome region 1p36 (Figure 1.11) and plays an important role in the transforming growth factor (TGF)-β signalling pathway. This may occur partly through interaction with FoxO3a/FKHRL1, both of which are indispensable for the activation of the pro-apoptosis protein Bim (Bcl-2-interacting mediator of cell death), or by cooperating with SMAD protein family members to induce the TGF-β/SMAD pathways in other ways. The RUNX3 is regulated by three mechanisms: loss of heterozygosity (LOH), protein mislocalization, and promoter methylation (FAN et al. 2011).

RUNX3 was found to be the most ancient transcription factor among its own family members as its function seems to be in the primitive animals such as
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hydra involved in neurogenesis of the monosynaptic reflex arc (LEVANON et al. 2003).

![Figure 1.11: (A) Physical map of human chromosome 1 spanning 2.7 Mb around the RUNX3 gene, based on public database information (http://genome.ucsc.edu). All of the genes indicated are transcribed in the same orientation as RUNX3. (B) Genomic structure of the human RUNX3 gene. The locations of the distal P1 and proximal P2 promoter regions are indicated. Vertical arrow, P2-associated CpG Island (STEWART et al. 2002).]

The evolution of RUNX genes begins with one gene, mostly like RUNX3 in invertebrates, progresses to four genes in Drosophila, and to three in vertebrates. RUNX3 establishes epigenetic silencing in CD4-CD8+ cytotoxic T-cells by binding the CD4 silencer core sequence. RUNX3 is a tumor suppressor gene with hemizygous deletion of one allele and hypermethylation of the other, resulting in gastric adeno-carcinoma. Invertebrates generally have one RUNX gene, most similar to RUNX3, the smallest of the vertebrate genes. RUNX3 is required for CD8+ T-cell development during thymopoiesis; has a primary role in determining the dorso-ventral projection pattern of proprioceptive and cutaneous sensory neurons; has a role in bone formation and especially in chondrocyte maturation; is found in the mesenchymal elements; controls the proper development of gastric endothelial cells by apoptosis. RUNX1 and RUNX3 are both expressed in the developing dorsal root ganglia, but in different classes of sensory neurons. They are also expressed in hematopoietic system, especially in mature T-cells, but at different stages during T-cell development with RUNX1 playing the major role.
RUNX2 plays the major role in bone and cartilage development. RUNX1 and RUNX3 are also expressed in developing bone and cartilage in some similar and some different areas, but at variable levels of expression.

One of the RUNX family members i.e. RUNX1 can physically interact with proteins of Ets family and together binds on DNA, thereby facilitating the activation of gene expression (ERMAN et al. 1998; GU et al. 2000; KIM et al. 1999; MAO et al. 1999; SUN et al. 1995; WHEELER et al. 2000; WOTTON et al. 1994).

1.5.3. LAT

Linker for activation of T-cells (LAT) plays a central role in T-cell activation by nucleating signalling complexes that are critical for the propagation of T-cell signals from the plasma membrane to the cellular interior. A large number of studies have revealed that LAT-based complexes catalyse critical TCR-mediated signalling reactions and lead to the induction of multiple downstream pathways that direct almost all TCR-initiated cellular responses (BALAGOPALAN et al. 2011). LAT is a transmembrane adaptor protein and plays an important role in the development of T-cells in the thymus and for the activation of mature T-cells to evoke an immune response (FINCO et al. 1998). Homeostatic proliferation and cell survival are the two major components which constitute and make the T-cell homeostatic. It was shown by Shudan Shen et al. that T-cells which are deficient of LAT are unable to survive for a long time and lymphopenia driven homeostatic proliferation (SHEN et al. 2010). Whitten et al. showed in 2008 that the promoter region of LAT is highly enriched with 5’-GC content which helps in the contribution of the LAT promoter activity (WITTEN et al. 2008). As shown in Figure 1.12, the 5’ promoter region of LAT gene contains binding sites for Ets and RUNX transcription factors.
Figure 1.12: A 200bp region of the human LAT promoter. The DNA sequence extending from -450 to -250 relative to the start site for the human LAT gene is shown. Mapped transcription start sites are indicated by arrows. Previously identified binding sites for Ets and RUNX transcription factors are indicated, as are the 5 GC-rich Sp sites.

It was proposed that similar to other regulatory regions, Runx1 and Ets-proteins cooperatively interact at the LAT promoter to activate gene expression. The tissues where LAT seems to be expressed are thymus, peripheral blood, and at a low level in spleen. The LAT gene expresses itself only in T-cells, NK cells and mast cells, while its expression is not seen in B-cells or monocytes (GRIFFITH et al. 1998).

1.6. EFFECT OF MUTATIONS ON STRUCTURE AND FUNCTION OF RUNX1

Functional transcription factor, RUNX1 consists of two subunits, CBF (Runx1) and CBFβ, the later protein has no DNA binding domain. The α-subunits of CBF recognize a specific sequence (TGT/cGGT) in the regulatory regions of their target genes in order to bind to DNA directly, while in case of the β-subunit, it heterodimerizes with the α-subunit but does not interact directly with the DNA. The interaction with CBFβ stabilizes the Runx1-DNA complex (Ogawa et al. 1993; Wang et al. 1993) and protects the Runx1 proteins from ubiquitin-mediated proteolysis resulting in increased stability of the complex (Huang et al. 2001). Although both the interacting partners have auto-inhibitory domains, their interaction represses the effect of inhibition and
allows Runx1 to bind more efficiently with DNA (Lichtinger et al. 2010). Runx1 protein always exists in homodimeric or heterodimeric form. The self-interaction of Runx1 occurs through C-terminus region only, and if there is any removal or alteration due to translocation or missense or point mutation, then the dimerization gets interrupted, which leads to an alteration in the transcriptional activity of Runx1 (Li et al. 2007). The region from 372 to 411 of C-terminus plays an important role in the homodimerization (Li, Sinha et al. 2007). It is also documented that even when C-terminus domain is intact, the Runx1 protein is unable to bind DNA effectively if runt domain is mutated. Similarly, mutation or translocation in the C-terminus results in disruption of homodimerization. Binding of mutated Runx1 to its target DNA results in pleiotropic changes in gene expression and hence, abnormal accumulation of hematopoietic cells leading to leukemia.

RUNX1 is arguably the most frequent target of somatic mutations in leukemic states and is mutated in an autosomal dominant fashion affecting platelets which predisposes to the leukemic state. Mutations in amino acid residues involved in DNA binding could apparently abrogate its transactivation function while those in C-terminal may abrogate for protein-protein interaction. Since RUNT domain is known to be involved in its interaction with CBFβ, the molecular mechanism by which these mutants deregulate hematopoiesis is still not completely clearly understood.

Chromosomal aberrations, rearrangements, alterations etc. which involve RUNX1 or CBFβ (Lutterbach and Hiebert 2000) (Figure 1.13), somatic point mutations in RUNX1(Osato 2004) and amplification of RUNX1 (Roumier et al. 2003), all have been implicated, studied and described in acute leukemias. In addition to somatic alterations, germ-line point mutations in RUNX1 have been shown to be responsible for an autosomal dominant platelet disorder with a propensity to develop leukemia (FPD-AML, OMIM 601399) (Michaud et al. 2002; Song et al. 1999). It is interesting to note that the measure of Runx1 protein seems to orchestrate a significant role in the fortitude of the leukemic phenotype. Nevertheless, negative regulation or
down-regulation of RUNX1, resulting from haploinsufficient or dominant negative mutations, lead to the development of myeloid leukemia (Michaud et al. 2002; Osato et al. 1999; Song et al. 1999), whereas amplification of RUNX1 expression gene is more often observed in lymphoid leukemia, particularly paediatric ALL (Mikhail et al. 2002).

Figure 1.13: Schematic AML1b and several AML1 fusion proteins. Runt, Runt domain; transactivation, transactivation domain; PST, proline/Serine/threonine-rich domain; NHR, Nervy homology region; ZFD, zinc finger domain; AD, acidic domain; PNT, pointed domain. Arrow heads indicate the breakpoint in each fusion protein. The amino acid numbers are given below the diagram (Lutterbach and Ebert 2000).

In spite of such divergent structure and biochemical features, almost all of the RUNX1 mutants show a loss of normal RUNX1 trans-activation potential, suggesting that RUNX1 mutants might fundamentally act through a loss-of-function mechanism and simple haploinsufficiency may contribute to some of the myeloid malignancies such as MDS/AML. However, the in-frame mutations in N-terminal runt domain (Ni-type) and the short truncated mutations in C-terminal region (Ct-type) of RUNX1 mutants show not only a loss of trans-activation potential, but also an inhibitory effect on the trans-activation activity of wild-type RUNX1 in a dose-dependent fashion. These RUNX1 mutants, although not as strong as CBFβ-MYH11, which is a well-
established negative regulator of RUNX1, could act as dominant-negative inhibitors of wild-type RUNX1. This suggests that such mutants may have some oncogenic potential in addition to the loss of normal RUNX1 function (DING et al. 2009). Figure 1.14 shows the most commonly known mutations prevalent in acute myeloid leukaemia patients.

A numbers of studies have suggested that although RUNX1 is involved in the initial steps of leukemia development, additional somatic mutations are indispensable and probably the determinant for the leukemic phenotype (Figure 1.15). Principle reasons to support this hypothesis are: Firstly, the predisposition to develop leukemia in FPD-AML patients shows that germline RUNX1 mutations are not sufficient for the development of the disease (SONG et al. 1999). Secondly, those somatic translocations are not able to induce leukemia in mouse cells on their own (RHOADES et al. 2000). And third, that the translocation t(12;21), which fuses ETV6 (TEL) to RUNX1, can arise in-utero but does not trigger leukemia until later in childhood, with as much as nine years latency (FORD et al. 1998). Thus additional mutations are likely to occur in molecules involved in the same biological pathways as RUNX1, as hemizygous loss of several molecules in the pathway (e.g. RUNX1 and SPI1) is thought to be almost as tumorigenic as homozygous loss of one molecule.
(e.g. homozygous RUNX1 mutation in AML-M0) (Cook and McCaw 2000). Therefore, identification of downstream targets of RUNX1, with care to the model systems including species and cell type of origin, would be of great interest in order to identify novel candidate molecules involved in leukemogenesis. The identification of the biological pathways regulated by RUNX1 is also of importance to shed light on its in-vivo function and role in leukemic development. A remarkable study has shown that RUNX1 knockout mice lack perfect hematopoietic maturation and die at embryonic stage 12 from hemorrhages in the central nervous system. This means that RUNX1 plays a vitally decisive and important role during hematopoietic development (Okuda et al. 1996; Wang et al. 1996). Moreover severity of the leukemia disease can also be varied, the chronic myeloid leukemia (CML) can also get transformed into acute myeloid leukemia (AML) if there would be any alteration in any one of the important differentiating regulators e.g. C/EBPα (Tenen 2003).

Characterization of its in-vivo and in-vitro functions may provide insight into the mechanisms leading to the development of leukemia, and will provide new candidate genes that are responsible for leukemic phenotypes. It is not, however, imperative to think that as a transcription factor and as a master regulator of hematological cancers, RUNX1 might alter the function of only one oncogenic molecule, or multiple molecules in the same pathway.

Using genomic and bioinformatic approaches Scherr and Eder (2005) identified the biological pathways and genes regulated by RUNX1. Using cells derived not only from patients harbouring a RUNX1 mutation but who have not yet developed leukemia (a characteristic preleukemic stage) allowed observing effects, largely due to changes in RUNX1 dosage. Moreover using overexpression system in HeLa as well as the knockout mouse embryos approach, highly significant correlation between the genes identified in the FPD-AML cells, the overexpression system and the clinical data on AML samples so obtained supports the hypothesis that a large number of genes are in reality regulated by RUNX1 (Pozner et al. 2007).
Figure 1.15: Clinical, cytogenetic and molecular data of the 51 patients with AML1 point mutations (ROUMIER et al. 2003). The repression of AML-ETO is not just because of the translocation but the coiled coil form of ETO can also associate with itself and get oligomerized with AML-ETO protein, which recruits a nuclear repressor, N-CoR protein, and thus represses the transcription of the AML1 targeted genes, thereby altering the differentiation of primary hematopoietic precursors (TANAKA et al. 1998; WESTENDORF et al. 1998).
Genes identified so far as differentially expressed following dysregulation of RUNX1 expression level in these AML samples may prove to be good candidates for targets of secondary hits during leukemogenesis downstream of RUNX1 mutations (Smith et al. 2005). In order to get insight in the in-vivo role of RUNX1, bioinformatics tools had been applied to identify the biological domains and processes that were changed following alteration of RUNX1 expression level. Further, genes involved in megakaryopoiesis tend to be differentially expressed in the FPD and CBF datasets demonstrating that a large number of the differentially expressed genes may play a role in platelet formation. These data validate the integrative approach as they confirm studies published using transgenic mice expressing the fusion proteins CBFβ-MYH11 (Cao et al. 1997) and RUNX1-ETO (Rhoades et al. 2000), both of which act in a dominant negative fashion over the wild type protein. These mice show a decrease in both lymphoid and myeloid cell proliferation. This observation also correlates with mouse data showing that RUNX1 promotes cell cycle progression from G1 to S phase (Strom et al. 2000). An anti-proliferative effect of a RUNX1 mutant protein may have an oncogenic effect due to an improper balance between proliferation and differentiation. In other words, overexpression of RUNX1 usually results in ALL while complete or partial loss of RUNX1 results in the development of AML. It is however, not clearly understood how mutations in RUNX1/AML1 regulate expression of its family members and of other target genes. It is also not explicitly shown whether accumulation of blast cells is a consequence of disruption of differentiation along a hematopoietic lineage or accumulation of cells due to a failure to undergo programmed cell death or both. An understanding of the mechanism of action of RUNX1/AML1 and mutants of RUNX1 in regulation of gene expression will give us a better insight into hematopoiesis and diseases associated with it.