Lessons from models of murine erythroleukemia to acute myeloid leukemia (AML): proof-of-principle of co-operativity in AML

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The models of acute erythroleukemia caused in mice by the Friend retrovirus SFFV (spleen focus forming virus) and the Spi-1/PU.1 transgenesis provide considerable information to help to understand the molecular mechanisms underlying the multistage nature of leukemia. Leukemogenesis in these murine models is initiated from an acute hyperplasia of erythroid progenitor cells followed later on by a blastic crisis. This review highlights recent findings demonstrating the key roles of the co-operation of two mutations occurring during leukemic progression, a mutation interfering with differentiation and a mutation conferring a proliferative advantage to cells. Through their multistep evolution, these mouse erythroleukemia models resemble the two phases of human acute myeloid leukemia (AML). The findings we discuss provide evidence for similar molecular mechanisms involved in the evolution of leukemia in mice and men.

Key words: leukemia, Friend, Spi-1/PU.1, Kit, co-operativity.

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Leukemias are disorders of blood-forming tissues in which the maturation and differentiation programs of distinct hematopoietic lineages originating from primitive stem cells with multilineage potential are affected. These disorders result from acquired somatic mutations targeting a variety of transcription factors and signaling molecules. An important insight into the molecular mechanisms responsible for hematopoietic malignancies arose from studies of leukemia induced in animals by retroviruses, in part because these models enabled the identification of several proto-oncogenes that were transduced in the genome of the retroviruses. In this review, I shall only address the avian E26 virus[1] and the avian erythroblastosis AEV virus[2] that transduce the viral oncogenic counterparts of the Myb and Ets proto-oncogenes for E26, and ErbA and ErbB proto-oncogenes for AEV. In addition, because retroviruses integrate efficiently into the cellular genome, they activate proto-oncogenes adjacent to their integration sites by providing strong transcriptional promotion or enhancement. In this way, the genes encoding the transcription factors Spi-1,[3] Fli-1[4] and Evi-1[5] were originally identified in murine leukemia as common sites for retroviral integration.

The purpose of this review is to summarize the currently available data about murine acute erythroleukemia. These murine models are unusual for their notably short latency and striking homogeneity. They represent powerful working models of a disease whose evolution is very similar to that of human acute myeloid leukemia (AML). They provide several lines of evidence indicating that leukemia proceeds via multiple steps characterized by genetic alterations driving the progression of a normal hematopoietic cell towards malignancy.

The Friend erythroleukemia

The Friend virus was isolated by Charlotte Friend in 1957.[6] When inoculated into susceptible mouse strains, it causes a rapidly developing erythroblastosis evident as early as 10 days after injection. This erythroblastosis is characterized by acute hyperplasia of the erythroid progenitor compartment and massive production of differentiated erythroid cells.[7] The Friend virus is a retroviral complex of a replication-defective spleen focus forming virus (SFFV) that is the pathogenic component responsible for the erythoblastosis and a replication-competent Friend murine leukemia virus (F-MuLV) that supplies the replicative functions defective in SFFV.[8,9] Two different strains of SFFV have been identified which reproducibly lead to either polycythemia ($SFFV_P$) or anemia ($SFFV_A$) due to a hemodilution.[10] The target cell in which both SFFV express their pathogenic effect is an erythropoietin (Epo)-responsive progenitor cell identified as a late BFU-E or a CFU-E.[11] The number of these SFFV-infected erythroid progenitors increases dramatically, but the cells do not acquire significant self-renewal potential in vitro and are not tumorigenic *in vivo*. Characteristically,

erythroid progenitors differentiate in the absence of Epo during SFFVp infection whereas erythroid precursors infected with SFFV$_A$ need Epo for proliferation and differentiation.[12,13]

Physiopathological studies established that the early erythroblastosis is rapidly followed by an acute transformation phase.[14-16] Twenty to 30 days after infection, tumorigenic proerythroblasts arrested in differentiation at the CFU-E stage can be isolated. These cells are serially transplantable *in vivo* and can be established as permanent cell lines *in vitro*. The tumorigenic Friend cells do, however, remain able to mature into hemoglobinized cells after exposure to a variety of chemical inducers such as dimethylsulfoxide (DMSO) and hexamethylene-bisacetamide (HMBA), providing a model which has been successfully used for studying terminal erythroid differentiation *in vitro*.[17]

Molecular events during Friend erythroleukemia

Expression of the viral gp55

The Friend virus complex differs from most acutely oncogenic retroviruses in that it does not carry a mutated cellular proto-oncogene. The pathogenicity of SFFV is determined by the product of the viral *env* gene, a glycoprotein with a molecular mass of 55k Da (gp55$_P$ and gp55$_A$).[18] gp55 derives from a deleted and mutated F-MuLV *env* gene, making the protein resident in the infected cells and unable to be processed in the viral particle. An important insight into the role of gp55 was provided by the demonstration that gp55 could interact with the receptor for erythropoietin (EpoR).[19] This interaction occurs through the respective transmembrane domains of EpoR and gp55$_P$ or gp55$_A$ at the surface of erythroid cells. A third partner contributing to the effect of gp55 in erythroid hyperplasia is the naturally occuring short form of the stem-cell kinase receptor (sf-Stk). Stk is a tyrosine kinase receptor of the Met family encoded by the *Fv2* gene, long known as one of genes determining the susceptibility of some mouse strains (Fv2s/s) to Friend disease.[20-22] Transcription of *sf-Stk* is initiated from an internal promoter within the *Stk* gene that is lacking in Friend-resistant mice (Fv2r/r). sf-Stk lacks the extracellular ligand-binding domain but retains the transmembrane and the tyrosine kinase domain.[23-25] The binding of gp55$_P$ to EpoR and sf-Stk promotes the Epo-independent proliferation and terminal differentiation of the erythroid precursors through the activation of signal-transducing proteins, including the Janus protein tyrosine kinases Jak1 and 2, the transcription factors STAT1, 3 and 5, the downstream effector AKT of PI3kinase and the mitogen-activated protein kinases.[26-30] Rare amino acid differences in the transmembrane domains of gp55$_P$ and gp55$_A$ are sufficient to minimize Jak2/STAT5 signaling making gp55$_A$ unable to promote Epo-independent erythroid proliferation.[25]

Transforming events

Although SFFV is an acutely leukemogenic retrovirus, it induces transformation of erythroid cells by a mechanism of insertional mutagenesis usually employed by non-acute leukemogenic viruses. The identification of integrated SFFV proviruses in the genome of various Friend tumor cells was the first molecular signature of their clonal nature. The consistent rearrangement of a genomic locus by SFFV insertion, the so-called Spi-1 standing for *SFFV proviral integration-1,* indicated that this rearrangement was crucial for the clonal expansion of a single cell.[3,31] The SFFV insertion activates the transcription of the *spi-1* gene by introducing LTR transcriptional enhancers able to by-pass the tissue-specific activity of the *spi-1* genomic promoters.[3,32,33] The Spi-1 protein is identical to the transcription factor PU.1 identified through its ability to recognize purine-rich DNA sequences in the genome.[31,34] Spi-1 is not mutated, implying that aberrant overexpression is responsible for the oncogenic function. The total incidence of *spi-1* mutation in Friend leukemia suggests a specific co-operation between gp55 and Spi-1 overexpression. This was verified in a heterologous model of self-renewal and differentiation of chicken primary erythroid progenitors in which it was shown that the effects of Spi-1 on survival, proliferation and arrest in differentiation were dependent on the co-expression of Spi-1 with an EpoR activated either by gp55 or by a mutation on residue R129C which mimics EpoR/gp55 activation.[35-37]

Another recurrent genetic alteration in Friend tumor cells is mutation in the tumor suppressor *p53* gene. Allelic deletions or missense mutations lead to loss of its tumor suppressive function.[38,39] Friend leukemia develops more rapidly in transgenic mice expressing a mutant allele of p53 or in p53-null mice than in normal mice.[40,41]Although ectopic expression of a normal p53 protein in Friend cells induces apoptosis and hemoglobin production,[42] the loss or mutation of p53 seem to accelerate the acquisition of other mutations, promoting growth and survival rather than specifically altering erythoid differentiation.

In conclusion, the multi-step evolution of SFFV-induced erythroleukemia indicates that gp55 induces a preleukemic stage, but that Spi-1 overexpression determines the onset of leukemia (Figure 1).

Erythroleukemia in spi-1 transgenic mice

To specify the role of Spi-1 in erythroleukemia, *spi-1* transgenic mice were engineered by germinal insertion of a *spi-1* transgene driven by a SFFV LTR. These animals develop an acute erythroleukemia that evolves as a two-step process.[43] The first step is characterized by large hepatosplenomegaly associated with severe anemia. The spleen and liver are infiltrated by proerythroblasts arrested in differentiation at a basophilic stage. Permanent erythroblastic cell lines are easily established

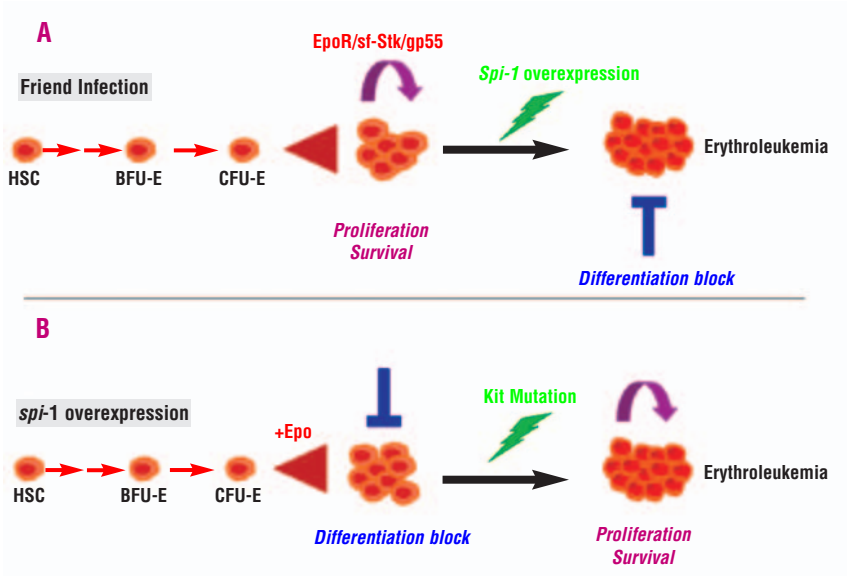

Figure 1. (A). Molecular mechanisms involved in the pathogenesis of murine acute erythroleukemia. The early stage of Friend acute erythroleukemia is characterized by an expansion of erythroid progenitors as a consequence of the activation of EpoR and sf-STK by viral gp55. The blastic crisis results in clonal expansion of erythroid progenitors whose differentiation is blocked. The arrest in erythroid maturation is associated with overexpression of the transcription factor Spi-1/PU.1 caused by SFFV integration. (B). In the leukemia that progress in *spi-1* transgenic mice, the initial event is overexpression of Spi-1/PU.1 by germinal mutation. This results in expansion of erythroid progenitors whose differentiation is blocked. The blastic crisis characterized by the autonomous expansion of proerythroblasts is associated with activating mutations in the *Kit* gene.

from diseased hematopoietic tissues, but only in the presence of Epo. Accordingly, the *in vivo* disorder is tightly controlled by the level of endogenous Epo as demonstrated by the rapid regression of hepatosplenomegaly and disappearance of circulating blasts following repeated red blood cell transfusions. The anemic state creates hypoxic conditions and induces stress erythropoiesis. Under these conditions, the massive expansion of the erythroid compartment destined to compensate for the deficit in mature red blood cells takes place in the presence of stem cell factor (SCF) and Epo in the splenic microenvironment.[44] Indeed, SCF cooperates with Epo to maintain the survival and proliferation of the *spi-1* transgenic proerythroblasts *in vitro* when Epo is used at limiting concentrations.[45] Finally, since these *spi-1* transgenic proerythroblasts are unable to induce tumors when engrafted *in vivo* they are considered as preleukemic cells. This disease clearly shows that ectopic expression of Spi-1 in the proerythroblast contributes to the maturation block of erythroid precursor cells without removing their dependence on growth factors.

Later, during the acute expansion of the proerythroblastic compartment, blastic cells emerge: these cells are are characterized by both Epo-independent growth *in vitro* and tumorigenicity *in vivo*. This evolution to a terminal blastic phase undoubtedly reflects the selection of a malignant cell subpopulation having acquired genetic lesions. In conclusion, the erythroleukemia in *spi-1* transgenic mice evolves as a multi-step process indicating that the onset of leukemia depends on the occurrence of somatic mutations in addition to Spi-1 overexpression (Figure 1).

Molecular events during erythroleukemia in spi-1 transgenic mice

Overexpression of Spi-1/PU.1

The function of Spi-1/PU.1 in erythroid transformation appears multifaceted. The arrest in differentiation of proerythroblasts is associated with cell cycle disorders and increased proliferation (*P. Rimmaele, F.M.G. and C. Guillouf, unpublished data*). However, the molecular mechanisms that engender these effects of Spi-1/PU.1 are still subject to speculation. Some information stems from the role of PU.1 in normal hematopoiesis. Various targeted disruption models have shown that PU.1 supports hematopoiesis at different stages. PU.1 is a crucial player in controlling B lymphoid and macrophage development[46-50] and its down-regulation is required for normal erythroid as well as T lineage development.[51,52] In addition, PU.1 plays an early role in regulating the commitment of multipotent hematopoietic progenitors.[53,54] These functions of PU.1 are determined by distinct threshold levels, high levels driving precursors to a myeloid cell fate and moderate levels specifying B lymphoid development.[55]

Aberrations in PU.1 levels are leukemogenic. The first evidence of this was that overexpression of Spi-1 plays an oncogenic role in Friend erythroleukemia.[4] Recently, other murine models demonstrated that a reduction in the level of Spi-1/PU.1 can initiate transformation in other hematopoietic lineages. The knock-down of Spi-1/PU.1 by ablation of its distal regulatory enhancer[32] in adult mice generates animals in which PU.1 is expressed at 20% of the normal level. These mice ultimately develop myeloid and lymphoid leukemias. Myeloid leukemia are typically characterized by a maturation block in myeloid precursor cells indicating that the

reduced PU.1 level was sufficient to support the survival of myeloid progenitor cells but not to sustain their differentiation, possibly because of deregulation of the expression of some cytokine receptors.[56] These mice also develop B-cell proliferative syndromes as a consequence of the reduced PU.1 level in B progenitor cells and T lymphoma as a consequence of the maintainance of PU.1 expression in T precursor cells.[57] PU.1 was identified to have a role in the development of murine myeloid leukemia induced by radiation in two complementary studies reporting that one allelic deletion of PU.1 is associated with a specific, presumably hypomorphic, mutation in the remaining allele.[58,59] Recent studies have also shown that inactivation of PU.1 in adult mice can lead to the development of myeloid leukemia.[60] Thus, the modulation of Spi-1/PU.1 activity, i.e. an increase or a decrease, would be variations of a common mechanism contributing to the pathogenesis of leukemia. Spi-1/PU.1 overexpression in the proerythroblast probably changes the delicate balance of transcriptional activities required for normal erythropoiesis. An excess of Spi-1 may disrupt the function of an erythroid factor and, in this hypothesis, GATA-1 is a likely candidate. Several studies have reported that a direct interaction of Spi-1/PU.1 with GATA-1 may lead to the inhibition of their respective functions.[61-64] The concept that Spi-1 could inhibit the function of GATA-1 in erythroleukemic cells is supported by the reversal of tumorigenicity and reinitiation of a differentiation program when GATA-1 expression is ectopically imposed in a Friend tumor cell line.[65] Nevertheless, GATA-1 target genes, such as EKLF, NF-E2, β globin, EpoR and GATA-1 itself are expressed in both Friend and *spi-1* transgenic cells[43] (*our unpublished data*). Moreover, it is difficult to reconcile the inhibition of GATA-1 functions in leukemic cells with its activities during normal erythroid differentiation in which GATA-1 protects against apoptosis and controls cell proliferation.[66] Little is known about the downstream Spi-1 targets in erythroleukemic cells. As a first element, transcription of the *fli-1* gene, which encodes an ETS family transcription factor, is directly regulated by Spi-1 in Friend cells.[67] The finding that Fli-1 is involved in the blockage of erythroid differentiation during late erythroleukemia induced by the Friend MuLV[4] is compatible with the role of Spi-1 in differentiation arrest. Indeed, future studies using global transcriptome analysis of the preleukemic *spi-1* transgenic proerythroblasts may offer a valuable approach to elucidate the Spi-1/PU.1-induced modifications in gene expression programs leading to erythroleukemogenesis.

Transforming events

Inactivation of p53 by mutations is frequently observed in malignant *spi-1* transgenic proerythroblasts and disease progression on a p53-null background is greatly accelerated.[68] Nevertheless, proerythroblasts that remain Epo-dependent and non-tumorigenic can be isolated from diseased p53$^{-/-}$-*spi-1* transgenic mice indicating that p53 extinction is not sufficient to confer malignancy in this context.[69] Thus, a p53 abnormality may be a permissive event supporting the illegitimate survival of proerythroblasts harboring genetic aberrations, as reported in Friend erythroleukemia.

More specific with regards to the erythroleukemic process in *spi-1*-transgenic mice are mutations in the *Kit* gene, which we recently identified in 86% of tumors isolated late during the progression of leukemia.[45] Kit is the tyrosine kinase receptor for SCF. It is expressed in hematopoietic stem cells (HSC) and committed progenitor cells of different blood cell lineages and is activated by SCF binding.[70] The Kit mutations detected in *spi-1* transgenic leukemic cells affect amino acids located in the Kit catalytic domain (mainly codon 814 and occasionally codon 818) and confer ligand-independent tyrosine kinase activity to Kit. Accordingly, expression of mutated forms of Kit in preleukemic *spi-1* transgenic proerythroblasts render them growth factor-independent and tumorigenic. This phenotype is related to the constitutive activation of the ERK MAP kinase and PI3Kinase/AKT pathways.[71] This mouse model demonstrates that the combined effect of the *Spi-1* mutation, targeting differentiation, and the Kit mutation, targeting intracellular signaling, leads to the development of the malignant leukemic phase (Figure 1).

The target cell for the malignant transformation during murine erythroleukemia

The clonal origin of the malignant cells in Friend virus-infected mice and in *spi-1* transgenic mice provides evidence for a specific change associated with the blastic crisis.[68,72] The precise nature of the cell in which the leukemogenic event(s) occurs is yet to be defined. The restriction of the blast cell populations to the erythroid lineage suggests that the leukemic clone could emerge from the CFU-E pool. Alternatively, the genetic change leading to self-renewal and proliferation of the proerythroblast might occur in a primitive multipotent stem cell and its effects may only occur in a more mature downstream progenitor. The specificity for SFFV integration upstream of the *spi-1* gene during Friend disease is informative. The murine leukemia viruses show a preference for insertion in regions surrounding the trancriptional start site of actively expressed genes.[73] In most Friend tumors, SFFV is integrated in a region 15 to 25 kbp upstream of the *spi-1* transcription start site corresponding to the distal transcriptional promoter,[3,32] inducing the maintenance of *spi-1* transcription in downstream erythroid progenitors. The transcriptional profile of *spi-1* in myelopoiesis predicts that the chromatin structure of the distal *spi-1* promoter is in an open conformation in immature and com-

mitted multipotent progenitors. In contrast, during erythroid committment, *spi-1* transcription is shut off implying that chromatin is in a repressive structure. Thus, the selection pressure for *spi-1* insertional mutagenesis argues in favor of a primitive cell as the target cell for leukemic transformation.

In the model of *spi-1* transgenic leukemia, the high incidence of Kit mutations suggests that the association of Kit mutations with Spi-1 overexpression reflects cell-type specific constraints to clonal selection. SCF, together with Epo supports the proliferation and differentiation of normal erythoid progenitor cells and SCF is also effective in stimulating the proliferation of multipotent progenitor cells. If the presumption is that oncogenic mutations will target a gene in a cell type for which development depends on its normal activity, mutations in either the Epo/EpoR or the SCF/Kit pathways could be expected. No mutation in EpoR was identified in *spi-1* transgenic proerythroblasts. Alternatively, autocrine or paracrine stimulation of EpoR may play a role in leukemic cells as reported in other murine erythroleukemias induced by F-MuLV[74,75] and in rare human erythroleukemias.[76] Regarding *spi-1* transgenic tumors, 7% were autocrine for Epo. However, half of them harbored Kit mutations indicating that Epo autocriny is not a major selective process. According to its function in primitive progenitors, the finding that Kit mutations are preferentially selected might be seen as an indication that mutation occurs in immature progenitor cells. Strikingly, the clonal selection in this model did not involve mutations in other tyrosine kinases, such as FLT3, also expressed in multipotential hematopoietic progenitors and more frequently detected in human AML (*see below*). This suggests that Kit mutations certainly occur in a narrow window that excludes selection pressure on FLT3, possibly in a cell in which FLT3 expression is shut off whereas Kit expression is present. In this context, a BFU-E would appear to be a better potential candidate. Thus, these models show that immature progenitor cell compartments are most likely targeted by oncogenic events even if the differentiation blockage is lineage- and stage-specific.

What lessons can be learnt from the murine leukemia models of AML ?

Clinically, human AML are characterized by an accumulation of immature blasts which exhibit uncontrolled proliferation and failure to differentiate normally. The phenotypes of AML are heterogenous and are classified into sub-types according to the predominance of a particular myeloid lineage. Molecularly, AML are associated with multiple and various genetic alterations, including chromosomal translocations and mutations. Some genetic alterations may serve as fingerprints for a particular leukemia sub-type such as the chromosomal translocation t(9;22) encoding the tyrosine kinase BCR-ABL in chronic myeloid leukemia (CML) (present in up to 98% of patients),[77,78] the mutation in the pseudo-kinase domain of JAK2 in myeloproliferative disorders (present in 80% of polycythemia vera patients)[79-83] and the translocation t(15;17) encoding the promyelocytic leukemia-retinoic acid receptor α (PML-RARα) found in 99% of cases of acute promyelocytic leukemia (APL).[84,85] Studies on the genetic alterations found in AML have been the subject of several recent reviews and will not be described here.[86-90] One important observation is that the genetic alterations can be divided into two classes. One class includes transcription factors that play a regulatory role in hematopoietic development.[91] These most frequently result from chromosomal translocations and the resulting chimeric proteins inhibit differentiation in a particular hematopoietic cell lineage, specifying the leukemia sub-type. For instance, the two AML1 and CBF, sub-units of the heterodimeric core-binding factor (CBF) are altered by translocation t(8;21) or inversion inv(16) in 20-25% of cases of AML. They are expressed as AML1-ETO[88,92] and CBFβ-MYH11[93] chimeric proteins, both acting as dominant negative inhibitors for CBF functions. PML/RARα, which is expressed in APL, behaves as a transcriptional repressor for the hormonal receptor RARα.[85] Albeit more rarely, transcription factors can also be altered by small or point mutations in their coding region as detected in AML1[94] and in the granulocyte transcription factor C/EBPα.[95,96]

PU.1 mutations are rare in human AML, as shown by screening studies in different groups, in which PU.1 mutations were found in 1.5% of cases.[97-99] PU.1 mutations are heterozygous and missense. They render the mutant protein defective in its transcriptional regulator functions, predicting that the oncogenic mechanism underlying PU.1 mutation would be a dosage effect. When not mutated, Spi-1/PU.1 function can also be disrupted downstream of oncogene signaling pathways as for AML-ETO.[100]

In the second class, mutations affect proteins that act in intracellular signaling and that are involved in the control of cell survival and proliferation.[89] Point mutations frequently target tyrosine kinases including the growth factor tyrosine kinase receptors FLT3 (in approximately 40% of AML),[86,101-103] Kit (in 5% of AML)[104,105] and Ras signaling proteins affecting mainly N-Ras in 30% of cases.[106] Kinases are also altered through chromosomal translocations leading to expression of chimeric proteins with constitutive kinase activity, such as BCR/ABL.[78,107] These are gain of function that provide proliferative signals in AML.

Extensive studies of large cohorts of patients with AML provide evidence that the combinations of genetic changes from the two classes are heterogenous but mutations within a class are mutually exclusive. For example, the initial chronic phase of CML is associated

with the BCR-ABL translocation. The translocation products AML1/EVI1,[108] NUP98/HOXA9[109] or TLS-ERG[110] are additional genetic alterations in the clonal evolution of CML and all of them encode chimeric proteins with transcription factors properties. Conversely, 25%-30% of AML patients with AML1 translocations carry Kit mutations[104,105,111,112] and 38% of APL patients with the PML/RARα translocation bear FLT3 mutations.[103]

The concept emerging from this complexity is that an acute leukemia would arise from co-operation between a mutation interfering with differentiation and a mutation conferring a proliferative advantage.[113] This concept is also supported by murine models designed to display an oncogenic event, using retrovirally-transduced bone marrow cell transplantation or transgenesis. All cases indicate that neither fusion genes nor mutated kinases are able to induce acute leukemia on their own. Generally, they provoke a myeloproliferative disorder in which the progression to leukemia requires either a long latency, making the occurrence of a second mutation plausible, or additional treatment with mutagenic compounds. Functional evidence for oncogenic co-operation in the development of AML can be obtained from the co-expression of pairs of mutations such as AML1-ETO and FLT3 mutant,[114] BCR-ABL and AML1-EVI1[115] or PML-RARα and FLT3 mutant[116] in primary hematopoietic cell transplantation models .

Both the Friend and *spi-1* transgenic models demonstrate that leukemia development depends on the co-operation between a mutation that impairs differentiation and blocks maturation and a mutation that promotes autonomous cell growth (Figure 1). In this way, the murine models provide direct evidence for the *two-hit* model of leukemogenesis hypothesized from the heterogeneity of AML in humans.[113] A glimmer of this concept is naturally illustrated by retroviruses in birds. The avian erythroblastosis virus (AEV) transduces the oncogenic tyrosine kinase v-erbB together with the aberrant nuclear transcription factor v-erbA. v-erbA is a mutated thyroid hormone receptor α that is no longer responsive to thyroid hormone and causes an arrest of erythroid differentiation at the BFU-E/CFU-E stage. v-erbB encodes a mutated epidermal growth factor receptor that induces extensive erythroblast self-renewal.[117] The murine erythroleukemia models highlight that it is the complementation between the two classes of mutations rather than the order of their occurence that is important for leukemogenesis.

The specificity of the association of Spi-1 mutation with either gp55-dependent EpoR activation or Kit activation in these leukemia models suggests that there is an obligatory relationship between these alterations. In addition, the nature of the mutation might also be specific since all mutations affect the phosphotransferase domain of Kit, exclusive of other activating mutations known to target the juxtamembrane domain of Kit.[118] Such a specificity may be determined by the cellular lineage that will give rise to leukemia and the requirement for signaling pathways downstream of Kit mutants. A possible specificity for associations between alterations in transcription factors and mutations in tyrosine kinases is apparent in human AML. For example, mutations in the phosphotransferase domain of Kit are associated with CBF leukemias involving inv(16) or t(8;21) chromosomal translocations whereas internal tandem duplications (ITD) affecting the juxtamembrane domain of FLT-3 are found in APL with PML-RARα translocations. This raises the question of identifying the mechanism by which oncogenic kinases and transcription factors co-operate in leukemic self-renewal. Tyrosine kinases and transcription factors could act independently, co-regulating complementary sets of key genes required for self-renewal. Alternatively, the activity of transcription factors could be regulated by kinases that belong to common cell signaling pathways. The hypothesis of co-operating mutations that would target genes participating in the development of the same lineage may give some hints for identifying the second mutation in cases of AML in which only one genetic change is known.

Conclusions

Significant advances have been made in understanding the mechanisms of leukemia from studies on murine models of erythroleukemia. At first, these models were fruitful for establishing the multiple-step evolution of leukemia. Twenty-five years later, these models validate the concept of oncogenic co-operativity emerging from the diversity of genetic lesions that underlie the development of AML in human. Unraveling transcriptional regulatory and signaling networks is the major challenge for the future in order to gain better understanding of leukemogenesis. Further clarifications on the molecular pathways downstream of each of the co-operating oncogenic events may lead to the design of new protocols of targeted therapies. There is no doubt that murine models are still promising tools for these prospective studies.

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