Module 1

Biology and Pathophysiology of Chronic Myelogenous Leukemia

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Learning Objectives

After reviewing this module the participant will be better able to:

- Explain the molecular anatomy of the *BCR-ABL* rearrangement associated with the presence of the Philadelphia (Ph) chromosome
- Recognize the signal transduction pathways activated by constitutive BCR-ABL tyrosine kinase activity
- Understand the molecular causes of disease progression resulting from the genetic instability characterizing Ph-positive chronic myelogenous leukemia
- Describe the potential mechanisms responsible for the occurrence of the t(9;22) translocation and of the *BCR-ABL* rearrangement

1.1 Molecular Anatomy of the *BCR-ABL* Rearrangement

Chronic myelogenous leukemia (CML) is a myeloproliferative disease characterized by the presence of a specific cytogenetic abnormality, the Philadelphia (Ph) chromosome, which is the result of a reciprocal translocation, t(9;22)(q34;q11), between the long arms of chromosomes 9 and 22.[1,2] Chronic myelogenous leukemia exhibits a characteristic biphasic clinical course. The initial chronic phase (CP), which can last for several years, originates as an indolent disease, but it is invariably followed by an acute leukemia known as a blast crisis (BC). This is marked by the emergence within the clonal hematopoiesis of fully transformed subclones of either myeloid or lymphoid origin that have been arrested at an early stage of differentiation.[3] The finding of lymphoid as well as myeloid subclones provides evidence that the chromosome translocation occurs in a multipotent stem cell, able to give origin to cells of different hematopoietic lineages.

In the 1980s, it was established that the Ph chromosome results in the juxtaposition of parts of the *BCR* and *ABL* genes. These genes are normally located on chromosomes 22 and 9, respectively. The translocation forms a new hybrid gene, *BCR-ABL,* usually located on the derivative chromosome 22q- (the Ph chromosome).[4,5] A more detailed molecular dissection of the *BCR-ABL* genes present in CML and in other Ph-positive leukemias showed that hybrid genes with different types of junctions between *BCR* and *ABL* may be present, but that all the derived translated proteins share a common functional aspect: constitutive tyrosine kinase (TK) activity.[6] Through experiments with transfection of hematopoietic cells with retroviral vectors carrying *BCR-ABL* constructs in a mouse model, it was demonstrated that the expression of a functional BCR-ABL protein was the primary and necessary factor for leukemic transformation.[7]

The rearrangement between *BCR* and *ABL* shows a certain degree of variability at the molecular level, and the different types of *BCR-ABL* hybrid genes generate different types of fusion transcripts and proteins, which show a preferential but not exclusive association with different leukemia phenotypes.[8] In CML, the breakpoints on

chromosome 22 are predominantly restricted to a central region of the *BCR* gene called "M-*bcr*" (major breakpoint cluster region). Two different types of *BCR-ABL* transcript junction may be present: e13a2 junction (previously b2a2) or e14a2 junction (previously b3a2).[9] These fusion transcripts encode proteins of 210 kd in molecular weight (P210) in almost all cases of CML. In 70% of the Ph-positive acute lymphoid leukemias, a shorter BCR-ABL hybrid protein (P190) is detected.[10] In these cases, the breakpoint is located in the first large intron of the *BCR* gene (the minor breakpoint cluster region, or m-*bcr*). In this rearrangement, the sequences of the first *BCR* exon are joined to *ABL* exon 2 (e1a2 junction).[11] Finally, in a small proportion of patients with CML, a longer type of *BCR-ABL* transcript is found in which the breakpoint takes place at the 3' end of the *BCR* gene (at the micro breakpoint cluster region, or µ-*bcr*) and joins *BCR* exon 19 to *ABL* exon 2 (e19a2). This results in a fusion protein of 230 kd molecular weight (P230).[12] Additional rare types of *BCR-ABL* transcripts have been occasionally identified in patients with CML and patients with Ph-positive leukemia. In summary, whereas there is a clear preferential association between certain types of *BCR-ABL* transcripts and specific leukemia phenotypes, such an association is not absolute. In cases in which the same fusion occurs in different types of leukemia (ie, p210 in CML and ALL or p190 in ALL and CML), other unknown factors, such as different levels of expression, may play a significant role.[13]

1.2 Signal Transduction Pathways Activated by the Constitutive BCR-ABL Tyrosine-Kinase Activity

Normally regulated TK of the ABL protein is constitutively activated by the junction with the N-terminal portion of the *BCR* gene product. The presence of these sequences allows constitutive dimerization of the BCR-ABL protein, thus promoting a trans-phosphorylation process.[14] The initial trans-phosphorylation of a regulatory tyrosine (Tyr) residue at position 1294 in the kinase domain is followed by phosphorylation of another Tyr residue at position 177 of the BCR portion, that becomes a growth factor receptor-bound 2 (Grb2) binding site. Grb2 in turn binds Sos (son of sevenless) and Grb2-associated binding protein 2 (Gab2), and the formation of this complex, strictly dependent on phosphorylation at Tyr 177, leads to activation of Ras and recruitment of Src homology 2 (SHP2) and phosphatidylinositol 3-kinase (PI3K).[15,16]

Multiple signals initiated by BCR-ABL (in particular those transduced through the Ras and PI3K/Akt pathways) have simultaneous proliferative and anti-apoptotic consequences. Both these aspects may contribute to the expansion of a clone in which a proliferative stimulus is not counterbalanced (as generally happens in normal clones) by increased apoptosis. BCR-ABL acts on both fronts concurrently, eventually promoting the expansion of the transformed clone.

One of the critical signaling pathways constitutively activated in CML hematopoiesis is that controlled by Ras proteins and related proteins.[17] The TK activity of P210 maintains Ras p21 in an active state, bound to GTP (guanosine triphosphate). Ras activation results from interaction of P210 with other cytoplasmic proteins, which function as adaptor molecules, to create multiprotein signaling complexes. Finally, BCR-ABL works through Ras p21 to activate Raf1, MAPK kinase (MEK) 1, MEK2, and ERK (extracellular signal-regulated kinase) kinases. This activation elicits early nuclear events, such as transactivation of *Jun*.[18]

Another postulated nuclear "target" of the transforming activity of the P210 protein is the proto-oncogene *Myc,* which is expressed at high levels in CML cells. *Myc* activation, however, seems to be independent from the activation of the Ras pathway and, in fact, *in vitro* complementation studies reveal that the SH2 and the C-terminus regions of ABL are directly involved in the mechanism of *Myc* upregulation.[19]

Signal transducers and activators of transcription (*STAT)1* and *STAT5* are constitutively activated in *BCR-ABL*-positive cell lines and in primary cells from patients with CML.[20] P190 BCR-ABL is also able to activate STAT6, and this may be relevant for the preferential association with the lymphoid phenotype shown by this type of BCR-ABL protein. Demonstration of STAT activation by BCR-ABL led to the intriguing possibility that this could explain the cytokine independence characteristic of the *BCR-ABL* positive cells.

BCR-ABL can inhibit apoptosis at the cytoplasmic level, mainly by activating the PI3K/Akt pathway. Akt kinase is certainly an important effector of BCR-ABL–activated PI3K, and its activation is dependent on the PI3K products phosphatidylinositol 3,4 bisphosphate (PIP_2) and phosphatidylinositol 3,4,5 trisphosphate (PIP_3).[21,22] Once active, Akt exerts many cellular functions through the phosphorylation of downstream substrates such as mammalian target of rapamycin (mTOR), Bcl-2–associated death promoter (Bad), caspase 9, apoptosis signal-regulating kinase 1(Ask1), murine double minute 2 (Mdm2), and the forkhead transcription factor, FKHRL1.[23] The net result of BCR-ABL activation of this pathway is the deregulation of the apoptotic machinery, ultimately leading to a prolonged survival and expansion of the abnormal clone.

Activation of the transcription factor NF-kappaB/Rel by BCR-ABL has been demonstrated, but the mechanisms remain obscure. BCR-ABL induces NFkappaB/Rel nuclear translocation, which is at least partially due to increased IkappaBa degradation.[24] Recently, it has also been reported that the p38 mitogen-activated protein kinase (MAPK) pathway is altered in CML.[25] A hypothetic scenario is that this pathway, normally inhibited by BCR-ABL, could be restored by interferon-alpha, explaining the antiproliferative effect of this drug on CML cells. The precise roles of several other pathways activated by BCR-ABL in CML remain to be defined.

1.3 BCR-ABL and Genetic Instability and Disease Progression

The natural history of CML ends, in the great majority of cases, with an acute leukemia phase that is usually fatal within a few months. Understanding the mechanisms that trigger the transition from the chronic to the acute phase of the disease is of utmost importance, as the timing of this event is the major determinant of patient survival in CML. A number of factors have been implicated as causative agents of acute transformation. When the disease progresses to the acute phase, approximately 80% of patients show additional chromosomal abnormalities in their leukemic cells.[26] Furthermore, a number of molecular changes have been found, including p53 mutations, Rb deletion, and Ras activation.[27] However, only a few specific alterations have been observed so far, such as the deletions of genes like *Ikaros*, *PAX5*, and the *ARF* (also called p16 or CDK4N2) tumor suppressor gene. Recently, these have been found to be deeply implicated in lymphoid transformation.[28-30] On the other hand, it is apparent that important roles in the transition to the acute phase also are played by the duplication of the Ph-positive chromosome and an increase in the expression of the *BCR-ABL*

transcript, either of which have been reported to precede the progression of the disease in some cases.[31]

Overall, the genetic alterations observed suggest that a large spectrum of molecular mechanisms may be involved in clonal progression of CML. Thus, the BCR-ABL–transformed cell seems to be endowed with an intrinsic genetic instability, leading to a progressive accumulation of genetic lesions, which ultimately result in the appearance of an acute leukemia phenotype. From this perspective, the *BCR-ABL* rearrangement appears to be not only the causative agent of the chronic phase of CML, but also the primary mechanism involved in the progression of the disease. At the moment, however, it is not clear whether the genetic instability is caused by BCR-ABL only, or whether the *BCR-ABL* rearrangement can, in some cases, itself be the consequence of a previously existing genetic instability. The finding of several other cytogenetic abnormalities in Ph-negative cells reemerging in patients who achieve complete Ph-negativity after therapy with imatinib may support this second hypothesis.[32,33] However, several works demonstrate that the presence of BCR-ABL significantly enhances the chromosomal instability (aneuploidy, translocations, truncations) induced by reactive oxygen species (ROS) and g-irradiation. This is supported by reports that BCR-ABL–positive cells acquire more double strand DNA breaks (DSBs) than normal counterparts in response to endogenous ROS and genotoxic treatment, and that BCR-ABL stimulates the efficiency but decreases the fidelity of DSB repair mechanisms.[34,35]

1.4 Mechanisms Leading to the t(9;22) Translocation and to *BCR-ABL* Rearrangement

Although we know that leukemogenesis in humans results from a number of recurrent chromosomal translocations, we still lack any substantial information on the mechanisms involved in many of these translocations, including t(9;22), which gives rise to the Ph chromosome. The t(9;22) translocation and *BCR-ABL* fusion is probably a relatively frequent event that only very sporadically leads to the development of a leukemia, as suggested by the finding of small amounts of *BCR-ABL* transcripts in leukocytes from a high percentage of normal adult subjects.[36] At present, only hypothetical mechanisms have been proposed favoring the rearrangement between the *BCR* and *ABL* genes. The close proximity of the *BCR* and *ABL* genes in hematopoietic cells in interphase has been suggested to be a potential mechanism that may favor translocations between the 2 genes.[37] In addition, a 76 kd duplicon (region of chromosome-specific duplications) has been identified recently on chromosome 9 near the *ABL* gene and on chromosome 22 near *BCR*.[38] This duplicon could play a role simply by drawing together the specific chromosomal regions containing the *BCR* and the *ABL* genes and favoring exchanges between them. Sporadically, this exchange could result in the formation of a functional *BCR-ABL* rearrangement, encoding an oncogenic BCR-ABL protein able to confer a growth advantage to the Ph-positive clone and ultimately leading to its expansion.[38]

Furthermore, another important observation made on the high percentage of patients with CML able to reach partial or complete cytogenetic responses (CCR) to imatinib treatment was that karyotypic abnormalities were detectable in the Ph- negative cells of some (5% to 10%) of these patients, and that this phenomenon, in a minority of cases, was also associated with myelodysplastic features.[39] The mechanism behind the formation of cytogenetic abnormalities in Ph-negative cells is unclear. A possible

scenario is that imatinib simply unmasks the presence of clones with Ph-negative cytogenetic abnormalities, and that these may represent the consequence of a diffuse damage to the hematopoietic compartment or the collateral progeny of an abnormal stem cell with a predisposition to acquire additional mutations, including the formation of a Ph chromosome.[39] According to this hypothesis, the t(9;22) translocation is not the primary, but a subsequent event in the pathogenesis of CML. This model for CML development had been proposed several years ago by Fialkow and colleagues.[40,41]

In this case, the genetic instability characterizing the Ph-positive clone and determining blastic transformation of the leukemia could precede the acquisition of the *BCR-ABL* rearrangement. This scenario could explain the differences in the tendency to progress observed in some CML cases.

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