Genomic Instability: The Cause and Effect of BCR/ABL Tyrosine Kinase

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Genes encoding c-ABL kinase and BCR protein are targeted by yet-unknown mechanisms causing DNA double-strand breaks resulting in the generation of a chimeric gene encoding BCR/ABL fusion tyrosine kinase. BCR/ABL kinase displays transforming activity because of its constitutive kinase activity causing deregulated proliferation, apoptosis, differentiation, and adhesion. Moreover, BCR/ABL kinase is able to facilitate DNA repair, prolong activation of G2/M and S cell cycle checkpoints, and elevate expression of the antiapoptotic protein Bcl-X_L, making malignant cells less responsive to antitumor treatment. BCR/ABL may also stimulate generation of reactive oxygen species and enhance spontaneous DNA damage in tumor cells. Unfortunately, BCR/ABL kinase compromises the fidelity of DNA repair mechanisms, thus contributing to the accumulation of additional genetic abnormalities that lead to resistance to inhibitors such as imatinib mesylate and to malignant progression of the disease. Therefore, chronic myelogenous leukemia cells display mutator phenotype.

Introduction

There are 90 tyrosine kinase (TK) genes known to date [1], encoding a cohort of catalytically active molecules that are tightly regulated by interaction with their ligands and inhibitors and by phosphorylation/dephosphorylation [2]. TK genes are targeted chronically by genetic errors, which lead to the generation of mutated enzymes exerting uncontrolled activity. At least three mechanisms may lead to constitutive activation of the catalytic activity: chromosomal translocation, overexpression, and activating mutations [3]. These changes cause

perturbed signal transduction eventually resulting in neoplastic disease.

BCR/ABL is a result of a t(9;22) reciprocal translocation of a segment of the *c-ABL* gene from chromosome 9 to a portion of the *BCR* gene locus on chromosome 22. It is present in chronic myelogenous leukemia (CML) and in a cohort of patients with acute lymphoblastic leukemia (ALL) [4].

BCR/ABL Formation

Genetic anomalies, resulting from unfaithful repair of elevated levels of DNA double-strand breaks (DSBs) may appear even before cancer is clinically diagnosed [5,6•]. Chromosomal translocations arise as a result of the fusion (misrepair) of two or more broken ends of chromosomes that occur in spatial proximity to one another [7]. Elegant studies by Richardson and Jasin [8] demonstrated that two DNA DSBs, each on different chromosomes, are sufficient to promote frequent reciprocal chromosomal translocations. Reactive oxygen species (ROS), ionizing radiation, genotoxic chemicals, and DNA replication stress are likely factors to consider as a source of DSBs. However, what induces the chromosome strands to finally break and why DNA repair mechanisms act improperly to produce chromosomal translocations is open to conjecture.

The cells of the human body must unremittently counteract constant assault by ROS such as superoxide anions (O₂⁻), hydroxyl radicals (•OH), singlet oxygen (¹O₂), and hydrogen peroxide (H₂O₂). It is estimated that about 104 oxidative DNA lesions occur per human cell per day [9]. ROS can be elevated by various physiologic stimuli such as hematopoietic growth factors. In addition, elevated release of ROS by leukocytes is observed during infections and inflammations, which are implicated in the induction of a quarter of all cancer cases worldwide. Moreover, the activation of cellular stress signals from unfavorable conditions such as aberrant oxygen tension may lead to the release of ROS from the mitochondria.

Unrepaired oxidative damage may result in the formation of DSBs [10]. The role of ROS in the generation of DSBs is supported by several observations: 1) cellular oxygen tension causes chromosome breaks [10]; 2) overexpression of c-Myc elevates ROS-mediated DSBs [11]; 3) treatment with H₂O₂ induces DSBs [12];

4) ROS-dependent DNA single-strand interruptions in replicating chromosomes generate DSBs [13]; and 5) chromosomally unstable cells display persistent oxidative stress [14]. Moreover, translocation breakpoints are GC-rich [15] and oxidative damage is predominantly detected in GC-rich sequences [16]. Thus, it is tempting to speculate that clustered oxidative damage creates multiple DNA lesions in a short DNA fragment, which pose a serious obstacle for DNA repair machinery, resulting in a DSB.

DNA replication stress also can induce DSBs independently of ROS [5]. Common chromatin structures at the breakpoint regions may lead to DSBs and chromosomal translocations [17]. Chromatin structural elements, including topoisomerase II (topo II), DNase I cleavage sites, and scaffold-associated regions (SARs), closely associate with BCR and ABL breakpoints. Topo II and scaffold protein II are essential for chromosome condensation. For example, topo II is a key protein with enzymatic and structural functions, responding to torsional stress of DNA. Therefore, the aberrant activity of these elements may result in DSBs.

Moreover, there is strong evidence of radiation-induced risks for CML. Experimental data, as well as cancer incidence records of atomic bomb survivors, indicated that *BCR/ABL* translocation might occur after irradiation [18].

Specific recombination mechanisms responsible for generation of the t(9;22) chromosomal translocation are currently unknown. Localization of DSBs may be important for the formation of chromosomal translocations. It has been estimated that approximately 6% to 13% of genomic DNA consists of repetitive *Alu* sequences. DNA sequences of surrounding breakpoint regions involved in the formation of *BCR/ABL* often revealed homology to *Alu* [19]. However, *Alu* sequences are not always close to the sites of recombination, so their direct role in chromosomal translocations is not clear. *Alu* elements may work as structural modifiers of chromatin organization, because translin-binding sites were identified within the *Alu* consensus [19]. Translin protein binds chromosomal breakpoint sequences and may promote illegitimate conjunctions to favor chromosomal translocations.

Mammalian cells have multiple pathways to repair DSBs that represent a "clear and present danger" to survival and genomic integrity: homologous recombination repair (HRR), nonhomologous end-joining (NHEJ), and single-strand annealing (SSA) [20]. Experimental data implicate NHEJ, involving small deletions and microhomology, in the formation of chromosomal translocations [21••]. In accordance, microhomology at the *BCR/ABL* junction region has been detected [22]. HRR does not appear to be involved in the overwhelming majority of cancer-associated translocations, as breakpoint junctions lack extensive homology, and translocations involving this pathway are not recovered in model systems [23••]. However, another homology-driven pathway, SSA, efficiently generates translocations in experimental models [23••].

Consequences of BCR/ABL Kinase Expression

BCR/ABL kinase exhibits two complementary roles in cancer. The first role is stimulation of signaling pathways that contribute to malignant transformation, including proliferation in the absence of growth factors, protection from apoptosis in the absence of external survival factors, and invasion [24]. The second role of *BCR/ABL* kinase in hematologic malignancies is the modulation of responses to DNA damage, rendering cells resistant to genotoxic therapies and causing genetic instability [25].

Drug resistance

BCR/ABL-transformed cells demonstrate an enhanced ability to survive genotoxic stress, probably because of enhanced DNA repair, inhibition of proapoptotic pathways, and prolonged S and G2/M checkpoints for extended repair [25,26]. These three factors may work in concert to provide the necessary protection from DNA damage-induced apoptosis in tumor cells transformed not only by *BCR/ABL* but also by other oncogenic kinases [27]. Leukemia cells surviving genotoxic attack may harbor DNA aberrations due to the unfaithful DNA repair, resulting in genomic instability [28,29••,30].

Drug resistance depends on the high expression levels of *BCR/ABL* suggesting amplification or hyperactivation of signaling pathways, DNA repair mechanisms relevant for response to genotoxic stress, or both [31]. Because drug resistance depends on *BCR/ABL* kinase activity, it is most likely not due to clonal evolution arising from accumulation of additional genomic aberrations. The expression/activity of *BCR/ABL* kinase may increase in the accelerated phase and blast crisis; therefore, it seems reasonable to speculate that at the later stages of the disease, high levels of *BCR/ABL* kinase trigger more pronounced resistance. Thus, if selection is involved in the development of resistance to apoptosis induced in CML cells by DNA damage, then it should be associated with the emergence of clones displaying elevated *BCR/ABL* kinase expression or activity. However, as CML progresses, cells can also accumulate additional genetic abnormalities, which further increase the resistance of these cells to genotoxic treatment; this effect may no longer depend on *BCR/ABL* catalytic activity.

DNA repair

DNA lesions emerge in various forms and require different repair mechanisms (Table 1). Damaged bases, mismatches, and adducts are repaired by base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR). *BCR/ABL*-positive myeloid leukemia cells exhibited elevated NER activity in response to UV radiation [32].

DSBs can be induced by genotoxic drugs, replication forks encountering lesions, or irradiation and are lethal if unrepaired. Three distinct mechanisms repair DSBs: HRR, NHEJ, and SSA [20]. HRR and NHEJ can play a pivotal role in genotoxic drug resistance. HRR involves

Table 1. Genotoxic agents, the DNA lesions they induce, and the DNA repair mechanisms required

Inducing agent	DNA lesions	DNA repair
Replication errors	Mismatch, insertion, deletion	MMR
X-rays, oxygen radicals, alkylating agents	Abasic site, 8-oxoG, single-strand break	BER
Ultraviolet light	Photoproduct, pyrimidine dimer	NER
X-rays, cytostatic agents, replication forks encountering lesions	Interstrand cross-link, double-strand break	HRR, NHEJ, SSA
BER—base excision repair; HRR—homologous recombination repair; MMR—mismatch repair; NER—nucleotide excision repair; NHEJ—nonhomologous end-joining; SSA—single-strand annealing.		

RAD51-driven invasion and pairing of a single-stranded DNA to the homologous template. Elevated levels of RAD51 have been observed in various tumors and were positively associated with genotoxic-therapy resistance [33]. Leukemias and lymphomas expressing BCR/ABL-related fusion tyrosine kinases (FTKs), such as TEL/ABL, TEL/PDGFR, TEL/JAK2, and NPM/ALK display elevated levels of RAD51 and enhanced HRR activity [30,34,35]. Although Deutsch et al. [36] indicated that DNA-PKcs, an important kinase in NHEJ, may be down-regulated in CML cells, subsequent reports employing various *in vitro* and *in vivo* methods detected enhanced activity of NHEJ in BCR/ABL-transformed leukemia cells and CML patient cells [29••,30,37,38].

Inhibition of apoptosis

In addition to modulation of DNA repair mechanisms, BCR/ABL also activates another line of defense against genotoxicity. BCR/ABL kinase may stimulate anti-apoptotic proteins such as BCL2 and BCL-X_L and inhibit pro-apoptotic proteins BAD and BAX, thus preventing the release of cytochrome *c* from the mitochondria and activation of caspase-3 [27].

Checkpoint activation

The time allotted for repair is as crucial as the repair mechanisms themselves. Cell cycle checkpoints are responsible for the mediation of phase transitions, activation of repair mechanisms, and the movement of DNA-repair proteins to lesion sites. Studies from our laboratory and others have demonstrated that cells transformed by BCR/ABL exhibit extended activation of the S and G2/M cell cycle phase checkpoints when exposed to chemotherapeutic drugs and γ -radiation [26,34,39]. We have recently reported that BCR/ABL leukemia cells might display enhanced stimulation of the ATR-Chk1 axis, which plays an important role in the activation of intra-S-phase checkpoint and drug resistance [26]. This observation is in accordance with another report indicating elevated activation of Chk1 kinase in BCR/ABL cells after γ -irradiation [40]. However, using tetracycline-induced BCR/ABL expression, Dierov et al. [41] reported inhibition of ATR kinase, abrogation of Chk1 phosphorylation, and disruption of the intra-S-phase checkpoint resulting

from translocation of BCR/ABL kinase to the nucleus in response to genotoxic treatment. We did not observe nuclear relocation of BCR/ABL kinase in response to DNA damage caused by various drugs and irradiation [26]. The reason for this discrepancy is not known but the effect described by Dierov et al. [41] may depend on the BCR/ABL-inducible expression system.

Facilitation of genomic instability

It is well known that cancers exhibit chromosomal abnormalities such as translocations, deletions, insertions, and amplifications. Such chromosomal anomalies have been observed at various stages of tumor progression, particularly in the later phases such as CML blast crisis [42]. Genomic instability in BCR/ABL-induced leukemias is manifested by the accumulation of chromosomal aberrations and mutations leading to malignant progression of the disease and acquired resistance to small-molecule inhibitors such as imatinib mesylate (Gleevec®, Novartis Pharmaceuticals, East Hanover, NJ) [28,43•].

Genetic aberrations leading to malignant progression of the disease

CML cells accumulate genetic abnormalities during the course of the disease. The aberrations associated with the progression of BCR/ABL-positive CML chronic phase to the aggressive blast crisis include additional chromosomes (+Ph, +8, +19); isochromosome 17q (associated with the loss of p53); reciprocal translocations (3;21, associated with the expression of AML-1/EBV1, and 7;11, associated with the expression of NUP98/HOXA9 fusion proteins); other translocations and inversions associated with acute myelogenous leukemia and myelodysplasia, including inv(3) and t(15;17); loss of heterozygosity at 14q32; homozygous mutations/deletions of pRBB and p16/ARF; and mutations in p53 and RAS [43•].

Mutations usually result from enhanced DNA damage, deregulated mechanisms of DNA repair, or both. Reports from our laboratory and others have shown that BCR/ABL leukemia cells display higher levels of spontaneous DNA damage, such as oxidized bases and DSBs caused by the elevation of ROS [29••,37]. In addition, they acquire more DNA lesions after genotoxic treatment [26]. As indicated earlier, BCR/ABL may modulate DNA repair

Figure 1. Mechanisms of genomic instability dependent on reactive oxygen species (ROS) in BCR/ABL-positive leukemia cells. Signals from ligand (L)-activated growth factor receptor (R) and other extracellular and intracellular sources stimulate the production of ROS, which cause oxidative DNA damage. Base excision repair (BER), nucleotide excision repair (NER), and mismatch repair (MMR) are involved in the repair of oxidative DNA lesions and prevention of the formation of double-strand breaks (DSBs). DSBs can result from incomplete repair of two oxidative lesions located on different strands at a distance of seven or fewer base pairs, or may occur if the replication fork encounters a nicked oxidative lesion. In leukemia cells, BCR/ABL enhances the production of ROS, resulting in elevated numbers of oxidative DNA lesions, including DSBs. Overexpression of DNA pol β can cause base misincorporation during BER and/or homologous recombination repair (HRR), resulting in point mutations. In addition, large deletions are generated during nonhomologous end-joining (NHEJ).

by HRR, NHEJ, and NER, which, in combination with cell cycle arrest and protection from cytochrome *c*-dependent apoptosis, eventually promotes survival [25]. Unfortunately, the repair mechanisms are unfaithful. For example, BCR/ABL stimulates HRR and NHEJ, but large deletions and numerous point mutations, respectively, were found in the repair products [29••,30,38]. Reasons for repair infidelity in BCR/ABL-positive cells are currently unknown.

RAD51 plays a fundamental function in HRR, which is generally an accurate process that repairs DSBs without intermediary deletions, insertions, or chromosomal arrangements [44]. However, increased RAD51 expression not only enhances HRR efficiency to cause drug resistance, but also promotes crossing over, involving gene conversion associated with an exchange of flanking markers leading to chromosomal translocations [45••]. Increased RAD51 also promotes aneuploidy and multiple chromosomal rearrangements. In addition, error-prone DNA polymerases, such as polymerase β , which exhibits elevated expression in BCR/ABL cells [46], may eventually replace other polymerases and introduce mismatches during HRR. Interestingly, base misincorporations made during DSB repair in *Saccharomyces cerevisiae* were not substrates for the MMR machinery [47]. Therefore, mismatched bases incorporated into HRR sites might not be removed efficiently, causing mutations in the recombination products. In conclusion, these data provide a link between elevated RAD51 protein levels, genomic instability, and tumor progression.

The molecular explanation for extensive degradation of DSBs preceding NHEJ in BCR/ABL cells is unknown.

Aberrantly regulated exonucleases like ExoI, Mre11, and Artemis could be suggested as potential mediators of this effect [48]. Moreover, NER in p210 BCR/ABL-positive cells was associated with mutations in the *HPRT* gene. Altogether, these observations implicate an excess of DNA lesions and faulty DNA repair mechanisms in malignant progression of CML (Fig. 1).

Mutations in BCR/ABL leading to resistance to kinase inhibitors

Imatinib mesylate (IM), a selective inhibitor of the ABL kinase activity, revolutionized the treatment of CML [49]. However, clinical and experimental observations reveal that resistance to the drug has become a rising problem [28]. Resistance may be achieved by enhanced expression of the kinase, blocking of the drug, reduction of cellular concentration of the drug, or mutations in the *BCR/ABL* gene. *BCR/ABL* gene mutations appear to play a major role in IM resistance of leukemias positive for the Philadelphia chromosome. Novel inhibitors are being generated to overcome IM resistance, but mutations causing resistance against new drugs are likely to emerge. The mechanisms causing mutations are poorly characterized.

Our recent studies implicate ROS-induced oxidative DNA damage as a source of mutations in the *BCR/ABL* kinase domain encoding IM resistance [50••]. Interestingly, a GC-rich region is present in the *BCR/ABL* kinase and is a “hot spot” for ROS-induced DSBs. Moreover, we showed that point mutations might be introduced during HRR and NER in BCR/ABL-

positive cells [29••,30,32]. Therefore, clustered oxidative damage in a GC-rich region may cause a DSB in the sequence encoding BCR/ABL kinase, whereas DNA polymerase-mediated mispairing opposite to an oxidized base during HRR-dependent DNA replication may be responsible for the mutagenic effect. We hypothesize that ROS work in concert with unfaithful DNA repair mechanisms to introduce mutations into the sequence encoding BCR/ABL kinase. Although ROS appear to play a major role in BCR/ABL-mediated mutagenesis, other factors may be important, too. For example, DNA polymerase β is overexpressed in BCR/ABL cells, which may diminish the fidelity of BER and HRR [46]. Altogether, we postulate that excessive oxidative DNA damage and unfaithful DNA repair mechanisms contribute to point mutations leading to resistance to IM and other small-molecule inhibitors.

In conclusion, we postulate that reciprocal chromosomal translocations carrying the *BCR/ABL* gene result from unfaithful repair of DNA DSBs. BCR/ABL kinase, the product of a hybrid gene, is able to initiate malignant transformation and further accelerate genomic instability, leading to malignant progression of the disease, and can induce resistance to genotoxic treatment and kinase-targeted small-molecule inhibitors.

Conclusions

The t(9;22) reciprocal chromosomal translocation results from faulty repair of DNA DSBs, so it could be postulated that appearance of the translocation is a consequence of a pre-existing condition of genomic instability. However, the presence of BCR/ABL kinase is associated with elevation of DNA damage induced by ROS or genotoxic agents. Leukemia cells are protected from apoptosis by BCR/ABL kinase and are capable of repairing more DNA lesions, but the repair is usually unfaithful. Therefore, BCR/ABL-positive cells, which survive genotoxic stress, may carry additional genetic errors leading to resistance to IM and malignant progression of the disease. CML cells thus display mutator phenotype.

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