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BCR/ABL promotes accumulation of chromosomal aberrations induced by oxidative and genotoxic stress

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BCR/ABL-induced chronic myeloid leukemia (CML) is a stem cell-induced but progenitor cell-driven disease, which usually starts as a relatively benign chronic phase (CML-CP), eventually progressing to a fatal blast crisis (CML-BC).[1] The frequency of additional chromosomal abnormalities is approximately 7% in CML-CP and increases to 40–70% in the advanced phases.[2] In addition, chromosomal aberrations have also been found in leukemia cells resistant to dasatinib and/or imatinib, and in patients relapsing after hematopoietic transplantation.[3,4]

The most frequently noticed chromosomal errors involve numerical gains and losses of chromosomes, isochromosome i(17q) causing loss of p53, reciprocal translocations 3;21 and 7;11 generating AML-1/Evi-1 and NUP98/HOXA9 fusion proteins, respectively, and other translocations and inversions associated with AML/myelodysplasia, such as inv(3) and t(15;17).[5] Clinical and experimental observations strongly suggest that these aberrations may contribute to malignant progression/relapse of the disease.[2,6-10]

BCR/ABL kinase results from unfaithful repair of two DNA double-strand breaks (DSBs) generating t(9;22), which may represent a random event or result from preexisting conditions associated with genomic instability.[5,11] Therefore, additional chromosomal aberrations accumulated during the course of CML may be promoted by preexisting condition responsible for t(9;22) and/or by BCR/ABL. The latter statement is supported by reports that BCR/ABL-positive cells acquire more DSBs than normal counterparts in response to endogenous reactive oxygen species (ROS) and genotoxic treatment[12-15] and that BCR/ABL stimulates the efficiency but decreases the fidelity of DSB repair mechanisms.[13,16,17] However, the former speculation cannot be ruled out because chromosome abnormalities were detected in t(9;22)-negative metaphases appearing during imatinib therapy in patients with newly diagnosed CML-CP.[18,19]

To test the hypothesis that BCR/ABL facilitates the accumulation of ROS-induced chromosomal aberrations, freshly established 32Dcl3-BCR/ABL and parental 32Dcl3-*neo* cells were continuously cultured *in vitro* for 8 weeks in the presence or absence of an antioxidant, vitamin E (VE) as described before,[20] and then analyzed by SKY (spectral karyotype analysis) to detect acquired chromosomal aberrations. Although parental cells did not contain consistent chromosomal aberrations, one or two additional chromosomes 12 (+12, +12×2) were detected in all metaphases of 32Dcl3-BCR/ABL cells maintained in

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Note added in proof

Dr Martin Carroll (University of Pennsylvania, Philadelphia, PA, USA) submitted the manuscript indicating that BCR/ABL induces chromosomal aberrations after genotoxic stress.

regular medium (Table 1, VE *in vitro*, P-C and B/A-C, respectively). Inhibition of ROS by VE was associated with approximately twofold reduction of the number of cells displaying +12 (Table 1, VE *in vitro*, B/A-VE, respectively). In addition, one or two der(X)t(X;8) chromosomal translocations (Figure 1a) were present in 9 of 10 metaphases of BCR/ABL cells, but the translocation was not detected if ROS was inhibited by VE. It is likely that BCR/ABL-positive cells containing +12×2 and der(X)t(X;8)×2 represent a clone derived from the +12, der(X)t(X;8) cell because they display 82, XY karyotype.

SCID mice were injected with freshly established 32Dcl3-BCR/ABL cells and fed with control or VE-rich diet to reduce ROS.[20] All analyzed metaphases of 32Dcl3-BCR/ABL leukemia cells obtained from mice fed with control chow contained an additional chromosome 12 and one or more chromosomal translocation, such as der(X)t(X;8), der(14)t(8;14), der(X)t(X;14) and der(X)t(X;9) (Table 1, VE *in vivo*, B/A). However, leukemia cells from the mice fed with VE-rich diet displayed no more than one of the chromosomal translocations, such as der(X)t(X;8), der(14)t(8;14) or der(12)t(12;16), and 75% of metaphases displayed +12 (Table 1, VE *in vivo*, B/A-VE). Metaphases containing some of the described aberrations are presented in Figures 1b and c. The relatively weaker antimutagenic effect of VE *in vivo* than *in vitro* may be due to less efficient inhibition of ROS in mice in comparison to tissue culture.

To test whether BCR/ABL promotes chromosomal aberrations after genotoxic treatment cells were allowed to recover after irradiation to detect errors which do not exert a negative impact on cell survival/proliferation as described by Libura *et al*.[21] These aberrations may exert an effect on the disease path.

32Dcl3-*neo* cells did not display any consistent chromosomal abnormalities (Table 1, γ-irradiation, P). In sharp contrast, all metaphases of 32Dcl3-BCR/ABL cells contained close to 80 chromosomes and carried der(4)t(4;12) chromosomal translocation and metacentric chromosome 17, which is a fusion of the centromeres of two chromosome 17s (Table 1, γ-irradiation, B/A, Figure 1d). Moreover, two additional translocations der(12)t(5;12) and der(9)t(9;13) were detected in individual metaphases.

In addition, BCR/ABL-transformed murine bone marrow cells (bmc-B/A) surviving irradiation acquired numerous chromosomal defects (Table 1, γ-irradiation). Basically, del(6) is consistent in all 15 metaphases, but it does not appear to result from preexisting aberration because C67Bl/6 mice display normal karyotype. t(4;X) and der(6)t(X;6) is present in 10 of 15 and 2 of 15 metaphases, respectively. Other aberrations listed in Table 1 and shown in Figures 1e and f are detected in only one cell each representing individual breaks and exchanges due to irradiation. Cells expressing empty plasmid did not resume enough proliferation potential during the experiment to allow SKY analysis. This effect is in concordance with the report that normal murine hematopoietic stem cells are very sensitive to irradiation in comparison with BCR/ABL-positive counterparts.[22]

The experimental conditions applied here reflected two clinical situations where chromosomal instability is acquired spontaneously during the disease course (ROS-induced) and after high-dose chemo/radiotherapy treatment before hematopoietic transplantation (irradiation-induced) to address the dilemma whether the accumulation of additional chromosomal aberrations in CML is driven by BCR/ABL and/or by already preexisting mechanism(s) responsible for t(9;22). Our work demonstrated that the presence of BCR/ABL significantly enhanced chromosomal instability (aneuploidy, translocations, truncations) induced by ROS and γ-irradiation; however, we cannot exclude the possibility that preexisting conditions, which generate t(9;22), may work in concert with BCR/ABL to destabilize chromosomes. In addition, although viral sequences were integrated in both

parental and BCR/ABL-positive cells, the possibility that viral integration combined with BCR/ABL kinase may exert some mutagenic effect in leukemia cells cannot be eliminated.

BCR/ABL-dependent mechanisms facilitating chromosomal aberrations are not known. BCR/ABL-positive cells in comparison with normal counterparts contain more DSBs induced by ROS and γ-irradiation, and demonstrate increased capability to survive DNA damage.[12,13,22] In addition, leukemia cells have enhanced probability to accumulate chromosomal errors due to a negative effect of BCR/ABL kinase on the fidelity of DSBs repair.[5] The potential role for unfaithful DSB repair mechanisms can be suggested, because BCR/ABL kinase enhances the expression of RAD51 and downregulates DNA-PKcs, key elements in two major mechanisms of DSBs repair, homologous recombination repair and non-homologous end-joining, respectively.[17,23] Interestingly, overexpression of RAD51 and the inhibition of DNA-PKcs promoted aneuploidy and multiple chromosomal rearrangements.[24,25] The negative effect of BCR/ABL kinase on the fidelity of DSB repair should be further elucidated to attenuate malignant progression and relapse of CML.

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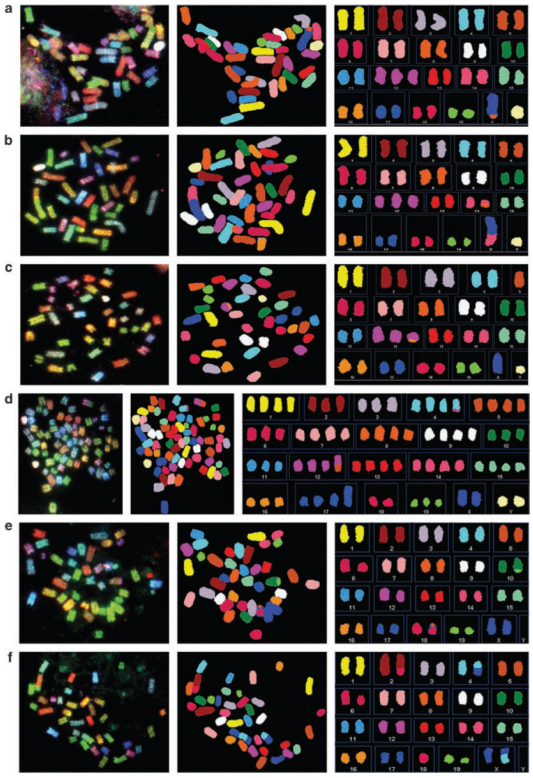

Figure 1.

Representative examples of SKY analyses of chromosomal aberrations described in Table 1: VE *in vitro* (**a**), VE *in vivo* (**b**, **c**) and γ-irradiation (B/A in **d** and bmc-B/A in **e**, **f**). Metaphases with spectral colors (left panel), metaphases with classified colors (middle panel), and metaphase panels (right panel) are shown. Chromosomal instability is demonstrated by the karyotype as follows: (**a**) 41,Y,der(X)t(X;8),+12; (**b**) 41,Y,der(X)t(X;14),der(14)t(8;14),+12; (**c**) 40,XY,der(12)t(12;16),-5,+12; (**d**) 71, XXYY, der(4)t(4;12), met(17)t(17;17), der(12)t(5;12); (**e**) 40, XX, del(6), der(10)t(10;19); and (**f**) 39, XX, del(6), t(X;4), dic (2;18).

Table 1

BCR/ABL promotes accumulation of ROS and radiation-induced chromosomal aberrations *in vitro* and *in vivo*

Conditions	Cells (metaphases analyzed)	Additional (+) and missing (−) chromosomes	T and T	T and T per metaphase	Aberrations per metaphase
VE *in vitro*	P-C (10)	0	0	0	0
	B/A-C (10)	+12 [7], +12 × 2 [3]	der(X)t(X;8) [6], der(X)t(X;8)x2 [3]	1.2±0.6*	2.5±1.1**
	B/A-VE (13)	+12 [7]	0	0	0.5±0.5
VE *in vivo*	B/A-C (13)	+12 [13]	der(X)t(X;8) [6], der(14)t(8;14) [6], der(X)t(X;14), [6], der(X)t(X;9) [1]	1.5±0.5*	2.5±0.5**
	B/A-VE (12)	+12 [8], −5 [1],−Y [1]	der(X)t(X;8) [8], der(14)t(8;14) [1], der(12)t(12;16) [3]	1.0±0.0	1.8±0.6
γ-irradiation	P(10)	0	0	0	0
	B/A (9)	71–78, <4N>XXYY [9]	der(4)t(4;12) [9], met(17)t(17;17) [9], der(12)t(5;12) [1], der(9)t(9;13) [1]	2.2±0.4*	N.C.
	bmc-B/A (15)	−18 [2], −2 [1], −4 [1], −11 [1], −13 [1], −X [1]	del(6) [15], t(X;4) [10], der(6)t(X;6) [2], dic(2;7) [1], +der(4)t(4;11) [1], der(4)ins(4;11) [1], dic(2:18) [1], dic(5:18) [1], der(X)t(X;1) [1], +der(1)t(1;8) [1], der(3)t(3;13) [1], der(8)t(8;14) [1], der(14)t(3;14) [1], der(10)t(10;19) [1], del(11) [1], del(2) [1]	2.8±1.1	3.3±1.9

Abbreviations: IL-3, interleukin-3; NC, not calculated; ROS, reactive oxygen species; SCF, stem cell factor; T and T, translocations and truncations; VE, vitamin E.

Metaphase spreads were analyzed by the Columbia University SKY core facility using the Applied Spectral Imaging software. Results indicate consistent chromosomal aberrations detected by SKY (number of metaphases displaying the chromosomal aberrations), and the statistical significance was probed by unpaired Student's *t*-test.

VE *in vitro*: freshly established mixtures of 32Dcl3 clones expressing p210BCR/ABL (B/A) or empty plasmid (P) were obtained as described before (no chromosomal aberrations detected)[20] and maintained in the continuous culture for 8 weeks in the absence (C) or presence of 200 μM VE (VE). VE decreased the level of ROS and did not affect cell proliferation rate in tissue culture.[20]

VE *in vivo*: BCR/ABL-positive cells were harvested from leukemic SCID mice after 8 weeks of feeding with regular (B/A-C) or VE-rich (B/A-VE) chow to reduce ROS as described before.[20]

γ-Irradiation: 32Dcl3-*neo* (P) and 32Dcl3-BCR/ABL (B/A) cells were described above. Bone marrow mononuclear cells from C57Bl/6 female mice (The Jackson Laboratory, Bar Harbor, ME, USA) were infected with BCR/ABL-IRES-GFP retroviral particles as described.[17]. GFP+ BCR/ABL-positive cells obtained by sorting were expanded for 2 weeks in the presence of pre-tested minimal concentrations of SCF+IL-3 required to maintain proliferation (bmc-B/A). Cells were irradiated from ^{137}Cs source with 2, 3, and 4Gy in a weekly intervals to allow recovery of proliferation.[13] One week after the last irradiation cells were analyzed by SKY.

*$P<10^{-6}$ in comparison with P-C and B/A-VE group

**$P<10^{-6}$ and $P<10^{-5}$ in comparison with P-C and B/A-VE groups, respectively.

*$P = 0.005$

**$P = 0.009$ in comparison with corresponding VE groups.

*$P<10^{-6}$ in comparison to P group.