TEL-fusion oncogenic tyrosine kinases determine leukemic cells response to idarubicin

Ireneusz Majsterek[a], Artur Slupianek[b] and Janusz Blasiak[a]

The family of BCR/ABL-related fusion tyrosine kinases (FTKs) is reported to participate in drug resistance in leukemogenesis. Our recent studies revealed a novel potential mechanism of resistance in *FTK*$^+$ cells underlined by the stimulation of DNA repair. In this work we examined a role of TEL family fusion oncoproteins in the response to idarubicin. We used murine pro-B lymphoid cell line BaF3, and its *TEL/ABL*, *TEL/JAK2* and *TEL/PDGFβR*-transformed clones. The transformed cells, in contrast to their non-transformed counterparts, exhibited resistance to idarubicin in the range 0.01–1 μM. The drug at 0.3 and 1 μM induced DNA damage in the form of strand breaks or/and alkali-labile sites in both transformed and control cells as evaluated by the alkaline Comet assay. The transformed cells removed the damage within 60 min, while the control cells required 120 min to recover. The results obtained suggest that TEL-related FTKs may stimulate the repair of DNA damaged by idarubicin and be relevant to the resistance of the leukemic cells to this drug. *Anti-Cancer Drugs* 14:625–631 © 2003 Lippincott Williams & Wilkins.

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Introduction

Several fusion tyrosine kinases (FTKs) have been detected in human hematopoietic malignancies, one of the most extensively studied being BCR/ABL, considered as the pathogenic principle of Philadelphia (Ph) chromosome-positive human leukemias [1]. The BCR/ABL fusion generated by a t(9;22) translocation mediates its biological effects through deregulated, constitutively active tyrosine kinase activity [2]. BCR/ABL was found to stimulate multiple signaling pathways responsible for the protection from apoptosis, induction of growth factor-independent proliferation, transformation, and also for the resistance to therapeutic drugs and γ-radiation [3–11].

Translocated Ets Leukemia (TEL) protein is a novel member of the ETS family of transcription factors whose genes are frequently rearranged in human leukemias [12,13]. Its N-terminal region has been shown to undergo translocation to the catalytic domain of three tyrosine kinases, c-ABL [14], PDGFβR [15,16] and JAK2 [17–19], which results in constitutive activation of each kinase. TEL/JAK2 fusion protein was characterized as a product of t(9;12) translocation which was found in acute lymphocytic leukemias (ALL) [12,13,20]. TEL/ABL resulted from t(9;12) translocation and consists of the N-terminal fragment of TEL domain fused in-frame with exon 2 of cytoplasmic kinase c-ABL [14]. TEL/ABL was detected in ALL, acute myeloid leukemias and atypical chronic myeloid leukemias (CML) [20]. TEL/PDGFβR is associated with t(5;12) translocations which juxtapose the N-terminal region of TEL with the transmembrane and tyrosine kinase domains of the platelet-derived growth factor receptor β (PDGFβR) [15]. TEL/PDGFβR was found in chronic myelomonocytic leukemia [15,20]. Since normal ligand-induced activation of PDGFβR depends upon ligand-induced dimerization [21], it has been suggested that the TEL-derived sequence might contribute to the oncogenic properties of TEL/PDGFβR by favoring constitutive dimerization and activation of the protein kinase activity of the PDGFβR moiety of the fusion protein [15]. Likewise, TEL-induced oligomerization might activate the protein kinase activity and transforming properties of TEL/ABL in a way similar to the BCR-induced oligomerization of the BCR/ABL fusion protein of Philadelphia-positive human leukemias [22,23].

Drug resistance in tumor cells, which can be considered as one of the main obstacles in cancer therapy, can be mediated by several different mechanisms including: (i) overstatement of P-glycoprotein family of membrane transporters, which decrease the intracellular accumulation of the drugs, (ii) changes in the statement of cellular proteins involved in detoxification or activation of the chemotherapeutic drugs, (iii) increase of the level of anti-

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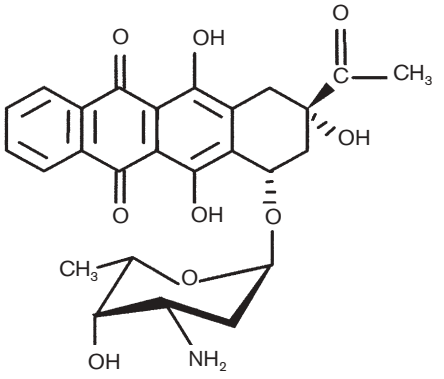
Fig. 1



Structure of idarubicin.

apoptotic proteins, (iv) transient G_2/M arrest, and (v) increased efficiency of DNA repair [24–27]. BCR/ABL and other oncogenic tyrosine kinases, such as v-SRC and HER-2/*neu*, can induce resistance to DNA-damaging drugs, but the exact mechanism(s) underlying this feature is unclear [28–30]. Although inhibition of apoptotic pathways and activation of the G_2/M cell cycle checkpoint seem to play an important role in survival of BCR/ABL-positive leukemia cells after DNA damage [31–34], our findings indicated that the repair of drug-induced DNA lesions may also be essential for drug resistance.

Idarubicin (4-demethoxy-daunorubicin, Fig. 1) is a member of the anthracycline antibiotics group, whose anti-proliferation activity originates from the ability to diffuse across the cell membrane, and to intercalate between DNA base pairs and target topoisomerase II [35]. Idarubicin shows features rendering this drug unique among anthracyclines. The higher lipophilicity leads to faster accumulation in the nuclei, superior DNA-binding capacity and consequently greater cytotoxicity compared to daunorubicin. Idarubicin also has, at least in part, an ability to overcome multidrug resistance [36].

In the present work we investigated the ability of the murine pro-B lymphoid cell line BaF3 and its *TEL/ABL*, *TEL/JAK2* and *TEL/PDGFβR* transformants to survive and repair DNA after treatment with idarubicin.

Materials and methods

Chemicals

Idarubicin was obtained from Pharmacia & Upjohn (Milan, Italy). Tris, RPMI 1640 medium, agarose, low-melting-point agarose, phosphate-buffered saline (PBS), DAPI (4′,6-diamidino-2-phenylindole), fetal bovine serum (FBS) and MTT [3-(4,5-dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide] were obtained from Sigma (St Louis, MO), WEHI 3B was obtained from Dr Jovani Rovera (Wistar Institute, University of Pennsylvania, PA).

Cells

Murine growth factor-dependent pro-B lymphoid BaF3 cells expressing TEL/JAK2 or TEL/ABL or TEL/PDGFβR were obtained from Dr Tomasz Skorski (Temple University, Philadelphia, PA). Cell lines were maintained in RPMI 1640 supplemented with 10% FBS and 15% WEHI-conditioned medium (the growth medium). The viability of cells was measured by Trypan blue exclusion and was about 99%. The final concentration of the cells was adjusted to $1–3 \times 10^5$ cells/ml by adding the growth medium to the single-cell suspension.

MTT assay

Idarubicin at final concentrations 0.01–1 μM was added to the cells (1.5×10^6/ml) in the growth medium. Four days later the viability of cells was evaluated by the MTT assay [36]. Briefly, cells were plated onto 96-well plates in 200 μl of growth medium and 20 μl of 10 mg/ml MTT was added to each well. After incubation at 37°C for 4 h, the supernatant was removed and 200 μl of a solution containing 10% SDS and 0.04 N HCl was added to dissolve the water-insoluble formazan salt. One hour later, the difference $OD_{650\ nm} - OD_{570\ nm}$ was measured with an ELISA reader (Bio-Rad, Hercules, CA). Drug resistance is presented as percentage of viable cells in suspension culture exposed to idarubicin in comparison to the untreated control group.

DNA repair

To examine DNA repair, BaF3 and BaF3-transformed by *TEL*-fused FTKs cells (1.5×10^6/ml) were treated with idarubicin at 0.3 and 1 μM, washed and re-suspended in fresh, drug-free growth medium. Aliquots of cell suspensions were harvested immediately (time 0), and after 30, 60 and 120 min, and placed on ice to stop the repair reactions. Cells exposed to 10 μM hydrogen peroxide for 5 min at 4°C served as positive control.

Comet assay

The Comet assay was performed under alkaline conditions essentially according to the procedure of Singh *et al.* [37] with some modifications [38]. A freshly prepared cell suspensions in 0.75% low-melting-point agarose dissolved in PBS was placed onto microscope slides pre-coated with 0.5% normal melting agarose. The cells were then lysed for 1 h at 4°C in a buffer consisting of 2.5 M NaCl, 100 mM EDTA, 1% Triton X-100, 10 mM Tris, pH 10. After the lysis, the slides were placed in an electrophoresis unit, DNA was allowed to unwind for 40 min in an electrophoretic solution consisting of 300 mM NaOH, 1 mM EDTA, pH > 13. Electrophoresis was conducted at 4°C (the temperature of the running

buffer not exceeding 12°C) for 30 min at an electric field strength of 0.73 V/cm (30 mA). The slides were then neutralized with 0.4 M Tris, pH 7.5, stained with 2 μg/ml DAPI and covered with cover slips. To prevent additional DNA damage all steps were conducted under a dimmed light or in the dark.

Comet analysis

The slides were examined at × 200 magnification in a Eclipse fluorescence microscope (Nikon, Japan) attached to a COHU 4910 video camera (Cohu, San Diego, CA) equipped with a UV filter block containing an excitation filter (359 nm) and barrier filter (461 nm), and connected to a personal computer-based image analysis system, Lucia-Comet version 4.51 (Laboratory Imaging, Prague, Czech Republic). Fifty images were randomly selected from each sample and the Comet tail moment (a product of fraction of DNA in tail and tail length) was measured. Two parallel tests with aliquots of the same sample of cells were performed for a total of 100 cells and the mean Comet tail moment was calculated. The Comet tail moment is positively correlated with the level of DNA breakage in a cell [39]. A mean value of tail moment in particular sample was taken as an indicator of DNA damage in this sample.

Statistical analysis

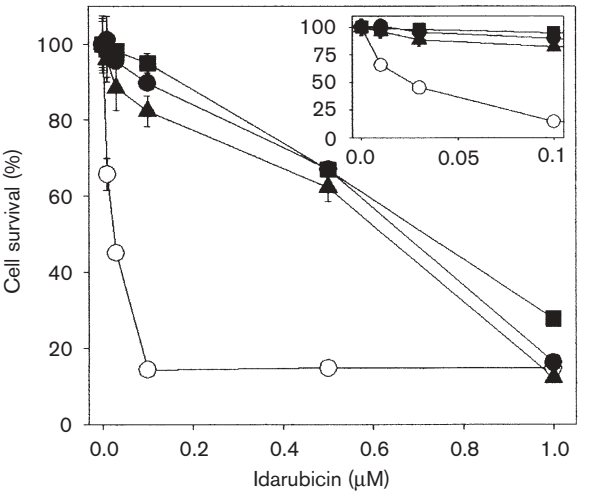
All the values in this study were expressed as mean ± SEM from two separate experiments. If no significant differences between variations were found, as assessed by the Snedecor–Fisher test, the differences between means were evaluated by applying Student's *t*-test. Otherwise, the Cochran–Cox test was used. The date was analyzed using the STATISTICA (StatSoft, Tulsa, OK) statistical package.

Results

MTT assay

The results of MTT assay of the survival of BaF3 cells and their *TEL*-fusion-transformed counterparts after treatment with idarubicin at different concentrations are displayed in Figure 2. MTT assay revealed that the *TEL*-transformed cells displayed therapeutic drug resistance to idarubicin. *TEL*-transformed cells survived better after treatment with idarubicin at the concentration range of 0.01–1 μM in comparison to control BaF3 cells. Idarubicin from the concentration range 0.01–0.1 μM (Fig. 2, inset) effected the viability decrease of *TEL*-transformed cells only to 20% ($p < 0.001$), but over 80% of control BaF3 cells were dead after idarubicin treatment ($p < 0.001$). The higher survival of *TEL/ABL*- or *TEL/PDGFβR*-transformed cells than control Baf3 cells was observed in the concentration range of idarubicin up to 0.5 μM ($p < 0.001$), *TEL/JAK2*-transformed cells had the higher cell viability even at 1 μM idarubicin ($p < 0.001$).

Fig. 2



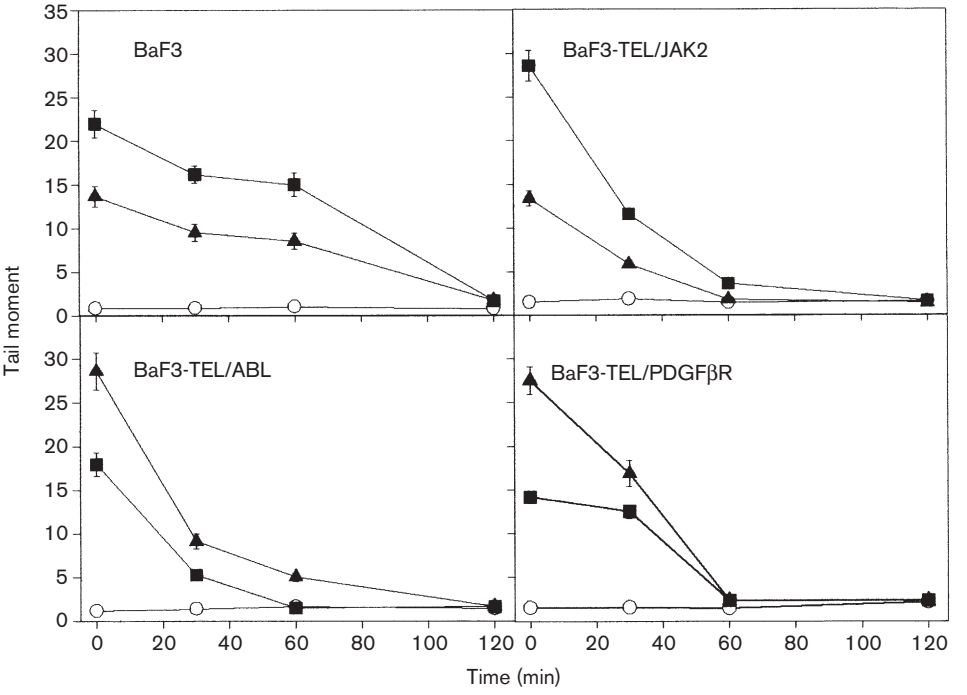
The survival of murine growth factor-dependent pro-B lymphoid cells BaF3 (○) and BaF3 cells transformed by (●) *TEL/ABL*, (■) *TEL/JAK2* and (▲)*TEL/PDGFβR* in the presence of idarubicin as evaluated by the MTT assay. The cell viability after idarubicin treatment in the concentration range 0.01–0.1 μM is displayed in the inset. Results represent three independent experiments; error bars denote SEM.

DNA repair

Figure 3 shows the Comet tail moment of BaF3 and BaF3-transformed cells exposed to idarubicin and analyzed immediately, as well as 30, 60 and 120 min thereafter. The BaF3 cells transformed by *TEL/PDGFβR* or *TEL/JAK2* for idarubicin at 0.3 and 1 μM as well as BaF3-*TEL/ABL* cells for idarubicin at 0.3 μM repaired DNA damage in 60 min, while BaF3 control cells needed an additional 60 min to recover. The Comet tail moments of the untreated cells were constant, indicating that preparation and subsequent processing of the cells did not introduce significant damage to their DNA. Cells exposed for 5 min at 0°C to 10 μM hydrogen peroxide (positive control) repaired DNA damage within 60 min of incubation (data not shown).

The distribution of cells exposed to idarubicin according to their tail moment is displayed in Figure 4. The cells of the control group (no idarubicin) had a Comet tail moment smaller than 10. Idarubicin treatment resulted in a similar distribution profile of BaF3 control and transformed cells. A gradual decrease of the fraction of the cells with a Comet tail moment greater than 10 was observed during the repair incubation time. After 60 mins of incubation BaF3 cells transformed by *TEL/PDGFβR* or *TEL/JAK2* for idarubicin at 0.3 and 1 μM had Comet tail moments smaller than 10. The BaF3 cells transformed by *TEL/ABL* for idarubicin at 0.3 μM had also tail moments smaller than 10 and for idarubicin at 1 μM only 4% of the total BaF3-*TEL/ABL* cells fraction had tail moments

Fig. 3



Time course of the repair of DNA damage in murine growth factor-dependent pro-B lymphoid cells BaF3, and BaF3 cells transformed by *TEL/ABL*, *TEL/JAK2* and *TEL/PDGFβR* treated with idarubicin at 0.3 (■) and 1 μM (▲) compared with untreated control (○). One hundred cells were analyzed per each point. Results represent three independent experiments; error bars denote SEM.

higher than 10, but control cells required 120 min to repair DNA damage to this level.

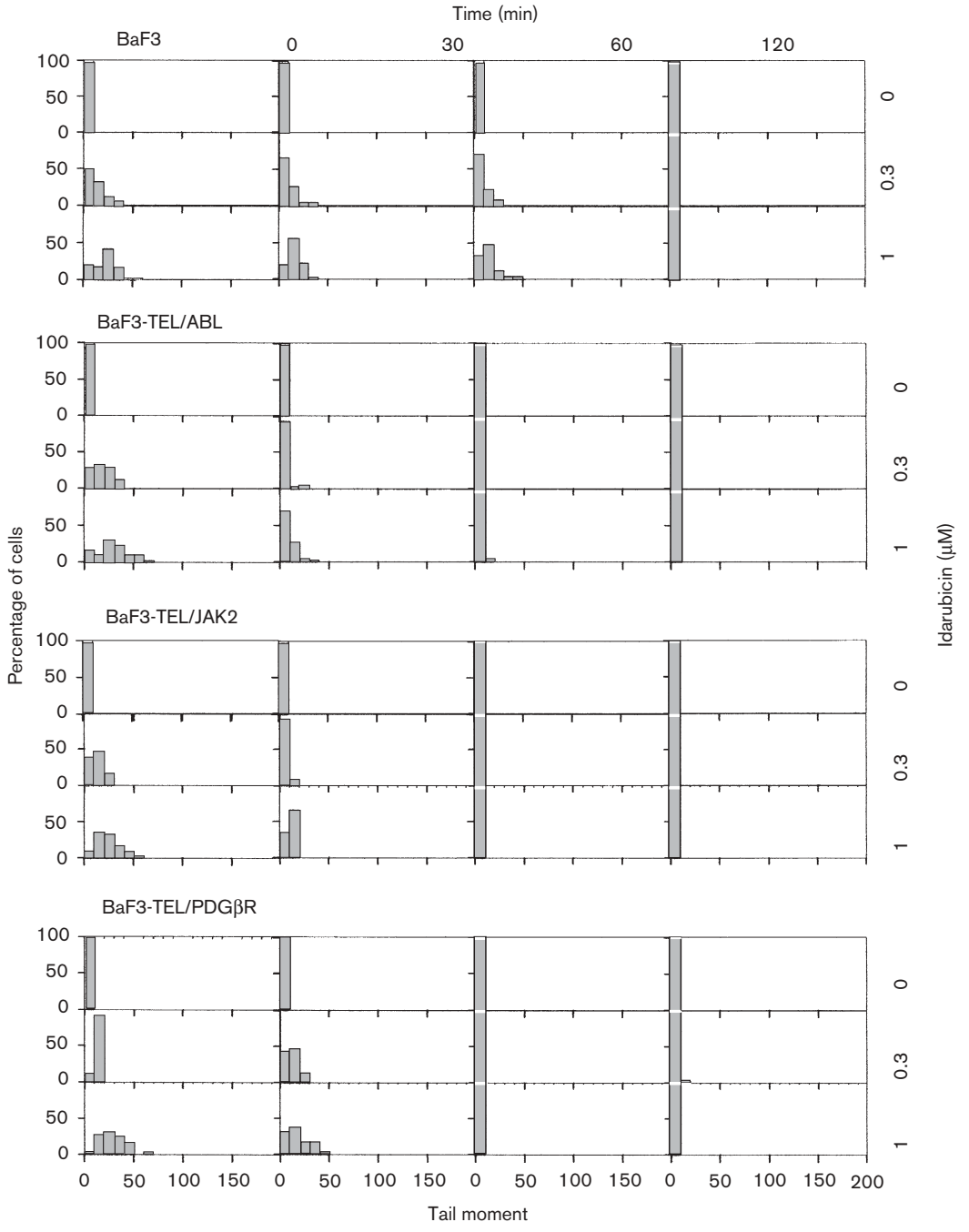
Discussion

Members of the BCR/ABL-related fusion tyrosine kinases family may induce therapeutic drug resistance. Studies of cellular resistance to DNA-damaging agents induced by BCR/ABL oncogenic tyrosine kinase implicated the elevation of Bcl-x_L and pronounced activation of the G_2/M checkpoint [31–34]. Recent studies revealed a novel mechanism of resistance in BCR/ABL-positive cells: stimulation of DNA double-strand break repair by homologous recombination [40,41]. This phenomenon is dependent on *BCR/ABL*-induced elevation of the level of RAD51, the mammalian homolog of *Escherichia coli* RecA protein, which plays an essential role in homologous recombination [40]. RAD51 in conjunction with Bcl-x_L increase and G_2/M arrest seems to be responsible for the drug resistance in *BCR/ABL*-positive cells [41]. In our previous work we demonstrated for the first time that deregulation of DNA repair mechanisms could be involved in drug resistance in BCR/ABL-positive leukemias [42,43]. The present work extends this observation by the demonstration that *TEL* FTKs accelerate repair DNA damage caused by idarubicin.

Idarubicin is a very effective drug in the treatment of hematological malignancies. The main mechanism underlying its cytostatic effect is topoisomerase II poisoning [44]. Moreover, anthracycline drugs have the ability to generate reactive oxygen species that arise during their metabolism [45]. Because of the structural alternations of DNA caused by idarubicin, the drug may activate base and nucleotide excision repair as well as homologous recombination repair (HRR) and/or non-homologous end-joining (NHEJ) repair pathways [44]. Therefore, the exact mode of the involvement of DNA repair in the resistance of the cells transformed by FTKs to idarubicin is not known. Our studies showed that idarubicin-induced DNA lesions detectable by the Comet assay were removed more efficiently in the presence of *TEL*-FTKs. Moreover, the increase in effectiveness of DNA repair was positively correlated with resistance to idarubicin. This phenomenon supported the hypothesis that the increase in the effectiveness of DNA repair in *BCR/ABL*-related FTK-positive cells might be involved in the resistance to idarubicin.

We demonstrated that the base of the molecular mechanisms of therapeutic drug resistance stimulated by *TEL* FTKs might be cellular response to DNA damage. Based on our previous findings implicating

Fig. 4



Histograms of the distribution of Comet tail moments in murine growth factor-dependent pro-B lymphoid BaF3 cells, and BaF3 cells transformed by *TEL/ABL*, *TEL/JAK2* and *TEL/PDGFβR* treated with 0.3 and 1 μM idarubicin for 1 h at 37°C and incubated for repair. One hundred cells were analyzed per each point.

RAD51 in drug resistance of BCR/ABL cells we can speculate that HRR may play a major role in accelerated removal of idarubicin-induced double-strand breaks in BaF3 cells transformed by FTKs. It seems unlikely that NHEJ repair is a significant factor in this process because BCR/ABL down-regulates DNA-PKcs [46], playing an essential role in NHEJ [47].

Usually drug resistance arises as a result of selection of tumor cell clones, which are able to develop protective

mechanisms and to survive genotoxic treatment [24–27]. However, malignancies induced by oncogenic tyrosine kinases such as BCR/ABL, v-SRC and HER2/*neu* display early drug resistance [28,29]. The phenomenon of early resistance to radiation and cytostatic drugs is observed in the untreated chronic phase of CML [48], thus investigation of mechanism(s) underlying this feature seems to be of a special importance. Altogether, our previous and present findings indicate that accelerated repair of drug-induced DNA lesions represents an important mechanism in drug resistance of FTK-positive leukemias. Further studies are required to validate the therapeutic potential of this observation.

Acknowledgments

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References

1 Lugo TG, Pendergast AM, Muller AJ, Witte ON. Tyrosine kinase activity and transformation potency of *bcr–abl* oncogene products. *Science* 1990; **247**:1079–1082.
2 Gotoh A, Broxmeyer HE. The function of BCR/ABL and related proto-oncogenes. *Curr Opin Hematol* 1997; **4**:3–11.
3 Nowell P, Hungerford D. A minute chromosome in human chromic granulocytic leukemia. *Science* 1960; **132**:1497.
4 Zou X, Calame K. Signaling pathways activated by oncogenic forms of Abl tyrosine kinase. *J Biol Chem* 1999; **274**:18141–18144.
5 Raitano AB, Whang YE, Sawyers CL. Signal transduction by wild-type and leukemogenic Abl proteins. *Biochim Biophys Acta* 1997; **1333**:201–216.
6 Sattler M, Salgia R. Activation of hematopoietic growth factor signal transduction pathways by the human oncogene BCR/ABL. *Cytokine Growth Factor Rev* 1997; **8**:63.
7 Cotter TG. BCR–ABL: an anti-apoptosis gene in chronic myelogenous leukemia. *Leuk Lymphoma* 1995; **18**:231–236.
8 Wang JY. Regulation of cell death by the Abl tyrosine kinase. *Oncogene* 2000; **19**:5643–5650.
9 Daley GQ, Baltimore D. Transformation of an interleukin 3-dependent hematopoietic cell line by the chronic myelogenous leukemia-specific P210bcr/abl protein. *Proc Natl Acad Sci USA* 1988; **85**:9312–9316.
10 Sirard C, Laneuville P, Dick JE. Statement of *bcr–abl* abrogates factor-dependent growth of human hematopoietic M07E cells by an autocrine mechanism. *Blood* 1994; **83**:1575–1585.
11 Skorski T. BCR/ABL regulates response to DNA damage: the role in resistance to genotoxic treatment and in genomic instability. *Oncogene* 2002; **21**:8591–8604.
12 Lacronique V, Boureux A, Valle VD, Poirel H, Quang CT, Mauchauffe M, *et al.* A TEL–JAK2 fusion protein with constitutive kinase activity in human leukemia. *Science* 1997; **278**:1309–1312.
13 Peeters P, Raynaud SD, Cools J, Wlodarska I, Grosgeorge J, Philip P, *et al.* Fusion of TEL, the ETS-variant gene 6 (ETV6), to the receptor-associated kinase JAK2 as a result of t(9;12) in a lymphoid and t(9;15;12) in a myeloid leukemia. *Blood* 1997; **90**:2535–2540.
14 Golub TR, Goga A, Barker GF, Afar DE, McLaughlin J, Bohlander SK, *et al.* Oligomerization of the ABL tyrosine kinase by the Ets protein TEL in human leukemia. *Mol Cell Biol* 1996; **16**:4107–4116.
15 Golub TR, Barker GF, Lovett M, Gilliland DG. Fusion of PDGF receptor to a novel *ets*-like gene, *tel*, in chronic myelomonocytic leukemia with t(5;12) chromosomal translocation. *Cell* 1994; **77**:307–316.
16 Jousset C, Carron C, Boureux A, Quang CT, Oury C, Dusanter-Fourt I, *et al.* A domain of TEL conserved in a subset of ETS proteins defines a specific oligomerization interface essential to the mitogenic properties of the TEL–PDGFR oncoprotein. *EMBO J* 1997; **16**:69–82.
17 Ho JM-Y, Beattie BK, Squire JA, Frank DA, Barber DL. Fusion of the *ets* transcription factor TEL to Jak2 results in constitutive Jak–Stat signaling. *Blood* 1999; **93**:4354–4364.
18 Schwaller J, Frantsve J, Aster J, Williams IR, Tomasson MH, Ross TS, *et al.* Transformation of hematopoietic cell lines to growth-factor independence and induction of a fatal myelo- and lymphoproliferative disease in mice by retrovirally transduced *TEL/JAK2* fusion genes. *EMBO J* 1998; **17**:5321–5333.
19 Lacronique V, Boureux A, Monni R, Dumon S, Mauchauffe M, Mayeux P, *et al.* Transforming properties of chimeric TEL–JAK proteins in Ba/F3 cells. *Blood* 2000; **95**:2076–2083.
20 Kolibaba KS, Druker BJ. Protein tyrosine kinases and cancer. *Biochim Biophys Acta* 1997; **1333**:217–248.
21 Heldin CH. Dimerization of cell surface receptors in signal transduction. *Cell* 1995; **80**:213–223.
22 Papadopoulos P, Ridge SA, Boucher CA, Stocking C, Wiedemann LM. The novel activation of *ABL* by fusion to an ets-related gene *TEL*. *Cancer Res* 1995; **55**:34–38.
23 McWhirter JR, Galasso DL, Wang JY. A coiled-coil oligomerization domain of Bcr is essential for the transforming function of Bcr–Abl oncoproteins. *Mol Cell Biol* 1993; **13**:7587–7595.
24 Goldie JH. Modelling the process of drug resistance. *Lung Cancer* 1994; **10**(**suppl 1**):91.
25 Hampson R. Selection for genome instability by DNA damage in human cells: unstable microsatellites and their consequences for tumourigenesis. *Radiat Oncol Invest* 1997; **5**:111–114.
26 Harrison DJ. Molecular mechanisms of drug resistance in tumours. *J Pathol* 1995; **175**:7–12.
27 El-Deiry WS. Role of oncogenes in resistance and killing by cancer therapeutic agents. *Curr Opin Oncol* 1997; **9**:79–87.
28 Masumoto N, Nakano S, Fujishima H, Kohno K, Niho Y. v-Src induces cisplatin resistance by increasing the repair of cisplatin–DNA interstrand cross-links in human gallbladder adenocarcinoma cells. *Int J Cancer* 1999; **80**:731.
29 Pietras RJ, Fendly BM, Chazin VR, Pegram MD, Howell SB, Slamon DJ. Antibody to HER-2/*neu* receptor blocks DNA repair after cisplatin in human breast and ovarian cancer cells. *Oncogene* 1994; **9**:1829–1838.
30 Skorski T. Oncogenic tyrosine kinases and the DNA-damage response. *Nat Rev Cancer* 2002; **2**:351–360.
31 Bedi A, Barber JP, Bedi GC, el-Deiry WS, Sidransky D, Vala MS, *et al.* BCR–ABL-mediated inhibition of apoptosis with delay of G_2/M transition after DNA damage: a mechanism of resistance to multiple anticancer agents. *Blood* 1995; **86**:1148–1158.
32 Amarante-Mendes GP, McGahon AJ, Nishioka WK, Afar DE, Witte ON, Green DR. Bcl-2-independent Bcr–Abl-mediated resistance to apoptosis: protection is correlated with up regulation of Bcl-x_L. *Oncogene* 1998; **16**:1383–1390.
33 Nishii K, Kabarowski JH, Gibbons DL, Griffiths SD, Titley I, Wiedemann LM, *et al.* ts BCR–ABL kinase activation confers increased resistance to genotoxic damage via cell cycle block. *Oncogene* 1996; **13**: 2225–2234.
34 Amarante-Mendes GP, Naekyung KC, Liu L, Huang Y, Perkins CL, Green DR, *et al.* Bcr–Abl exerts its antiapoptotic effect against diverse apoptotic stimuli through blockage of mitochondrial release of cytochrome *c* and activation of caspase-3. *Blood* 1998; **91**:1700–1705.
35 Borchmann P, Hubel K, Schnell R, Engert A. Idarubicin: a brief overview on pharmacology and clinical use. *Int J Clin Pharmacol* 1997; **35**: 80–83.
36 Siu WY, Arooz T, Poon RYC. Differential responses of proliferating versus quiescent cells to adriamycin. *Exp Cell Res* 1999; **250**:131–141.
37 Singh NP, McCoy T, Tice RR, Schneider EL. A simple technique for quantitation of low levels of DNA damage in individual cells. *Exp Cell Res* 1988; **175**:184–192.
38 Klaude M, Eriksson S, Nygren J, Ahnstrom G. The comet assay: mechanisms and technical considerations. *Mutat Res* 1996; **12**:89–96.
39 Ashby JA, Tinwell H, Lefevre PA, Browne MA. The single cell gel electrophoresis assay for induced DNA damage (comet assay): measurement of tail length and moment. *Mutagenesis* 1995; **10**: 85–90.
40 Slupianek A, Schmutte C, Tombline G, Nieborowska-Skorska M, Hoser G, Nowicki MO, *et al.* BCR/ABL regulates mammalian RecA homologs, resulting in drug resistance. *Mol Cell* 2001; **8**:795–806.
41 Slupianek A, Hoser G, Majsterek I, Bronisz A, Malecki M, Blasiak J, *et al.* Fusion tyrosine kinases induce drug resistance by stimulation of homology-dependent recombination repair, prolongation of G_2/M phase, and protection from apoptosis. *Mol Cell Biol* 2002; **22**:4189–4201.

42 Majsterek I, Blasiak J, Hoser G, Skorski T. Is *BCR/ABL*-mediated increase in the effectiveness of DNA repair involved in the cancer cells drug resistance? *Cell Biol Int* 2002; **26**:363–370.
43 Blasiak J, Gloc E, Pertynski T, Drzewoski J. DNA damage and repair in BCR/ABL-expressing cells after combined action of idarubicin STI571 and amifostine. *Anticancer Drugs* 2002; **13**: 1055–1060.
44 Binaschi M, Capranico G, Dal Bo L, Zunino F. Relationship between lethal effects and topoisomerase II-mediated double-stranded DNA breaks produced by anthracyclines with different sequence specificity. *Mol Pharmacol* 1997; **51**:1053–1059.
45 Horenstein MS, Vander Heide RS, L'Ecuyer TJ. Molecular basis of anthracycline-induced cardiotoxicity and its prevention. *Mol Genet Metab* 2000; **71**:436–444.
46 Deutsch E, Dugray A, Abdulkarim B, Marangoni E, Maggiorella L, Vaganay S, *et al.* BCR–ABL down-regulates the DNA repair protein DNA-PKcs. *Blood* 2001; **97**:2084–2090.
47 Lees-Miller SP. The DNA-dependent protein kinase, DNA-PK: 10 years and no ends in sight. *Biochem Cell Biol* 1996; **74**:503–512.
48 Chan TA, Hwang PM, Hermeking H, Kinzler KW, Vogelstein B. Cooperative effects of genes controlling the G_2/M checkpoint. *Genes Dev* 2000; **14**:1584–1588.