

Mitochondrial mutagenesis in BCR-ABL1-expressing cells sensitive and resistant to imatinib

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Imatinib revolutionized the treatment of chronic myeloid leukemia (CML) with the expression of the BCR-ABL1 tyrosine kinase, but imatinib resistance is an emerging problem. Imatinib can hinder the inhibitory effects of BCR-ABL1 on mitochondrial apoptotic pathway, so mitochondrial mutagenesis can be important for its action. To explore the mechanisms of imatinib resistance we created a mouse-derived CML model cells consisting of parental 32D cells (P) and cells transfected with the BCR-ABL1 gene (S cells) or its variants with the Y253H or T315I mutations (253 and 315 cells, respectively), conferring resistance to imatinib. A fraction of the S cells was cultured in increasing concentrations of imatinib, acquiring resistance to this drug (AR cells). The 253, 315 and AR cells, in contrast to S cells, displayed resistance to imatinib. We observed that the T315I cells displayed greater extent of H₂O₂-induced mtDNA damage than their imatinib-sensitive counterparts. No difference in the sensitivity to UV radiation was observed among all the cell lines. A decrease in the extent of H₂O₂-induced mtDNA damage was observed during a 120-min repair incubation in all cell lines, but it was significant only in imatinib-sensitive and T315I cells. No difference in the copy number of mtDNA and frequency of the 3,867-bp deletion was observed and genotoxic stress induced by H₂O₂ or UV did not change this relationship. In conclusion, some aspects of mtDNA mutagenesis, including sensitivity to oxidative stress and DNA repair can contribute to imatinib resistance in BCR-ABL1-expressing cells.

Key words: Imatinib, chronic myeloid leukemia, BCR-ABL1 gene

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INTRODUCTION

Imatinib (imatinib mesylate, STI571, Gleevec), a tyrosine kinase inhibitor (TKI), has revolutionized the therapy of chronic myeloid leukemia (CML) and is a paradigm for cancer targeted therapies (Druker 2008). It targets the ATP-binding site in the BCR-ABL1 tyrosine kinase, present in CML cells and responsible for cancer phenotype, inhibiting phosphorylation of its substrates. The main mode of anti-leukemic action of imatinib is inhibition of the pro-survival signaling pathways activated by BCR-ABL1. Although imatinib is not free of unwanted side effects, the main problem with its use is the resist-

ance, which can be primary or secondary. Primary resistance is present at the moment of CML diagnosis and can be augmented by mechanisms, which are poorly known (reviewed in Bixby & Talpaz, 2011). Secondary resistance appears after initial responsiveness to imatinib and can be associated with mutations in the BCR-ABL1 gene. The problem of imatinib resistance has been only partly resolved by second- and third- generations of TKIs and supplementation of imatinib-based therapy with other drugs modulating pro-survival pathways activated by BCR-ABL1 (reviewed in Lipton *et al.*, 2015).

Although BCR-ABL1 confers CML phenotype by inhibiting apoptosis and stimulating cell proliferation, the mechanisms of progression of the disease from long-lasting, often syndrome-free chronic phase (CP) through acceleration phase (AP) to usually fatal blast crisis (BP) are not fully known. The final stage of the disease progression is associated with acquiring TKIs resistance. Therefore, the mechanism of imatinib resistance, or more generally — TKIs resistance, can be a key mechanism of CML relapse and recurrence.

CML cells, like almost all other cancer cells, display genomic instability. We and others showed that this instability is associated with the production of reactive oxygen species (ROS) and unfaithful DNA repair stimulated by BCR-ABL1 (Brady *et al.*, 2011; Griaud *et al.*, 2012; Nieborowska-Skorska *et al.*, 2012, 2013, 2014; Slupianek *et al.*, 2013). Moreover, genomic instability of CML cells increases with the disease progression, so it can be associated with imatinib resistance. We showed that increased ROS production by mutated BCR-ABL1 could be linked with imatinib resistance (Koptyra *et al.*, 2006).

The term genomic instability is usually related to the nuclear genome. However, if the instability of CML cells is associated with an increased ROS production, and we showed that Rac2 GTPase altered the mitochondrial electron transport and generated ROS in both stem and progenitor leukemic cells, then mtDNA can be in front-line of ROS attack and its instability may significantly contribute to genomic instability of CML cells (Nieborowska-Skorska *et al.*, 2012). It is particularly interesting in the context of imatinib resistance as the drug blocks

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Abbreviations: AP, acceleration phase; Bcl-xL, B-cell lymphoma extra large; BER, base excision repair; BCR, the breakpoint cluster region gene; BCR-ABL1, fusion tyrosine kinase; BP, blast phase; c-ABL1, the Abelson proto-oncogene; CCK-8, cell counting kit; CML, chronic myeloid leukemia; CP, chronic phase; ETC, electron transport chain; NER, nucleotide excision repair; ROS, reactive oxygen species; TKI, tyrosine kinase inhibitor

BCR-ABL1 in anti-apoptotic pathways, so mitochondria, along with mtDNA, can be important for this effect. In our previous work we showed that imatinib resistance was associated with increased ROS production and DNA damage induced by doxorubicin, an anticancer drug inducing DNA double-strand breaks and other DNA damage (Blasiak *et al.*, 2015). Moreover, we showed also that the expression of some apoptosis-related mitochondrial genes could be different in imatinib-sensitive and -resistant cells exposed to UV radiation (Synowiec *et al.*, 2015). In turn, imatinib-resistant cells were reported to display several mitochondrial dysfunctions associated with an increased ROS production in mitochondria (Kluza *et al.*, 2011). These extra ROS could damage mtDNA, which can accelerate the mitochondrial viscous cycle, leading to genomic instability. All these data suggest that mutagenesis of mtDNA can play an important role in imatinib resistance in CML cells. To explore this subject further, in the present work we investigated the response of mtDNA to H₂O₂ and UV in BCR-ABL1-expressing cells sensitive and resistant to imatinib. H₂O₂ produces ROS, which can damage mtDNA contributing to genome instability. The main DNA damage induced by UV are cyclobutane pyrimidine dimers and (6–4) photoproducts, which are primarily removed by nucleotide excision repair (NER) in the nucleus, but this DNA repair system is considered not to operate in mitochondria. Therefore, analysis of UV-induced DNA damage in mitochondria can facilitate the assessment of the general role of NER in imatinib resistance mechanism.

MATERIALS AND METHODS

Cells, viability and treatment. Murine 32D cells were transfected with the *BCR-ABL1* gene or its mutated Y253H or T315I variants as described previously (Slupianek *et al.*, 2002). Some cells expressing non-mutated *BCR-ABL1*, were cultured in increased concentrations of imatinib to acquire resistance to this drug up to 1.0 mM. This was achieved by S cells incubation with 0.01 µM imatinib for 3 weeks with survival rate about 80%. The incubation was continued with 0.05 µM imatinib and this process was repeated with growing concentrations of imatinib until not less than 50% of cells survived at 1 mM imatinib. Consequently, we used parental 32D cell line (P) and four 32D BCR-ABL1-transfected cell lines: cells sensitive to imatinib (S), imatinib-resistant cells with the mutation in the *BCR-ABL1* gene (Y253H and T315I) and cells with acquired resistance to imatinib (AR). The cells were cultured in IMDM medium supplemented with 2 mM L-glutamine, 100 U/ml penicillin, 100 µg/ml streptomycin, 10% fetal bovine serum and maintained at 37°C in 5% CO₂ atmosphere at 100% humidity. The medium of parental cells was supplemented with 1 ng/ml of interleukin-3.

To assess sensitivity/resistance to imatinib, the cells were incubated at 37°C with imatinib for 24 h and cell viability was determined with a tetrazolium salt using the Cell Counting Kit-8 (CCK-8) (Sigma-Aldrich).

Total DNA from the cells was isolated with QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The concentration and purity of DNA were determined spectrophotometrically.

To evaluate mtDNA damage and repair as well as mtDNA copy number during genotoxic stress, the cells were incubated for 30 min at 37°C with 50 µM H₂O₂ or were irradiated with 254 nm UV radiation at room

Table 1. Sequence of primers used to amplify fragments of mitochondrial DNA by semi-long real-time PCR

Fragment (length)	Primers (5'→3', forward and reverse)
Shorter (82 bp)	AACCCACGATCAACTGAAGC GTACGATGGCCAGGAGGATA
Longer (1054 bp)	ATCCTCACCTCAGCCAACAA TGAGGACTGGAATGCTGGTT
Reference (82 bp)	AGAATGGGAAGCCGAACATA CCGTTCCTCAGCATTTGGAT

temperature using UVC-6-12 lamp (NeoLab, Heidelberg, Germany) with dose rate of 0.12 Wm⁻²s⁻¹.

Mitochondrial DNA damage and repair and data analysis. Mitochondrial DNA damage was quantified by semi-long real-time quantitative PCR as described elsewhere (Ballinger *et al.*, 1999; Rothfus *et al.*, 2010). In this reaction, two mtDNA fragments of length about 1000 and 80 bp are amplified. The longer amplicon is the target for DNA-damaging agents, whereas the shorter one is unlikely to be affected by DNA damage and enables normalization of the amount of PCR product to relative copy number. In this reaction, any mtDNA lesion causing block of DNA polymerase results in a decrease in the amount of amplicons of the target fragment in mtDNA. The primer sequences are given in Table 1. All primers were synthesized by Sigma. An 82 bp fragment of the mouse beta-2 microglobulin gene was used as a reference.

Each reaction was carried out in a thermal cycler model CFX96 Real-Time PCR Detection System (Bio-Rad Laboratories, Hercules, CA, USA) in a total volume of 10 ml with 1 × Maxima SYBR Green QPCR Master Kit (Thermo Scientific, West Palm Beach, FL, USA) with 500 ng of each primer and 100 ng of DNA template. The amplification reaction consisted of the preinitial denaturation step at 95°C for 10 min followed by 40 cycles of 15 s at 95°C/30 s at 62°C/75 s at 72°C for the long product, whereas for the short products amplification had a preincubation phase at 95°C for 10 min and 40 cycles of 15 s at 95°C/30 s at 59°C/30 s at 72°C. Each sample was analyzed in triplicate.

Repair of mtDNA damage was determined by the measurements of the extent of mtDNA damage at time 0 (the beginning of the repair incubation) and 30, 60 and 120 min thereafter.

DNA damage in cell exposed to H₂O₂ or UV radiation was compared to control, non-exposed cells. Relative mtDNA damage (*D*) was expressed as the number of mtDNA lesions per 10 kb DNA and was measured by calculating the difference Δ*C_t* for longer and shorter fragments for the 2^{-ΔΔ*C_t*} method (the comparative *C_t* method) in correlation with the size of longer fragment after amplification according to the equation:

$$D = \frac{1 - 2^{-\Delta_i - \Delta_s}}{L}$$

where Δ_i and Δ_s are Δ*C_t* for longer and shorter fragments, respectively, *L* is the size of longer fragment in bp (Schmittgen & Livak, 2008; Hou *et al.*, 2010).

The 3,867-bp deletion in mtDNA. Quantitative detection of the 3,897-bp deletion was performed by quantitative real-time PCR with TaqMan fluorescence quenching probes essentially as described by Kazachkova *et al.* (Kazachkova *et al.*, 2013). Briefly, the assay is based on the amplification of fragments of all mtDNA mol-

Table 2. Sequence of primers used to determine the number of mtDNA molecules with the 3,867-bp deletion by real-time PCR

Fragment specificity	Primers (5'→3', forward and reverse)
All mtDNA molecules	TCGCCACTTCTATAACAGCTATG AATGCTAGGCGTTTGATTGG
mtDNA molecules without the 3,867-bp deletion	TGTACCCACGCATTCTTCAA AATGCTAGGCGTTTGATTGG

ecules (primers specific to a region outside the deletion) and molecules without deletions (primers within the deletion). The former, after normalization to the nuclear *GAPDH* gene, provides information on the number of copy of mtDNA. The difference between the number of all mtDNA molecules and of molecules without the deletion indicates the number of molecules with the 3,867-bp deletion. The sequence of primers are listed in Table 2.

Each reaction was run on the CFX96 thermal cycler in a total volume of 10 ml containing 10 ng template DNA, 5 pmol of each primer and probe, 1 U of DNA polymerase, 1.25 mM MgCl₂ and 0.2 mM of each dNTP. Amplification conditions were: initial 10 min denaturation at 95°C, followed by 40 cycles at 95°C for 15 s and at 60°C for 60 s. Each sample was run in triplicate.

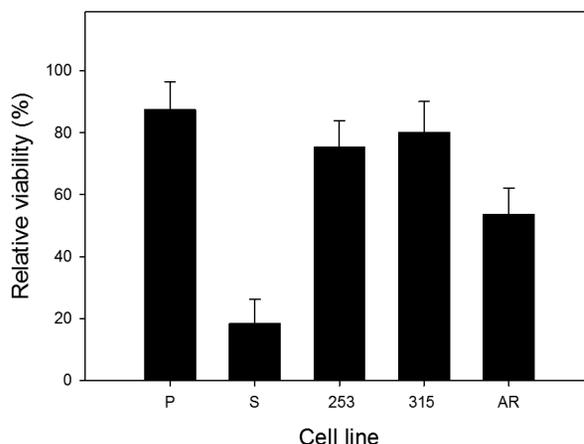
Statistical analysis. All values in this study were expressed as mean ± S.E.M. and the differences between means were evaluated by the Student's *t* test. The data were analyzed using the STATISTICA (StatSoft, Tulsa, OK, USA) statistical package.

RESULTS

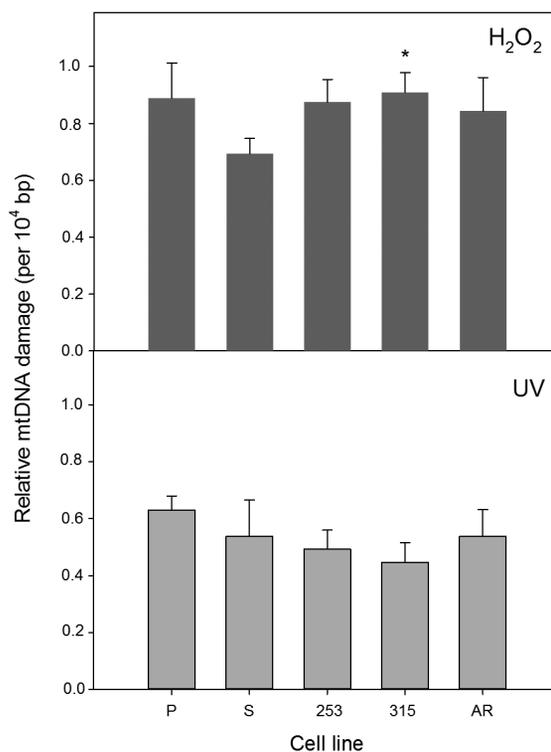
Imatinib resistance. The parental 32D cells (P) were almost insensitive to 1 μM imatinib as their relative viability was about 87% (Fig. 1). For cells transfected with non-mutated *BCR-ABL1* (S) the survival rate after imatinib treatment was less than 20%, so they were further considered as imatinib-sensitive. The viability of cells with the Y253H and T315I mutations in the *BCR-ABL1* gene was over 70% and of the AR cells — about 50%, so we considered these cells as imatinib-resistant.

Damage to mtDNA. Neither H₂O₂ nor UV radiation changed substantially the viability of the cells (results not shown). H₂O₂ evoked pronounced damage to mtDNA in all cell lines (Fig. 2). There was no difference between the extent of mtDNA damage in parental and *BCR-ABL1*-expressing cells. All imatinib-resistant cell lines displayed higher extent of DNA damage than their imatinib-sensitive counterparts, but this relationship was rather of border-line significance. All cell lines presented similar, moderate sensitivity to UV radiation. Although the sensitivity to imatinib seemed to be negatively correlated with H₂O₂-induced mtDNA damages and the T315I cells appeared to be the least sensitive to UV, these relationships are not statistically significant.

Repair of mtDNA. The kinetics of mtDNA repair in the studied cell lines exposed to H₂O₂ or UV radiation is presented in Fig. 3. There was an essential difference in the reaction of all cell lines to H₂O₂ and UV. The extent of mtDNA damage in cells exposed to H₂O₂ decreased after a 120-min repair incubation, although the difference between DNA damage at 120 min and zero was not always statistically significant (a border-line significance for P (*p* = 0.061) and 253 lines (*p* = 0.58)). There was not any difference in the extent of mtDNA dam-

**Figure 1. Relative viability of mouse 32D cells incubated for 24 h with 1 μM imatinib at 37°C.**

Parental cells (P) were transfected with the *BCR-ABL1* gene in its normal form (S) or with Y253H (253) and T315I (315) mutation. Some S cells were cultured in a growing concentration of imatinib (AR) prior to viability determination. The viability was determined with Cell Counting Kit-8. Each measurement was performed in triplicate; error bars denote S.E.M.

**Figure 2. H₂O₂- and UV-induced damage to mtDNA in mouse 32D cell lines: parental (P) and transfected with the *BCR-ABL1* gene: sensitive (S) and resistant to imatinib (253, 315, AR).**

The resistance resulted either from the Y253H or T315I mutation in the *BCR-ABL1* gene (253 and 315, respectively) or from incubation of sensitive cells with increasing concentration of imatinib (AR). Cells were incubated 30 min at 37°C with H₂O₂ at 50 μM or were irradiated with UV light at 35 J/m² at room temperature with a dose rate of 0.12 Wm⁻²s⁻¹ and mtDNA damage was quantified by semi-long real-time PCR. Error bars denote S.E.M., n = 3 for each measurement; **p* < 0.05 as compared with S line.

age at the beginning and at the end of the repair incubation in cells exposed to UV radiation. The kinetics of mtDNA repair in H₂O₂-pretreated cells was similar and there were slight, statistically insignificant differences be-

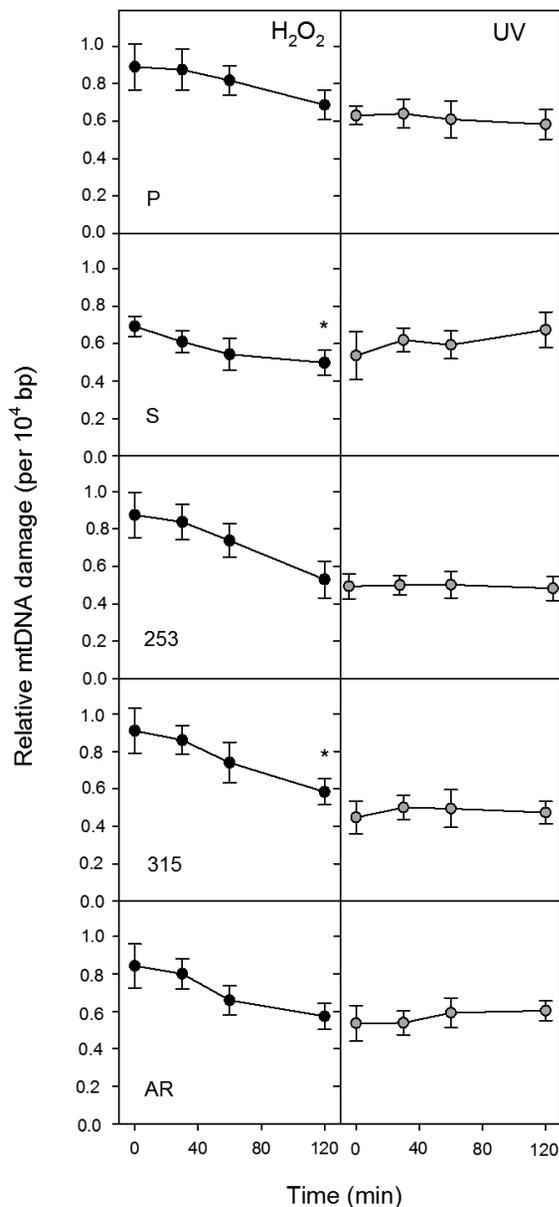


Figure 3. mtDNA repair in mouse 32D cell lines: parental (P) and transfected with the *BCR-ABL1* gene: sensitive (S) and resistant to imatinib (253, 315, AR).

The resistance resulted either from the Y253H or T315I mutation in the *BCR-ABL1* gene (253 and 315, respectively) or from incubation of sensitive cells with increasing concentration of imatinib (AR). Cells were challenged with H_2O_2 (30 min, $37^\circ C$) at $50 \mu M$ (left panels) or UV radiation with a dose rate $0.12 Wm^{-2}s^{-1}$ (right panels) and mtDNA damage was quantified by semi-long real-time PCR at 0 (immediately after the exposure), 30, 60 and 120 min thereafter. Error bars denote S.E.M., $n = 3$ for each measurement, * $p < 0.05$ as compared with the time 0.

tween imatinib-resistant and -sensitive cells, which is best illustrated in the case of 253 and S cells. Apparently, it seems that in the former the rate of the mtDNA repair is higher than in case of the latter.

Copy number of mtDNA. Figure 4 presents the relative mean copy number of mtDNA in cells exposed to H_2O_2 or UV radiation as compared with non-exposed cells. There was not any difference in the copy number between imatinib-resistant and -sensitive cells. Therefore, neither H_2O_2 nor UV did induce degradation of mtDNA in any kind of cells.

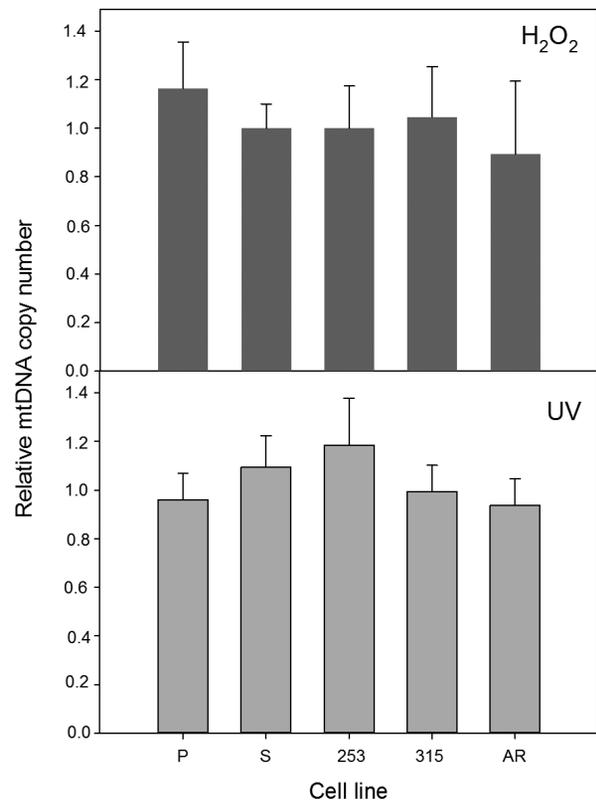


Figure 4. Relative mtDNA copy number in mouse 32D cell lines: parental (P) and transfected with the *BCR-ABL1* gene: sensitive (S) and resistant to imatinib (253, 315, AR).

The resistance resulted either from the Y253H or T315I mutation in the *BCR-ABL1* gene (253 and 315, respectively) or incubation of the sensitive cells with increasing concentrations of imatinib (AR). Cells were challenged with H_2O_2 (30 min, $37^\circ C$) at $50 \mu M$ (upper panel) or UV radiation with a dose rate $0.12 Wm^{-2}s^{-1}$ (lower panel) and mtDNA copy number was calculated by quantitative real-time PCR of 82-bp fragments of the mitochondrial NADH dehydrogenase, subunit 2 (*ND2*) gene and the nuclear beta-2 microglobulin (*B2M*) gene. The ratio of copy number in cells exposed to either H_2O_2 or UV radiation and unexposed cells is presented. Error bars denote S.E.M., $n = 3$ for each measurement.

The 3,867 deletion in mtDNA. No statistically significant difference in relative number of mtDNA copies with the 3,867-bp deletion between the cell lines was observed (Fig. 5). Neither the exposure to H_2O_2 , nor UV irradiation affected this number.

DISCUSSION

After the initial success of imatinib, resistance to this drug or, more generally, to TKIs, has become an emerging problem and is one of the most serious obstacles of imatinib-based targeted cancer therapy. Several mechanisms can underline imatinib resistance and some of them are partly known, stimulating modifications of CML therapy. As mentioned above, these modifications include first of all introduction of second- and third-generations of TKIs as well as supplementation of imatinib-based therapy with other drugs modulating imatinib effect. As *BCR-ABL1* enhances ROS production, DNA damage and repair is of a special significance in *BCR-ABL1*-positive cells. Because mitochondria primarily produce intracellular ROS due to the activity of electron transport chain (ETC), mtDNA is particularly exposed to ROS. As the repair of mtDNA takes place mainly in the immediate vi-

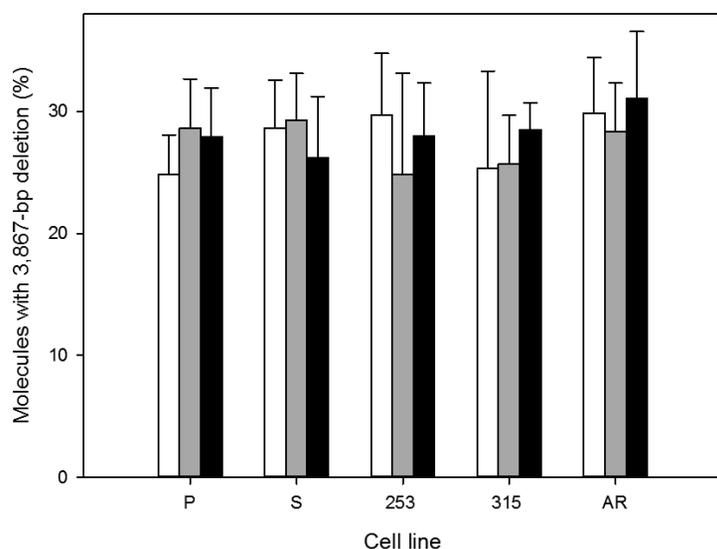


Figure 5. Relative mean number of mtDNA copies with the 3,867-bp deletion in mouse 32D cell lines: parental (P) and transfected with the *BCR-ABL1* gene: sensitive (S) and resistant to imatinib (253, 315, AR) exposed to H₂O₂ at 50 μ M for 30 min at 37°C (gray bars) or UV radiation with a dose rate 0.12 Wm⁻²s⁻¹ (black bars) as compared with non-exposed cells (white bars). Imatinib resistance resulted either from the Y253H or T315I mutation in the *BCR-ABL1* gene (253 and 315, respectively) or incubation of the sensitive cells with increasing concentrations of imatinib (AR). The number of mtDNA molecules with the deletion was determined by quantitative real-time PCR of fragments of mtDNA without the deletion and all mtDNA molecules. The number of mtDNA molecules without the deletion was then subtracted from the number of all mtDNA copies and divided by it. Error bars denote S.E.M., n = 3 for each measurement.

cinity of ETC proteins in the inner mitochondrial membrane, it can be error-prone due to the presence of ROS attacking DNA repair proteins (Gredilla *et al.*, 2010). The vulnerability of mtDNA to damage is also important in the light of imatinib mechanism of action — blocking the antiapoptotic action of BCR-ABL1. Because the mitochondrial apoptotic pathway is the key route of apoptotic signaling, damage to mtDNA can interfere with this process and thus modulate imatinib action.

Although the amount of mtDNA in a human cell can be quite high due to a high number of mitochondria in some kind of cells, the mechanisms of mtDNA repair are much less effective than those operating in the nucleus. The most striking difference between DNA repair in the nucleus and mitochondria is the lack of mitochondrial NER, at least the one operating in a nucleus-like fashion. Some reports suggest the presence of certain proteins in the organelle, including Cockayne syndrome proteins (CSA and CSB), which in the nucleus are involved in transcription-coupled NER (Kamenisch & Berneburg, 2013). Moreover, a possible link between NER-associated CS proteins and mitochondrial base excision repair (BER) is suggested, but this issue still needs detailed studies. It was shown that CSA and CSB proteins are associated with human mitochondrial 8-oxoguanine glycosylase, the crucial element of antioxidant defense (Kamenish *et al.*, 2010, Kamenisch & Berneburg 2013). Lastly, the presence of the XPD, an important NER protein, in the inner mitochondrial membrane was reported, but its involvement in putative mitochondrial NER is uncertain (Liu *et al.*, 2015).

If the extent of mtDNA damage exceeds the repair capacity of the cell, mtDNA is directed to degradation (Kang & Hamasaki, 2002). DNA damage tolerance mechanisms have not been reported in animal mitochondria. Therefore, if NER is not operating in mitochondria, induction of NER-specific DNA damages can lead

to the accumulation of mtDNA lesions, increase in genomic instability and eventually to degradation of mtDNA. That is why we chose UV radiation as the DNA-damaging factor, because its main products — pyrimidine dimers and (6–4)photoproducts, are removed in the nucleus by NER.

We used cells, which were derived from the 32D cell line. We modified the parental 32D cells to express the BCR-ABL1 kinase in its native type and two mutated forms, conferring imatinib resistance. Additionally, cells with non-mutated BCR-ABL1 were incubated in escalating doses of imatinib to acquire resistance to this drug. The status of the AR cells is rather uncertain in that sense that they form a highly dynamic system, so their usefulness in the investigation of the mechanisms of biochemical processes is rather limited. However, they were included in our project as they correspond to the situation when CML patients, initially sensitive to imatinib, become resistant due to mutations in the *BCR-ABL1* gene and other mechanisms. Therefore, we created a CML model cellular system, representing imatinib-sensitive and -resistant cases. Certainly, using a single cell system is a limitation of our study, but we do not know other such system, which would be available for us. Using primary cells from CML patients is an option, but it requires enrolling many patients and much time to collect adequate number of samples.

Our research can be viewed as an introduction to the field, along with other studies we recently performed (Blasiak *et al.*, 2015; Synowiec *et al.*, 2015).

In the present work we showed, that there was not any difference in the extent of mtDNA damage induced by UV radiation among the cell lines we used (Fig. 2). The assay we employed — synthesis of new DNA fragment on a template from exposed cells is sensitive to UV-induced damage, as both pyrimidine dimers and (6–4)photoproducts inhibit DNA polymerase movement along the template. Therefore, we can speculate that NER could not be involved in the mechanisms of imatinib resistance in BCR-ABL1-expressing cells. The imatinib-resistant T315I cells displayed an increased extent of mtDNA damage after H₂O₂ treatment compared to their imatinib-sensitive counterparts (Fig. 2). This can be related to increased genomic instability associated with imatinib-resistance. H₂O₂ induces a variety of DNA damages, but at moderate concentrations, oxidative modifications to the DNA bases are the most frequently occurring DNA lesions. These are removed by base excision repair (BER), a pathway which is likely the most effectively operating DNA repair system in mitochondria. However, we did not observe any significant difference in mtDNA damage induced by H₂O₂ between other imatinib-resistant cells and their sensitive counterparts.

The T315I and Y253H lines displayed similar resistance to imatinib in our experiments, but they represent two different mutations (Miething *et al.*, 2006). The T315I mutation, most frequently occurring in imatinib-resistant CML patients, directly impairs imatinib binding as it is located at the drug-contact side. In turn, the Y253H mutation is located at the nucleotide-binding loop of BCR-ABL1, impairing the optimal conformation for imatinib binding. Therefore, these two mutations can differently influence the conformation of the BCR-ABL1 kinase, which, in turn, can differently affect its role in DNA repair (Skorski, 2002).

Although there were some border-line significance differences in DNA repair after H₂O₂ treatment, all cell lines displayed similar kinetics of mtDNA repair and, in general, repaired their DNA during this 120-min incubation (Fig. 3). However, repair kinetics after H₂O₂ differed from that representing repair of UV-induced mtDNA damage, confirming no NER involvement.

Although no effective repair of UV-induced mtDNA damage was observed, we did not observe changes in the mtDNA copy number either (Fig. 4), therefore possible lack of NER did not result in mtDNA degradation due to inability to repair pyrimidine dimers and (6-4) photoproducts.

There was no difference in the occurrence of the common 3,867-bp deletion between resistant and sensitive cells and genotoxic treatment did not change this relationship (Fig. 5). We explain these results by relatively mild conditions of the treatment and the nature of this mutation, as the mechanism of its generation is different from the mechanisms underlying point mutations, most frequently associated with genomic instability of cancer cells.

In conclusion, BCR-ABL1 imatinib-resistant cells can display an enhanced instability of mtDNA in comparison to their sensitive counterparts, which is supported by an increased sensitivity of T315I cells to H₂O₂. Our results suggest also that NER cannot be involved in the mechanisms of imatinib resistance, as indicated by UV-induced mtDNA damage and its repair in resistant and sensitive cells. In summary, some aspects of mitochondrial mutagenesis can play a role in imatinib resistance in BCR-ABL1-expressing cells.

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Conflict of interest statement

The authors do not declare any conflict of interest.

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