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BCR-ABL Hits at Mitosis; Implications for Chromosomal Instability, Aneuploidy and Therapeutic Strategy

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1. Introduction

1.1 Genomic and chromosomal instability in CML

An unstable genome is a common hallmark of nearly all solid tumors and most of leukemias in contrast to normal, healthy cells which are able to maintein genome integrity (Negrini et al., 2010). Genomic instability could result from changes in chromosome structure and number as well as changes on the DNA level. Chromosomal instability (CIN) arises from unproper chromosome segregation as well as division defects and leads to aneuploidy (Foijer, 2010), whereas accumulation of mutations and DNA alterations usually is an effect of the defective repair systems and DNA damage response in cancer cells (Economopoulou et al., 2011).

Chronic myeloid leukemia (CML) cells expressing the BCR-ABL tyrosine kinase have been found to accumulate mutations as well as chromosomal abnormalities. One of the first indications that CML correlates with additional chromosome changes has been presented in 1987 (Alimena et al., 1987). Moreover, authors showed that the rate of chromosomal anomalies increased during the blastic transformation. In the next years this has been also confirmed by other authors (Hagemeijer, 1987; Johansson et al., 2002; Su et al., 1999; Suzukawa et al., 1997). Later, random aneuploidy rate between chromosomes 9 and 18 has been reported in CML patients – both, untreated as well as upon imatinib therapy (Amiel et al., 2006). In broader analysis of CML patients it was found that chromosomal instability caused by centrosomal aberrations significantly correlated with the disease progression (Giehl et al., 2005). In the chronic phase only one sample out of 18 showed additional karyotypic alterations, in contrast to blast crisis where 73% patients (11/16) displayed additional karyotype alterations. The observation that CML patients have karyotype aberrations was confirmed in other studies where complex chromosomal rearrangements (CCR) were investigated (Babicka et al., 2006). By using cytogenetics, the FISH, and multicolor FISH (mFISH) methods, a very high level of the genomic instability at the chromosomal level, in cells obtained from chronic myeloid leukemia patients was observed.

Altogether, it was shown that the aberrations associated with the progression of BCR-ABL-positive CML chronic phase to the aggressive blast crisis include additional chromosomes (Ph^1, +8, +19), isochromosome 17q (associated with the loss of p53), reciprocal

translocations, loss-of-heterozygosity at 14q32, homozygous mutations/deletions of pRb and p16/ARF, and mutations in p53 and RAS (Calabretta & Perrotti, 2004). The possible mechanisms participating in the BCR-ABL-mediated aneuploidy will be broadly described and discussed in the next paragraphs.

BCR-ABL has been also indicated as a promoter of secondary DNA mutations in CML (Burke & Carroll, 2010). This is the effect of the defective DNA damage response and DNA repair mechanisms found in CML cells. DNA damage can occur as single-nucleotide alterations, single-strand breaks (SSB), or double-strand breaks (DSB). Double-strand breaks are proposed to be the most mutagenic, as neither strand remains intact to serve as a template for repair. Single-nucleotide alterations are repaired by mismatch repair (MMR) or nucleotide excision repair (NER) mechanisms. Single or double-strand breaks are repaired by either high-fidelity homologous recombination repair (HRR) or non-homologous end-joining (NHEJ), when a sister chromatid is not available as a template. The last mechanism is error-prone and can lead to short deletions in the repaired strands.

Data from different laboratories collectively indicate that BCR-ABL promotes dysfunctions of nearly all mechanisms participating in the DNA repair. It is known that BCR-ABL cells treated with genotoxic agents present higher levels of DNA damage and aberrant repair systems, leading to the accumulation of DNA errors (Brady, 2003; Laurent et al., 2003; Slupianek et al., 2002). Studies from Skorski's group clearly showed that expression of BCR-ABL affects different mechanisms participating in the DNA repair. They found that BCR-ABL modifies the repair of DNA double-strand breaks (Koptyra et al., 2008; Nowicki et al., 2004; Slupianek et al., 2006). Briefly, CML cells produced increased rate of DSBs in S and G2/M phases of the cell cycle, as a result of oxidative DNA damage caused by BCR-ABL. These breaks were repaired, however with a high mutation rate and large deletions, as a result of defective HRR and NHEJ repair systems, respectively. Moreover, they found that BCR-ABL is able to inhibit both, mismatch repair (MMR) and inhibit apoptosis as well as to induce point mutations (Stoklosa et al., 2008). Upon this, CML cells were able to survive treatment leading to generation of the O(6)-methylguanine and O(4)-methylthymine recognized by the MMR system, however they displayed 15-fold higher mutation frequency than parental counterparts.

Deutsch et al indicated that DNA-PKcs, a protein involved in the NHEJ repair system, may be downregulated by BCR-ABL (Deutsch et al., 2001). This decrease was proteasome- and tyrosine kinase-dependent, as it was reversed by proteasome as well as tyrosime kinase inhibitors. Alternatively, the role of DNA-PKcs has been recently indicated to switch on the backup-NHEJ system, which is more error-prone (Poplawski & Blasiak, 2010). It was also shown that BCR-ABL upregulates the error-prone DSB repair pathways, particularly single-strand annealing and non-homologous end-joining due to an increased level of DNA-end-processing factor CtIP (Salles et al., 2011). Additionally, BCR-ABL also promotes the DNA DSB repair by using the highly mutagenic single-strand annealing (SSA) pathway which involves single repeats (Fernandes et al., 2009). This required the active Ras and PI3K pathways, acting downstream of the Y177 site of BCR-ABL, which is a major regulatory site for ROS induction and is necessary for the optimal activation of the PI3K and Ras pathways. Moreover, using stromal cell lines authors also showed that the stromal cell-conditioned media increased the SSA frequency, measured in K562 cells in the presence or absence of imatinib. This supported the hypothesis that microenvironment additionaly promotes mutagenesis in CML cells.

Altogether, there is no doubt, that defects in DNA repair mechanisms and genomic surveillance in CML cells are an effect of the expression of BCR-ABL itself. However, there was still an open question, whether occurrence of the genomic instability participates in the development of the blast crisis phase (Penserga & Skorski, 2007; Shet et al., 2002). This has been strongly indicated to play a significant role in the malignant progression of the disease by many authors (Burke & Carroll, 2010; Salleset al., 2011; Skorski, 2008; Skorski, 2011).
Till now, convincing data was presented and it seems clear, that genetic instability, accumulation of mutations and additional chromosomal alterations are the major factors involved in the CML progression and resistance to cell death. This leads to an accumulation of additional genetic aberrations and changes in gene expression, which result in the expansion of differentiation-arrested and increasingly malignant cell clones. Importantly, genetic instability of tyrosine kinase refractory cells, including leukemia stem cells (LSCs) has also recently been proposed as a reason for their fast transformation leading to the generation of additional resistant clones and transformation to a blast phase (Skorski, 2011). This mechanism could be responsible for clonal evolution and expansion causing finally relapse and malignant progression.
The current model of blastic transformation proposed recently by Perotti (Perrotti et al., 2010), indicates that acquiring of additional genetic and epigenetic changes by LSCs or their progeny causes leukemia transformation from the chronic phase to the advanced phases. This can explain the complexity of the disease progression and blast crisis as well as the inability to find common features of cells in blast crisis and specific secondary genetic aberrations. Most likely different mutations and aberrations are cumulated to obtain the critical point allowing the disease to progress. Thus, it will be very difficult to plan the therapeutic strategy against genetically unstable LSCs, resistant to tyrosine kinase inhibitors, with the already used agents and probably novel therapies need to be developed.

2. The role of aberrant divisions in CML cells

It has been known for more than a century that neoplastic cells could exhibit disturbances of the cell division process (Boveri, 1902, 1914). Boveri observed that sea urchin embryos manipulated to undergo mitosis in the presence of multipolar spindles produced aneuploid progeny and proposed that tumors arise from normal cells becoming aneuploid as a result of aberrant mitoses. Boveri's theory that division errors and aneuploidy could lead to cancer development has been revisited during the last decade (Duesberg et al., 2006; Holland & Cleveland, 2009; Weaver & Cleveland, 2006).
Today, it is commonly accepted that aberrant mitoses result in chromosomal instability (CIN), leading to the gain or loss of whole or large fragments of chromosomes, which are the main form of genomic instability in cancers. As it was mentioned in the previous chapter, it is fully convincing that expression of BCR-ABL leads to significant chromosomal aberrations. Moreover, these abnormalities increase along with the disease progression, participating in the blastic transformation. Below, we present current data concerning the role of BCR-ABL-mediated defects in the mechanisms controlling cell division as well as the role of BRCA1 in the development of aneuploidy in CML.

2.1 Centrosomal multiplication

Centrosomes are small organelles with a crucial role in the formation of bipolar mitotic spindle, which is necessary for the accurate segregation of chromosomes (Fukasawa, 2007;

Rusan & Rogers, 2009; Tanenbaum & Medema, 2010). Briefly, they are formed by paired centrioles surrounded by a protein matrix of pericentriolar material, including pericentrin. Their function is to nucleate and anchor microtubules to form an interphase cytoplasmic-microtubule network and mitotic spindle. During the cell division, each daughter cell receives one centrosome, thus the centrosome has to duplicate before the next mitosis. This takes place during the S phase and is driven at least partially by the Cdk2-cyclin E complex. Coordination of the DNA and centrosome replication is crucial to avoid their overduplication. Two mature centrosomes are generated at the late G2 phase and they become the spindle poles. It was shown that the DNA damage checkpoint proteins, such as ATM, ATR, Chk1 and Chk2 and others also localize at the centrosomes (Zhang et al., 2007). It seems that these proteins interact with gamma-tubulin and are involved in the controlling of microtubule kinetics during the DNA damage response. It was reported that DNA damage leads to centrosome amplification in the G2 phase as a result of cell cycle arrest (Inanc et al., 2010). Studies performed by Dodson and colleagues showed the involvement of ATM in the centrosome amplification in response to DNA damage, however gene targeting of *ATM* reduced, but did not abrogate completely centrosome amplification (Dodson et al., 2004). Alternatively, data from lymphoid gamma-irradiated cells showed that neither ATM nor ATR kinases are involved in this process, however Chk1-dependent signaling seems to be crucial (Bourke et al., 2007). This issue still needs to be clarified.

It is commonly accepted that the appearance of supernumerary centrosomes is associated with aberrant mitoses and chromosomal instability. Multipolar mitoses, lagging chromosomes or multinuclei are observed in cells with overduplicated centrosomes. Cells with three centrosomes usually undergo cytokinesis and some of the generated cells are viable, however aneuploid. Cells with multipolar (>3) spindles fail to undergo cytokinesis and can become polyploid if they are p53-deficient and are able to continue the cell cycle (Godinho et al., 2009).

Centrosome abnormalities are commonly observed in cancers and participate in the chromosomal instability and tumorigenesis (Carroll et al., 1999; Duensing & Duensing, 2010; Pihan et al., 2001). As mentioned before, mutlipolar mitosis as a result of centrosome overduplication can lead to gross chromosome missegregation and cell death. Thus cancer cells with supernumerary centrosomes possess the ability to suppress multipolar mitoses due to the inactivation, clustering or asymmetric segregation of extra centrosomes (Brinkley, 2001; Godinhoet al., 2009). This results in the formation of a bipolar, functional, however not symmetric mitotic spindle and so called mitotic stability of aneuploid cancer cells.

Abnormalities in the number of centrosomes were also found in leukemias. It was reported that defects in the number of centrosomes caused by the p53 mutation and cyclin E overexpression, detected in bladder cancers, led to centrosome amplification and chromosomal instability (Kawamura et al., 2004). Moreover, the centrosome aberrations were proposed as one of the main factors responsible for aneuploidy in acute myeloid leukemia (Kramer et al., 2003; Neben et al., 2003). Studies of CD34+ Ph+ cells isolated from chronic myeloid leukemia patients showed that centrosome aberrations correlate with the stage of the disease and aneuploidy (Giehl et al., 2005). In these studies freshly isolated cells from CML patients, in the chronic phase or blast crisis, were stained for pericentrin and gamma-tubulin to analyse the number as well as the structure of centrosomes. Moreover, they were studied for additional karyotypic abnormalities. Importantly, a strong correlation between the increase of centrosome aberrations, CML progression and blastic transformation was found. As centrosome defects were indicated as en early detectable

feature of CML, they have been proposed as a cause of karyotype instability and aneuploidy in CML progenitor cells as well as a valuable prognostic factor. In the long-term *in vitro* studies, using a cellular model of the chronic phase of CML, authors confirmed, that expression of BCR-ABL leads to significant centrosomal hypertrophy visible already after 4 weeks of BCR-ABL expression (Giehl et al., 2007). This increased upon the next 10 weeks of propagation and correlated with the clonal expantion of aneuploid cells.

We also found, using a mouse cellular model of CML, that the stable expression of low or high level of BCR-ABL in mouse progenitor 32D cells leads to the generation of cells with supernumerary centrosomes (Wolanin et al., 2010). This was accompanied by increased percentage of cells with aberrant mitoses, particularly multipolar spindles, lagging chromosomes and multinuclei. The presence of aberrant cells correlated with the level of BCR-ABL expression, indicating that the BCR-ABL itself is responsible for these abnormalities. Interestingly, Patel and colleagues presented that CML cells have defects in the centrosome-centriole cycle (Patel & Gordon, 2009). They showed that p210 (BCR-ABL1) and p145 (ABL1) are both, centrosome-associated proteins and form a complex with the pericentriolar protein, pericentrin. Numerical and structural centrosomal abnormalities were found in CML cell lines and in primary CD34+ cells from CML patients as a result of an increased level of separase participating in the abnormalities in the centrosome-centriole cycle. They also confirmed the previous data that abnormal centrosome distribution, amplification and loss are more evident in the advanced stages of CML.

Although the tyrosine kinase inhibitors are very potent, selective and successful therapeutic agents for treatment of leukemia as well as some solid tumors it can not be neglected that some reports indicated that they can lead to centrosome aberrations in cancer as well as normal cells (Fabarius et al., 2005; Fabarius et al., 2008; Giehl et al., 2010). This was caused by blocking cells in the G1/S transition and the inhibition of cell growth which was followed by centrosomal aberrations. This should be taken into consideration with regards to the potential side-effects as well as a possible reason of dangerous clonal chromosomal abnormalities observed in BCR-ABL-negative progenitor cells under imatinib therapy.

2.2 Mitotic checkpoint failure

The spindle assembly checkpoint (SAC) plays a major role in the division control and segregation of sister chromatids, preventing occurence of aneuploidy (Chin & Yeong, 2010; Kops, 2008; Logarinho & Bousbaa, 2008; Nezi & Musacchio, 2009). SAC proteins, including Mad1 (mitotic arrest-deficient protein 1), Mad2, Bub1 (budding uninhibited by benzimidazoles 1), BubR1 (Bub1-related kinase 1) and Bub3 are recruited to unattached or tensionless kinetochores, forming mitotic checkpoint complex, which inhibits the anaphase promoting complex (APC). This protects cells from preearly anaphase entry and unproper segregation of chromatids. In physiological conditions the mitotic checkpoint is temporarily activated until the mitotic spindle is properly formed, whereas in anticancer therapy it is activated upon treatment with a group of microtubule damaging agents, such as taxanes and vinca alcaloids. Both interfere with tubulin organization and spindle formation, leading to the cell cycle arrest in mitosis and eventually cell death.

It is known that the complete loss of the mitotic checkpoint function results in embryonic lethality, what was shown in *Caenorhabditis elegans* (Kitagawa & Rose, 1999) as well as in mammalian cells (Michel et al., 2001; Schliekelman et al., 2009). Alternatively, partial loss of its function leads to chromosomes missegregation and chromosomal instability (Bharadwaj

& Yu, 2004; Ito & Matsumoto, 2010). This was due to the inability to activate the mitotic checkpoint and to arrest in mitosis in response to some disturbances. Instead - further progression of mitosis eventually leads to aberrant divisions and unproper chromosomes segregation.

Dysfunctions of the mitotic checkpoint were reported in different types of cancers (Baker et al., 2005; Bannon & Mc Gee, 2009; Tanaka & Hirota, 2009). They correlated with aneuploidy, disease progression and the increase of aggressiveness. Interestingly, similar effects were observed in case of the upregulation or decreased expression of mitotic checkpoint members. For example, the Mad2 protein has been recently proposed as a critical factor leading to aneuploidy in cancers with defects in the Rb and p53 pathways (Schvartzman et al., 2011). Authors found that Mad2 expression is repressed by p53 *via* the Rb pathway, thus the cancer cells lacking the Rb protein require Mad2 upregulation leading to chromosomal instability and tumor progression *in vivo*. On the other hand, also Mad2 haplo-insufficiency caused chromosomal instability in human cancer cells and murine primary embryonic fibroblasts (Michelet al., 2001).

BubR1 dysfunctions has also been found as a cause of cancer-susceptible disorder mosaic variegated aneuploidy (MVA) (Suijkerbuijk et al., 2010). Similarly to Mad2, BubR1 can be also overexpressed in cancer, what was shown in hepatocellular carcinoma (HCC) (Liu et al., 2009). Authors suggest that BubR1 overexpression, which was found in 45% of patients correlated with later stages and was associated with worse prognosis, thus it can be used as a potential prognostic factor for HCC.

There were indications that CML cells could have a dysfunctional mitotic checkpoint, as their resistance to spindle poisons was reported previously. In the K562 and Lama-84 CML cell lines, microtubule disruption caused either by paclitaxel, nocodazole or novel microtubule-targeting agent PBOX-6 led to polyploidization without the presence of significant apoptosis (Greene et al., 2007). Imatinib treatment minimized the formation of polyploid cells and enhanced the apoptotic index upon treatment of CML cells with spindle poisons. Resistance to paclitaxel was also shown in K562 cells (Blagosklonny, 2001), but mitotic checkpoint competence was not investigated. All these data suggested that BCR-ABL could somehow affect the response to microtubule disruption; however this issue was not discussed by the authors.

We have shown for the first time that the expression of BCR-ABL in mouse 32D cells decreases the expression of SAC proteins, such as Mad2, Bub1, Bub3 and BubR1, as well as their mRNA levels, what was estimated by real time RT-PCR (Wolanin et al., 2010). Decreased levels of the mitotic checkpoint proteins were associated with dysfunctions in the mitotic checkpoint competence observed upon nocodazole and paclitaxel treatment as well as resistance to cell death induced by these agents. We found that the inhibition of the BCR-ABL kinase activity by imatinib reversed the observed phenotype confirming the crucial role of BCR-ABL.

2.3 Aberrant expression of mitotic kinases

Mitotic kinases have also been implicated in the regulation of the centrosome cycle, spindle checkpoint and microtubule-kinetochore attachment, as well as spindle assembly and chromosome condensation. The family of Aurora kinases consists of the following proteins: Aurora A, B and C. The whole family has serine/threonine kinase activity which modifies microtubules during chromosome movement and segregation. Aurora kinases have been

found at the centrosomes of interphase cells, at the poles of the bipolar spindle and in the midbody of the mitotic apparatus. All three Aurora kinases members are overexpressed in many human cancers. This correlated with chromosomal instability and clinically aggressive forms of disease (Fu et al., 2007; Meraldi et al., 2004). Aurora A is localized in centrosomes and is important for maturation, spindle assembly and metaphase I spindle orientation. It has two independent functions in centrosome maturation and asymmetric protein localization during mitosis. Ectopic overexpression of Aurora A was shown to induce oncogenic transformation (Katayama et al., 2003). Moreover, overexpression of Aurora A and aneuploidy have been proposed as predictors of poor outcome in serous ovarian carcinoma (Lassus et al., 2011). Also a high level of Aurora B has been reported to promote tetraploidy and tumorigenesis in the mouse Xenograft model (Nguyen et al., 2009).

High expression of Aurora A in leukemia cell lines and freshly isolated leukemia CML cells has been presented by Ochi T et al (Ochi et al., 2009). We also showed that the expression of BCR-ABL leads to the mislocalization of Aurora A in the chromosomal passenger complex (Wolanin et al., 2006). The importance of Aurora A-dependent signaling in CML has been shown in studies indicating that Aurora inhibitors seem to be very effective therapeutics for CML treatment (Gontarewicz et al., 2008), what will be discussed by us later.

Another family of tubulin-associated serine/threonine kinases, Polo-like, has also received significant attention regarding its participation in tumorigenesis. As far, in mammalian cells four members of this family have been identified (PLK1-4), and each one of them has a distinct function. PLK1 is essentially involved in the control of mitotic steps, PLK2 and PLK3 have been described as potential regulators of the G1 and early S phases of the cell cycle, PLK4 as a major centrosome duplication regulator. Polo-like kinase 1 (PLK1) is a key regulator of mitosis and participates in regulating this process from its entry to cytokinesis (Yuan et al., 2011). Transcription and translation of PLK1 is highly coordinated with cell cycle progression. *Plk1* mRNA and protein levels begin to accumulate in the S-phase and reach a peak at the G2/M transition and then decline upon mitotic exit (Lee et al., 1995). At the G2/M phase, PLK1 regulates the Cdk1/Cyclin B1 complex promoting mitotic entry and regulating mitotic progression due to regulation of phosphorylation of Cyclin B1, Cdk1, Myt1 and Cdc25C. PLK1 also plays a role in centrosome maturation by promoting increased recruitment of microtubules to the spindle pole bodies. It also regulates the localization of Aurora A to the centrosomes for proper maturation. It is known today that all mitotic kinases interplay with each other and form an extensive functional network, thus targeting any of them has tremendous consequences for cell physiology (Lens et al., 2010). Additionally, it was shown that PLK1 catalysis survivin priming phosphorylation at Ser20, what is necessary for survivin-mediated Aurora B docking to the centromere and activation (Chu et al., 2010). Expression of the non-phosphorylable survivin mutant prevented Aurora B activation and corrected spindle microtubule attachment. We also observed that silencing of survivin in CML cells significantly affected CPC function and mitosis as well as proper completion of cytokinesis leading to the formation of giant polyploid cells (Wolanin et al., 2006). PLK1 also regulates the spindle assembly checkpoint (Nezi & Musacchio, 2009) probably by phosphorylation of BubR1 and finally, regulates chromosome segregation, cytokinesis and mitotic exit.

PLK1, similarly to other mitotic kinases has been shown to be upregulated in cancers, including lymphomas. Studies of a big group of non-Hodgkin's lymphoma (NHLs) patients presented that the level of PLK1 expression was significantly lower in low-grade NHLs than

in high-grade and intermediate-grade NHLs. Moreover, PLK1 has been proposed as a valuable marker of proliferating cells, even better than the commonly used Ki67 (Mito et al., 2005). It was also described that PLK1 is overexpressed in AML cell lines as well as in primary cells and its inhibition preferentially targeted lymphoid cells, indicating an important role of the PLK1-mediated signaling (Renner et al., 2009). Importantly, healthy hematopoietic progenitor CD34+ cells were much less sensitive to growth inhibition caused by PLK1 targeting, indicating a high potential of this therapeutic strategy. This observation was confirmed by studies performed by Ikezoe and colleagues, who also found PLK1 overexpressed in a number of human leukemia cell lines and freshly isolated leukemia cells from individuals with acute myelogenous leukemia as well as acute lymphoblastic leukemia, in comparison with normal bone marrow mononuclear cells (Ikezoe et al., 2009). As previously, they indicated PLK1 inhibition as a potent way to inhibit proliferation and induce cell death in leukemia cells. Moreover, the functional link between PLK1 and mTOR pathway has been shown in AML cells (Renner et al., 2010). Abnormal growth of cells overexpressing the active form of PLK1 was reversed by rapamycin, a specific inhibitor of the TORC1 complex. This showed a novel aspect of PLK1's role in leukemia and opened new therapeutic possibilities.

In chronic myeloid leukemia, PLK1was found to be expressed in the phosphorylated form in the CML cell line K562 as well as in primary CML cells from patients (Gleixner et al., 2010). Studies presenting the potential of the PLK1 inhibitors in therapy against CML were performed and indicate an important role of PLK1 in CML development and progression. They will be discussed in a detailed way in the chapter dedicated to anti-mitotic therapies against leukemia.

3. BCR-ABL-mediated downregulation of BRCA1

BRCA1, a tumor suppressor isolated in 1994 (Miki et al., 1994) has been implicated in a broad range of cellular processes, including DNA repair, cell cycle checkpoint control, cell division and gene transcription (Linger & Kruk, 2010; Thompson, 2010; Wu et al., 2010; Yang & Xia, 2010). It is a known familiar ovarian and breast cancer-specific tumor suppressor, however today it seems that it is involved in the development of other types of cancers as well. The protein contains two motifs: a RING domain at the N-terminus and two tandem copies of BRCT domain at the C-terminus (Baer, 2001). *In vivo* it exists in a heterodimeric complex with the BRCA1-associated RING domain (BARD1) protein, which resembles BRCA1 (Wu et al., 1996).

The first observation that BRCA1 protein is nearly undetectable in leukemia cells from chronic myeloid leukemia (CML) patients has been made by Deutsch et al (Deutsch et al., 2003). They found a significant downregulation of BRCA1 in primary CD34+ cells obtained from both, the chronic phase and the blast crisis patients as well as in cell lines expressing BCR-ABL. This was not accompanied by a decrease of the *BRCA1* mRNA, what was studied by real-time RT-PCR in one of the investigated cell lines.

Our group studied the direct influence of BCR-ABL on the BRCA1 expression, using the previously mentioned mouse progenitor 32D cell line stably expressing with BCR-ABL, particularly in clones, expressing low and high BCR-ABL levels (Fig.1A), (Wolanin et al., 2010). We found that BCR-ABL expression leads to a strong decrease of BRCA1 at the protein level. This was reversed by treatment with imatinib, a specific inhibitor of the BCR-ABL tyrosine kinase, confirming dependence on the tyrosine kinase activity (Fig. 1B). The

lack of a significant decrease of mRNA confirmed the previous observation that BCR-ABL affects the posttranscriptional stages of protein expression. Incubation with the proteasome inhibitor MG132 did not lead to an increase at the BRCA1 protein level (Fig. 1C), thus excluding the possibility that increased degradation is responsible for the protein downregulation.

Recently, it was shown that BCR-ABL interferes with the Fanconi Anemia/BRCA1 pathway, thus increasing the predisposition to DNA repair errors and development of centrosomal and chromosomal aberrations (Valeri et al., 2010). The interference of BCR-ABL with the formation of BRCA1 and FANCD2 nuclear foci was observed in hematopoietic progenitors from CML patients. These authors also showed that the ectopic expression of BRCA1 reverted the generation of aberrant centrosomes induced by BCR-ABL. This suggests, however not directly studied, that overexpression of BRCA1 could antagonize also other effects of BCR-ABL expression, if they are mediated by BRCA1 downregulation, indeed.

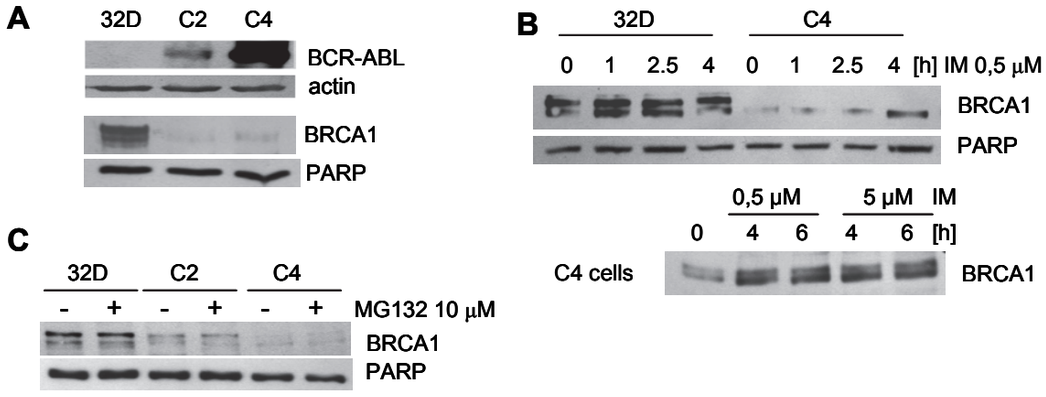

Fig. 1. The influence of BCR-ABL expression on the level of the BRCA1 Protein.
A. Expression of BCR-ABL leads to downregulation of the BRCA1 protein. The level of BRCA1 was determined by Western Blot in mouse progenitor 32D cells, control or stably expressing BCR-ABL at low (C2 cells) or high (C4 cells) level.
B. Imatinib treatment leads to upregulation of the BRCA1 protein level in cells expressing BCR-ABL. 32D and C4 cells were treated with 0.5 μM imatinib for 1, 2.5 or 4 hours (upper panel) or with 0.5 or 5 μM imatinib for 4 and 6 hours (lower panel) followed by estimation of the BRCA1 protein level.
C. BRCA1 downregulation caused by BCR-ABL is not a result of increased proteasomal degradation. 32D, C2 and C4 cells were treated with 10 μM proteasome inhibitor MG132 for 6 hours, followed by determination of the BRCA1 protein level by Western Blot.

Altogether, there are strong evidences indicating that the decrease of the BRCA1 protein and the BRCA1-dependent signaling is caused by BCR-ABL expression and is also specific for chronic myeloid leukemia, in addition to other types of tumors. There is a number of intracellular processes crucial for cell physiology controlled by BRCA1, including DNA damage response as well as activation of the cell cycle checkpoints, chromatin remodelling, apoptosis and mitosis. Aberrations in any of them, lead to the accumulation of mutations, genomic instability and finally an increased risk of cancerogenesis. Thus, we postulate that the decrease of BRCA1 caused by BCR-ABL could have tremendous consequences due to defective control of genomic stability. The role of BRCA1 in the regulation of the DNA

damage response and cell cycle checkpoint control has been already well explained (Huen et al., 2010; Kim & Chen, 2008; Wuet al., 2010; Yang & Xia, 2010; Zhang & Powell, 2005). The detailed role of BRCA1 in the regulation of mechanisms participating in the occurence of genomic instability as a result of mitosis dysfunctions, referred as a CIN (chromosomal instability) will be discussed in the next paragraphs.

4. The role of BRCA1 in mitosis

4.1 BRCA1 in the transcriptional regulation

Currently, there is a lot of evidence suggesting that BRCA1 is involved in the transcriptional regulation. This opens a new list of possible interactions with intracellular processes (Murray et al., 2007). It has been shown that BRCA1 is a component of the RNA polymerase II (pol II) holoenzyme (Scully et al., 1997). Authors developed a purification strategy for the mammalian pol II holoenzyme to search for specific transcription factors and they found that the wild-type BRCA1 protein was copurified. Moreover, immunopurification of BRCA1 complexes also contained TFIIF, TFIIE and TFIIH transcription factors, which were previously reported to form a complex with the pol II holoenzyme (Maldonado et al., 1996). This strongly suggested that one of the BRCA1 functions is to regulate genes expression.

Unlike many enhancer-specific activators, BRCA1 does not appear to require the specific DNA binding domain to stimulate gene transcription, what was shown by investigation of the p53-responsive promoter *MDM2* (Nadeau et al., 2000). BRCA1 interacts rather with multiple transcription factors. Among them we can name ATF1, a member of the cAMP response element-binding protein/activating transcription factor (CREB/ATF) family. BRCA1 stimulates its transcription from a natural promoter as well as reporter systems (Houvras et al., 2000). Moreover, BRCA1 significantly enhanced the transcription of NF-kappaB target genes due to the binding to p65/RelA, one of the two subunits of the transcription factor NF-kappaB (Benezra et al., 2003). Authors suggested that BRCA1 acts as a coactivator and proposed a model in which BRCA1 interacts physically with p65/RelA, CBP as well as with RNA polymerase II and enhances transcriptional activation of the NF-kappaB target genes. Additionally, MacLachlan reported that p53 can be stabilized by BRCA1 in response to DNA damage and by this selectively transactivated towards genes involved in the growth arrest and DNA repair (MacLachlan et al., 2002). The role of BRCA1 in the regulation of p53-dependent gene expression has been also shown by other groups (Ouchi et al., 1998; Zhang et al., 1998).

BRCA1 is also able to interact with components of the histone deacetylase complex, particularly with HDAC1 and HDAC2 (Yarden & Brody, 1999). It was shown to interact *in vitro* and *in vivo* with the Rb protein as well as with the RB-binding proteins, RBAp46 and RBAp48, which are components of the histone deacetylase complexes and are involved in chromatin remodelling. Involvement of BRCA1 in chromatin remodelling suggests its important role in the regulation of transcription, replication, recombination and others. BRCA1-mediated activation of specific genes may result from sequestration of histone deacetylases from DNA promoters. It was also reported that BRCA1 interacts with the hGCN5/TRAP histone acetyltransferase complex (Oishi et al., 2006), which co-activates the transactivation function of BRCA1.

More recently, BRCA1 has also been shown to play a role in the transcriptional repression by ubiquitin-dependent mechanism (Horwitz et al., 2007). It leads to ubiquitination of the transcriptional preinitiation complex, thus preventing the stable association of TFIIE and

TFIIH transcription factors and blocking the initiation of mRNA synthesis. Amphiregulin (AREG) and early growth response-1 (EGR-1) are examples of genes repressed by BRCA1 in breast cancers. This phenomen could be broader and may contribute to the BRCA1-mediated tumor suppression.

4.2 BRCA1 in the regulation of the mitotic checkpoint

The role of BRCA1 in the regulation of the mitotic checkpoint has been indicated. BRCA1 was identified as a mitotic target of the Chk2 kinase in the absence of DNA damage (Stolz et al., 2010). Accordingly, loss of BRCA1 or its Chk2-mediated phosphorylation led to defects in the spindle formation and chromosomal instability (CIN) due to generation of lagging chromosomes and chromosome missegregation. It was shown that MCF-7 cells transfected with BRCA1 siRNA display a reduced mitotic index followed by premature cyclin B1 degradation upon paclitaxel treatment. This suggested that BRCA1 depletion results in the inactivation of the spindle checkpoint (Chabalier et al., 2006). They presented that BRCA1 up-regulates the expression of the protein kinase BubR1, an essential component of the functional spindle checkpoint. This indicated that BRCA1 directly influences the expression of the mitotic checkpoint components. It was also shown that BRCA1, due to an interaction with the transcription factor OCT-1, mediates the transactivation of Mad2 (mitotic arrest deficient protein 2) (Wang et al., 2004). The studies of BRCA1 knock-down in human prostate and breast cancer cell lines, by using the microarray technique, showed that BRCA1 depletion caused downregulation of many genes involved in mitosis progression (Bae et al., 2005). Specifically, mitotic checkpoint components (Bub1, STK6), proteins involved in the chromosome segregation and centrosome function as well as cytokinesis (including PLK) and finally proteins regulating mitosis entry and progression, such as cyclin B1, Cdc2 and Cdc20 were downregulated.

The influence of BRCA1 on the expression of components of the mitotic checkpoint was also confirmed in our studies (Wolanin et al., 2010). We showed that the downregulation of BRCA1, caused either by BCR-ABL expression or by gene silencing using siRNA, resulted in the downregulation of Mad2 as well as BubR1 and Bub3 gene expression, which all belong to the mitotic checkpoint complex and undergo common regulation. Decreased levels of these proteins finally led to dysfunctions of the mitotic checkpoint and increased occurence of aberrant mitoses and chromosomal instability. Moreover, we observed the increased rate of supernumerary centrosomes as well as aberrant divisions in cells expressing BCR-ABL. We propose that decrease of the BRCA1 protein caused by BCR-ABL could be an important factor participating in the development of genomic instability due to the generation of chromosomally unstable cells. We added the regulation of mitotic checkpoint to the repertoir of BRCA1-mediated mechanisms participating in the development of aneuploidy in CML cells.

Due to its function in the regulation of mitotic checkpoint competence, BRCA1 has been shown to correlate with the sensitivity to spindle poisons (Byrski et al., 2008; Quinn et al., 2007). As mentioned before, cells ability to activate the mitotic checkpoint is necessary for the sensitivity to spindle poisons. BRCA1 downregulation resulted in resistance to microtubule damage due to the inability to efficiently activate the mitotic checkpoint, block cells in mitosis and induce apoptosis. In our studies, cells expressing BCR-ABL with a significantly decreased BRCA1 level were resistant to cell death activated by nocodazole or paclitaxel (Wolanin et al., 2010). This was reversed by imatinib treatment, resulting in

BRCA1 upregulation. In ovarian cancer it was suggested that BRCA1 can act as a predictive marker of response to chemotherapy (Quinn et al., 2009) and dysfunctional BRCA1 resulted in resistance to taxanes and other chemotherapeutics. On the other hand, reconstitution of BRCA1 into ovarian cancer cells, carrying BRCA1 mutation, reversed the resistance and sensitized cells to paclitaxel (Zhou et al., 2003). BRCA1 was also proposed as a predicive marker of drug sensitivity in breast cancer treatment (Mullan et al., 2006). As resistance to spindle poisons has been reported for CML cells, this supports the previously proposed idea, that the overexpression of BRCA1 diminishes some effects of BCR-ABL expression. In our opinion, BRCA1 level could serve as a prognostic marker of sensitivity to different therapies also those used in leukemias.

4.3 BRCA1 in the regulation of centrosome number and function

The first observation that BRCA1 localizes to centrosomes has been made by Hsu et al (Hsu & White, 1998), who showed that BRCA1 is associated with centrosomes during mitosis in a cell cycle-dependent manner. Moreover, they found that BRCA1 forms a complex with gamma-tubulin, which is preferentially associated with the hypophosphorylated form of BRCA1. Gamma-tubulin is a crucial component of centrosomes and is responsible for nucleation of microtubules. Therefore, this confirmed the idea that BRCA1 could play a role in the regulation of centrosome amplification and function and led to the later findings that a BF3 domain of BRCA1 (BRCA1 fragment no. 3, amino acids 504-803) is responsible for the gamma-tubulin binding (Hsu et al., 2001). Overexpression of the BF3 domain in COS-7 cells resulted in the accumulation of mitotic cells with supernumerrary centrosomes and abnormal spindles, what is known to lead to aneuploidization.

The role of BRCA1 in the regulation of centrosome number has been indicated by experiments using the mutated forms of BRCA1. Centrosomal amplification was shown in mouse embryonic fibroblasts carrying a targeted deletion of exon 11 of BRCA1 (Xu et al., 1999) and in a BRCA1-mutant breast cancer cell line HCC1937 (Schlegel et al., 2003). What is important, Waever et al showed that mouse embryonic fibroblasts carrying different BRCA1 defects show supernumerary centrosomes and other features similar to human breast cancer cells, indicating that the mechanisms are conserved between mice and humans (Weaver et al., 2002).

Moreover, immunohistochemical analysis of 50 samples from breast cancer patients showed that numerical centrosome aberrations were signicantly associated with the negative BRCA1 expression as well as with the BRCA1 germline mutation, whereas there was no significant correlation with the centrosome aberrations in size (Shimomura et al., 2009). This suggests that BRCA1 plays a role rather in the regulation of centrosome duplication and defects in its expression or function result in numerical aberrations. Very recently, direct studies of 14 different missense mutations in the RING domain of BRCA1 and their influence on the control of centrosome number were performed (Kais et al., 2011). Authors showed that only 2 out of the 14 BRCA1 variant proteins were neutral in the centrosome duplication assay. The others were either very effective and resulted in mutated BRCA1 proteins that caused centrosome amplification (C24R, C27A, C39Y, H41F, C44F, C47G, M18T and I42V) or had an intermediate, however still significant effect on centrosome duplication (I21V, I31M, L52F and D67Y).

Interestingly, we also observed a correlation between the loss of BRCA1 expression and increased percentage of cells with supernumerary centrosomes in murine lymphoid cells

expressing BCR-ABL oncogene (Wolanin et al., 2010). This was in contrast with the hypothesis that BRCA1 defects lead to centrosome amplification in breast cells but not in other types of cells (Starita et al., 2004). This idea has been based on the data obtained using the transient expression of the BRCA1-inhibiting BIF peptide in nine different cell lines, where four non-breast cell lines - prostate (PC3), cervix (HeLa), colon (DLD-1) and osteosarcoma (U2OS), did not accumulate extra centrosomes. However, lymphoid cells were not included in these studies. To date, there were other indications, apart from ours, that the loss or mutation of BRCA1 could affect the centrosome number also in other types of cells. Recently, it was shown that BCR-ABL intereferes with the Fanconi Anemia (FA)/BRCA pathway and the ectopic expression of BRCA1 in CD34+ progenitor cells reversed the appearance of aberrant centrosomes, thus confirming our previous observations (Valeri et al., 2010).

The direct mechanism of BRCA1-mediated control of centrosome number is still not fully clear, although the BRCA1-dependent ubiquitination of gamma-tubulin is proposed to be involved in the regulation of centrosome function (Staritaet al., 2004). Gamma-tubulin is an important protein involved in the initiation of microtubule nucleation by centrosomes. Gamma-tubulin's lysines 48 and 344 have been indicated as crucial in the regulation of centrosome duplication and microtubule nucleation function, respectively (Sankaran et al., 2005). Cells with mutated lysines on gamma-tubulin, unable to be ubiquitinated, were characterized by centrosome amplification. On the other hand, the same phenotype was observed after inhibition of the enzymatic activity of BRCA1 by transfection of the BRCA1 (I26A) ligase-defective mutant (Sankaran et al., 2006). Additionally, *in vitro* experiments using *Xenopus* extracts, purified centrosomes and BRCA1 together with ubiquitination factors confirmed that BRCA1 is involved in the microtubule nucleation. It seems that BRCA1 controls the centrosome number by preventing reduplication due to ubiquitination of lysines of gamma-tubulin, which needs to be phosphorylated to prevent reduplication (Ko et al., 2006). Loss of BRCA1 did not affect centrosome duplication in the early S phase but rather caused a second round of duplication just prior to mitosis. The model has been proposed, in which BRCA1 marks centrosomes as already duplicated *via* the BRCA1-mediated ubiqutination of gamma-tubulin (Wong & Stearns, 2003). This issue is still not fully clarified, however there is no doubt about the significant role of the BRCA1-mediated ubiquitination of gamma-tubulin in this process (Kais & Parvin, 2008). Altogether, this led to the conclusion that the E3 ubiquitin ligase activity of BRCA1 is crucial for the effects on the biology of centrosomes, and controls centrosome duplication as well as microtubules nucleation.

Recently, it was demonstrated that BRCA1 interacts with centrosomal protein Nlp (ninein-like protein) (Jin et al., 2009), which is a fast turnover protein and plays a role in the centrosome maturation and spindle formation (Casenghi et al., 2005). Authors found that Nlp is a BRCA1-associated protein and colocalizes with BRCA1 in different types of cancer cells, including HeLa and U2OS cells. Moreover, Nlp expression and stability depends on normal cellular BRCA1 function. A variety of different types of cells expressing the mutated BRCA1 or silenced for BRCA1 exhibited disrupted Nlp colocalization to centrosomes as well as enhanced Nlp degradation. This data was consistent with our observations concerning the role of BRCA1 in different types of cancers. The lack of Nlp protein led to centrosome amplification, aberrant chromosome segregation, cytokinesis failure and appearance of miltinuclei, thus resembling the phenotype upon BRCA1 disruption. Recent studies showed that Nlp is recruited by the Aurora B protein and localizes at the midbody during

cytokinesis, thus its depletion or increased degradation triggers aborted division and subsequently leads to multinucleated phenotypes (Yan et al., 2010).
Altogether, this data strongly supported the idea that BRCA1 is one of the key elements controlling mitosis and the loss of BRCA1 could result in very severe dysfunctions of cell division. We propose that this can significantly participate in the generation of aneuploidy, CML progression and blastic transformation. A proposed model showing the influence of BCR-ABL-mediated downregulation of BRCA1 on the occurrence of genomic instability and aneuploidy in CML cells is presented in Figure 2.

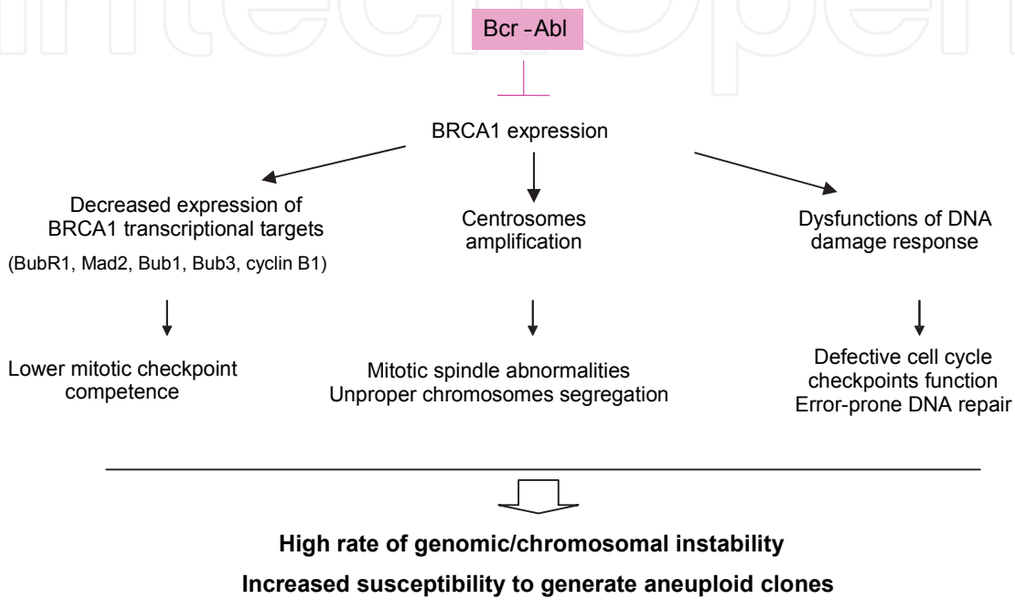

Fig. 2. Proposed scheme of the mechanisms influenced by BCR-ABL-mediated BRCA1 downregulation; role in the genomic instability and generation of aneuploid cells.

5. Therapeutic targeting of mitosis in CML cells

The effects of the improper control of mitosis in the development and progression of leukemias, including chronic myeloid leukemia has been already described above. The importance of these processes and their potential as targets for therapy is already obvious. In general, looking for new treatment options or combined therapies is still necessary to overcome the insensitivity or resistance to tyrosine kinase inhibitors, often developed in CML patients. In our opinion, targeting the chromosomal passenger complex and mitotic kinases is a very promising trend in the development of novel anti-leukemia therapeutic strategies. We will discuss the current data and implications for the future.

5.1 Chromosomal passenger complex and Aurora kinases

The chromosomal passenger complex (CPC) is a group of proteins, which are involved in the regulation of nearly all stages of mitosis (Vader et al., 2006; Vagnarelli & Earnshaw, 2004; Yanet al., 2010). In most organisms, the chromosomal passenger complex is formed by four main proteins: Aurora B kinase, INCENP, Survivin and Borealin/Dasra-B (Ruchaud et al., 2007). Other proteins, like telophase disk 60 kDa (TD-60) have been shown to interact with

the CPC proteins and have a typical localization, however they are not members of the core complex. The complex undergoes a characteristic scenario of translocations during mitosis – they localize at the inner centromeres in prometaphase and metaphase then, at anaphase onset, they leave the chromosomes and transfer to the kinetochores at the central spindle to finally move to the midbody at cytokinesis. It plays a crucial role in the regulation of chromatin condensation, kinetochore function, mitotic checkpoint competence as well as cytokinesis (Terada, 2001; Vaderet al., 2006). Very recently it was presented that the chromosomal passenger complex is essential for correcting the non-bipolar chromosome attachments and for cytokinesis (Becker et al., 2010). To do this, Aurora B and INCENP have to be localized to centromeres. This is a very important finding as it indicates the supportive role of the CPC complex in case of mitotic checkpoint failure. Moreover, the translocation of Aurora B and other CPC proteins from centromeres to the spindle midzone in anaphase is necessary to prevent mitotic checkpoint engagement at anaphase (Vazquez-Novelle & Petronczki, 2010).

Members of the CPC complex have been proposed as very potent therapeutic targets. Treatment of imatinib-resistant CML cells carrying the T315I mutation with small molecule inhibitor, PHA-739358, which selectively targets BCR-ABL and Aurora kinases led to strong antiproliferative and apoptotic effects (Gontarewicz et al., 2008). Moreover, this has also been observed in CD34+ cells derived from untreated CML patients and from imatinib-resistant patients in the chronic phase or blast crisis, including those harbouring the T315I mutation. Similar effects were obtained by combined treatment of imatinib resistant CML cells with vorinostat together with Aurora kinase inhibitor MK-0457 (Dai et al., 2008). Effectivity of this combined treatment has been shown against primary CD34+ CML cells, murine Ba/F3 cells with various BCR-ABL mutations (T315I, E255K, and M351T), as well as in imatinib-resistant K562 cells with BCR-ABL-independent, Lyn-dependent resistance. The same combination of therapy was presented also in other studies by Fiskus et al (Fiskus et al., 2008). Authors studied different CML cell lines, murine cells expressing BCR-ABL as well as primary CML and AML cells and came to the same conclusions. Novel inhibitors of Aurora kinases are still investigated in the anti-leukemia therapy of imatinib-resistant cells (Fei et al., 2010; Kelly et al., 2010). Usually they are proposed to be used together with tyrosine kinase inhibitors, such as imatinib or dasatinib.

Survivin has been also proposed as a universal target for anticancer therapy (Andersen et al., 2007). Several trials are currently undergoing, using different methodologies, from small molecule antagonists to immunotherapy (Kanwar et al., 2010). However, the development of survivin inhibitors is not as advanced as other therapeutic small inhibitors. It is important to point that anti-survivin therapy should be probably combined with other treatments, as it is known that survivin depletion uncovers the function of the mitotic as well as post-mitotic p53-p21-dependent checkpoints, which protect from polyploidization upon mitosis disturbances (Beltrami et al., 2004). In case of cancers with the defective p53 function, survivin silencing led to reduced mitotic arrest and enhanced polyploidy, what is a very unwanted and dangerous side-effect. Also in our studies, specific depletion of survivin by siRNA approach in CML cells with checkpoints defects, resulted in strong polyploidization and chromosomal instability (Wolanin et al., 2006). However, when we used a natural compound – curcumin, which has been shown as a broadly acting, very potent anticancer agent, we found that it affects the CPC proteins and induces mitotic catastrophe, however without polyploidization. Curcumin decreased the level of survivin and caused unproper localization of Aurora B, leading to perturbances in mitosis and defective cytokinesis.

Thus, the status of the mitotic checkpoint competence as well as p53 should be taken into consideration when anti-survivin therapy is proposed. Another natural compound, 16-hydroxycleroda-3,13-dien-15,16-olide (PL3), one of the clerodane diterpenoid compounds isolated from *Polyalthia longifolia*, induced degradation of Aurora B, mitotic checkpoint dysfunctions and finally led to cell death of CML cells, including the T315I-mutated BCR-ABL+ BA/F3 cells (Lin et al., 2011). Additionally, it reversed the sensitivity to imatinib of T315I-mutated CML cells in comparizon to treatment only with imatinib.
Recently, Aurora inhibitors were indicated as promising agents for acute myeloid and chronic myeloid leukemias (Moore et al., 2010). The most promising data was obtained for FLT3-mutated AML and imatinib-resistant Ph+ CML, particularly with the T315I mutation. Clinical trials investigating these agents have been already initiated (Cheung et al., 2011).

5.2 Polo-like kinases

Polo-like kinases are of strong interest according to potential anticancer therapy, as similarly to Aurora kinases, they can be targeted with selective small molecule inhibitors (Warner et al., 2008). Additionally, many natural compounds with the ability to prevent cancerogenesis, such as wortmanin, quercetin, thymoquinone, genistein, indirubin and others, have been shown to modulate PLK1 level or activity. It is proposed that naturally occurring PLK1 inhibitors with low or no toxicity should be considered as interesting agents in prevention as well as treatment of cancer (Schmit et al., 2010).
As described before, PLK1 has been upregulated in different cancers, including leukemias. Its inhibition or silencing resulted in cell cycle arrest, decrease of cell viability and induction of apoptosis in various cancer cells. Inhibition of PLK1 by different small molecule compounds in acute myeloid leukemia (AML) cells led to mitotic accumulation and apoptosis (Didier et al., 2008). Comprehensive studies of PLK1 silencing and inhibition using the novel selective inhibitor GW843682X in a broad range of different leukemia cell lines and primary cells led to the conclusion that PLK1targeting can be a promising strategy (Ikezoeet al., 2009). This observation was confirmed by other studies of leukemia primary cells (Renneret al., 2009). The potential of PLK1 inhibition to improve the chemotherapy or irradiation of resistant leukemia cells has been also investigated in primary patient cells as well as *in vivo,* in mouse Xenograft models of B-lineage ALL studies, with the same conclusions (Uckun et al., 2010). Currently, several PLK1 inhibitors are in different phases of clinical development for anticancer therapy (Chopra et al., 2010; Schoffski, 2009). Data from one of the first clinical trials indicated that the PLK1 inhibitor BI 2536 was well tolerated and showed antitumor activity in patients with advanced solid tumors and refractory or relapsed AML (Wasch et al., 2010). According to CML, this scheme of therapy was not very intensively studied till now, however it also seems to be very potent and prospective. PLK1 inhibitor BI 2536 in a low, nanomolar concentration was able to induce growth inhibition and mitotic arrest followed by apoptosis in CML cells, including cell lines and primary cells from patients (Gleixneret al., 2010). Importantly, this agent was very effective not only against imatinib-sensitive CML cells, but also imatinib-resistant cells carrying the T315I mutation. Treatment with BI2536 together with imatinib or nilotinib showed synergistic effect, indicating possibility of a combined therapeutic application.

6. Final conclusions

Taken together, it is already clear that BRCA1 due to its multifunctional nature, is one of the key molecules controlling mitosis on the different levels of organization. There is no doubt

that the decrease of BRCA1 caused by BCR-ABL in CML cells could be a critical factor determining the generation of supernumerary centrosomes, aberrant mitotic spindles, mitosis and cytokinesis failures, finally leading to aneuploidization. All data presented in this review convincingly show that activity of the BCR-ABL kinase is directly responsible for the promotion of chromosomally unstable phenotype. As chromosomal instability seems to play a crucial role in the disease progression, mitosis is a prospective target for treatment in CML. This opens new possibilities for therapeutical intervention based on the targeting processes involved in the control of mitosis. It can be an alternative strategy for alone or combined treatment of leukemia cells, which developed resistance to imatinib or second-generation tyrosine kinase inhibitors, such as dasatinib or nilotinib.

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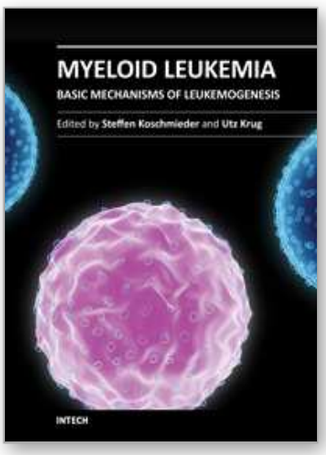

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The current book comprises a series of chapters from experts in the field of myeloid cell biology and myeloid leukemia pathogenesis. It is meant to provide reviews about current knowledge in the area of basic science of acute (AML) and chronic myeloid leukemia (CML) as well as original publications covering specific aspects of these important diseases. Covering the specifics of leukemia biology and pathogenesis by authors from different parts of the World, including America, Europe, Africa, and Asia, this book provides a colorful view on research activities in this field around the globe.

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