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REVIEW

While at Rome miRNA and TRAIL Do Whatever BCR-ABL Commands to Do

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Abstract It is a well-acclaimed fact that proteins expressed as a consequence of oncogenic fusions, mutations or amplifications can facilitate ectopic protein–protein interactions that re-wire signal dissemination pathways, in a manner that escalates malignancy. BCR-ABL-mediated signal transduction cascades in leukemic cells are assembled and modulated by a finely controlled network of protein–protein interactions, mediated by characteristic signaling domains and their respective binding motifs. BCR-ABL functions in a cell context-specific and cell type-specific manner to integrate signals that affect uncontrolled cellular proliferation. In this review, we draw attention to the recent progress made in outlining resistance against TRAIL-mediated apoptosis and diametrically opposed roles of miRNAs in BCR-ABL-positive leukemic cells. BCR-ABL governs carcinogenesis through well-organized web of antiapoptotic proteins and over-expressed oncomirs which target death receptors and pro-apoptotic genes. Set of oncomirs which inversely correlate with expression of TRAIL via suppression of SMAD is an important dimension which is gradually gaining attention of the researchers. Contrary to this, some current findings show a new role of BCR-ABL in nucleus with spotlight on apoptosis. It seems obvious that genetic heterogeneity of leukemias poses therapeutic challenges, and pharmacological agents that target components of the cancer promoting nano-machinery still need broad experimental validation to be considered competent as a component of the therapeutic arsenal for this group of diseases. Rapidly developing technologies are empowering us to explain the molecular "nature" of a patient and/or tumor and with this integration of personalized medicine, with maximized efficacy, cost effectiveness will hopefully improve survival chances of the patient.

Keywords BCR-ABL · TRAIL · miRNA · Apoptosis · Cancer

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Introduction

Leukemia is characterized by hallmark features including recurring chromosomal aberrations and gene mutations that are fundamental to disease progression and pathogenesis. A supplementary layer of complexity is the role of tumor suppressor microRNAs (miRNAs) and oncomirs in leukemias. It is becoming progressively more prominent by various documentations that BCR-ABL signaling network shows a modular and layered organization. Furthermore, this modular and phosphorylation-driven interaction network provides a distinct scaffold for the integration of pleiotropic signaling effects of BCR-ABL that trigger leukemic transformation.

Accumulating sophisticated findings suggest that oncogenic fusions reassemble the circuitry and nanomachinery. In addition, how fusion proteins modulate microRNAome in leukemic cells and dampening of TRAIL-mediated responses in cells has been less appreciated.

TRAIL and BCR-ABL: Unending Rivalries Unleashed

TRAIL is a death receptor ligand that has the capability to preferentially trigger apoptosis in cancerous cells with negligible toxicity to normal cells. It has emerged as an important regulator that has documented efficacy and minimal off target effects. Changes in endogenous TRAIL and TRAIL receptor expression during the leukemogenesis and the mode in which the expression pattern is affected by treatment are of great focus, and understanding the biological consequences of such changes will be vital to take full advantage of the potential of TRAIL-based therapeutics.

It is becoming increasingly apparent that BCR-ABL-positive cells are resistant to TRAIL-mediated apoptosis. Additionally, BCR-ABL utilizes various cytoplasmic and nuclear modulators which dampen the expression of pro-apoptotic genes or hamper the TRAIL-mediated signal transduction by enhancing the expression of negative regulators of signaling. In accordance with this interpretation, recent study indicated that PRAME is upregulated in BCR-ABL cells and negatively regulates expression of TRAIL and is tightly inter-related with disease aggressiveness in chronic myeloid leukemia (CML) patients. Notably, abrogation of PRAME or EZH2 by RNA interference in a BCR-ABL-positive cell line recapitulated TRAIL expression. Laboratory investigations unveiled the fact that there is an enrichment of EZH2 on the promoter region of TRAIL in a CML cell line; however, targeted inhibition of PRAME resulted in attenuation of binding affinity (De Carvalho et al. 2011).

Recently, a research group studied correlation between BCR-ABL expression and TRAIL responsiveness in three imatinib-resistant K562/R1, R2 and R3 variants with gradual loss of BCR-ABL from K562 cells to design therapeutic interventions for imatinib-resistant CML. Data derived from experimentations highlighted the fact that BCR-ABL-silenced K562 cells displayed suppression of c-FLIP and the consequent resensitization to TRAIL. Altogether, findings demonstrated that there is a positive correlation between BCR-ABL and cFLIP as the loss of BCR-ABL in imatinib-resistant cells led to the down-regulation of c-FLIP and consequent increase of TRAIL sensitivity (Park et al. 2009a).

Death receptor expression is repressed in leukemic cells and parallel decline in availability of these receptors on plasma membrane for TRAIL. Recent data suggest that Plumbagin exposure results in up-regulation of DR4 and DR5 in leukemic cells; however, plumbagin-induced increase in DR4 and DR5 is attenuated upon treatment with *N*-acetyl cysteine (Sun et al. 2011). It is worth mentioning that apicidin, a novel histone deacetylase inhibitor, could circumvent the TRAIL refractoriness in CML-derived K562 cells and concurrently suppressing expression of BCR-ABL (Park et al. 2009b). TRAIL has been documented to induce apoptosis in the Ph1-positive leukemia cell lines that are insensitive to a BCR-ABL-specific tyrosine kinase inhibitor imatinib mesylate (Uno et al. 2003).

Another study underscored comparative analysis of apoptotic effects of Apo-2L/TRAIL, with or without co-treatment with STI-571, in HL-60/neo, HL-60/Bcr-Abl, and K562 cells. It is interesting to note that as compared to HL-60/neo, HL-60/Bcr-Abl and K562 cells are relatively resistant to Apo-2L/TRAIL-induced apoptosis. However, combinatorial drug design might offer effective approach for enhancing sensitivity of resistant cells to TRAIL. Results demonstrated that cotreatment with STI-571 considerably enhanced Apo-2L/TRAIL-induced apoptosis irrespective of the levels of DR4, DR5, decoy receptors, or c-FLIP(L) (Nimmanapalli et al. 2001). However, there is a paradigm shift in the classical concept that BCR-ABL-positive cells desensitize cells to TRAIL-mediated apoptosis. Recently, it has been reported that Ph^+ leukemia cells frequently express DR4 and DR5 and exposure of cells to imatinib specifically downregulated DR4 and DR5 expression. Astonishingly, targeted inhibition of BCR-ABL also downregulated DR4 and DR5 expression in Ph^+ leukemia cells, and Ph^- leukemia cells reconstituted for BCR-ABL displayed considerable DR4 and DR5 expression, which was abrogated upon imatinib treatment (Kuroda et al. 2012). Parallel findings further provided evidence that cell lines and patients' samples of t(17;19)-ALL expressed death receptors for TRAIL, and recombinant soluble TRAIL-induced apoptosis in t(17;19)-ALL cell lines. Transient transfection of E2A-HLF into non-t(17;19)-ALL cell line triggered the expression of DR4 and DR5 and sensitized cells to TRAIL-mediated apoptosis (Zhang et al. 2012b).

It is worthwhile to evaluate that exposure of leukemia blasts to cytosine arabinoside and etoposide resulted in a decline in cell death marker lactate dehydrogenase and an increase in the nucleosomes in the supernatants at 0, 24, 48, 72, and 96 h after drug application. In addition, upon exposure of HepG2 cells to TRAIL also resulted in a corresponding increase in nucleosomes dose-dependently 24 h after exposure to TRAIL and reached a peak at 48 h (Holdenrieder et al. 2012).

HL-60 cells treated with 2′-nitroflavone displayed upregulation of both the TRAIL and its death receptor DR5 (Cárdenas et al. 2012). It has recently been reported TRAIL-resistant human leukemic K562 cells treated with Amurensin G, a novel SIRT1 inhibitor, are re-sensitized to TRAIL-induced apoptosis (Kim et al. 2012). Animal model studies indicated that in vitro treatment of xenograft cells with TRAIL notably reduced and delayed their engraftment

and enhanced animal lifespan (Castro Alves et al. 2012). Gemtuzumab ozogamicin is a well-known targeted therapeutic intervention in which an anti-CD33 antibody is chemically linked to an exceedingly cytotoxic calicheamicin derivative through a hydrolysable linker. Unfortunately, its use is linked with severe myelosuppression and hepatotoxicity. To minimize the off target effects and simultaneously targeting CD33, scFvCD33: sTRAIL was designed in which an anti-CD33 single chain fragment of variable regions (scFv) antibody fragment was genetically linked to soluble tumor necrosis factor-related apoptosis-inducing ligand (sTRAIL). Normal CD33-positive monocytes were fully resistant to prolonged treatment with scFvCD33:sTRAIL, while treatment with Gemtuzumab Ozogamicin resulted in considerable cytotoxicity and tissue toxicity (ten Cate et al. 2009).

The above-described contradictions make it complicated to derive solid conclusions on the diametrically opposed roleplay of the BCR-ABL fusion protein in terms of regulation of cell surface appearance of death receptors and decoy receptors. How BCR-ABL-positive cells decide to retrieve the death receptors back to the cytoplasmic compartments and are these proteins degraded and if not why these proteins are not docked to the plasma membrane. There is another important and outstanding question to be asked and answered: Are these BCR-ABL-driven leukemogenesis studies targeting the right cells? More importantly, how BCR-ABL-positive cells license and antagonize cell surface appearance of death receptors is still a mystery that needs detailed analysis.

It is remarkable that with the introduction of new and powerful investigative tools that enable the detection of cytogenetically cryptic rearrangements, this fraction is likely to increase to a large extent.

Micro Traffic Cops Remove "BCR-ABL Roadblocks" on TRAIL

It is a well-established fact that miRNA has irreconcilable roles in cellular activities. In the upcoming section, we discuss positive and negative regulation of target genes in BCR-ABL-positive cells.

miRNAs are fundamental elements in the post-transcriptional regulation of gene expression. Signalling pathways are well-studied models for miRNA-mediated regulation owing to the sharp dose-sensitive nature of their effects. Certainly, data points towards misrepresented miRNA signature that underlies dysregulated expression of target genes in BCR-ABL-positive cells. In the forthcoming section we discuss the modes by which miRNAs regulate nodes of signalling networks that mediate opposing cellular activities in BCR-ABL-positive cells.

Laboratory investigations suggest that cells reconstituted for miR-203 display considerable downregulation of ABL1 and BCR-ABL1 fusion protein levels. On a similar note, re-establishment of miR-203 expression reduces ABL1 and BCR-ABL1 levels and counteracts uncontrolled cellular proliferation (Bueno et al. 2008; Faber et al. 2008). An analysis of available data shows that miR-150 inhibition concomitantly restores the expression of c-MYB in BCR-ABL-positive cells, and may be involved in BCR-ABL driven leukemogenesis (Machová Poláková et al. 2011). BCR-ABL kinase activity negatively regulates expression of miR-451 (Lopotová et al. 2011). In contrast, another study added another dimension and showed increased miR-451 expression despite the presence of high number of Ph^+ cells and/or BCR-ABL transcripts. This was investigated during imatinib (IM) therapy and also in differential Dasatinib responsiveness in patients (Scholl et al. 2012). Although, these findings verify the reported inverse correlation between BCR-ABL and miR-451 levels, and introduce new data supporting the idea that miR-451 expression levels at diagnosis are interconnected with response to IM therapy. Simultaneously, it contributes to the concept of the intricacy of the mechanisms underlying TKI therapy resistance in CML, and to an improved knowledge of the dynamics of miR-451 expression during TKI treatment, thus reinforcing a putative role of miRNAs in the resistant phenotype.

Consistent with the same approach, inhibition of BCR-ABL1 resulted in de-repression of hsa-miR-150 and hsa-miR-151. Proof of the concept was provided by analysis of the expression of these miRNAs by Q-RT-PCR in the Mo7e and Mo7e-p210 cell line before and after treatment with imatinib. Intriguingly, Mo7e-p210 cells displayed a reduced expression of hsa-miR-150 and hsa-miR-151 in contrast to Mo7e cells as treatment with imatinib and induced a robust expression of both miRNAs in Mo7e-p210 cells (Agirre et al. 2008). In agreement with same notion, another finding suggested significant inverse correlation of miR-150 expression with BCR-ABL transcript level that existed among all patient samples analyzed. To check whether miR-150 is triggered by BCR-ABL, a Ph^+ cell line MOLM-7 was used and incubated with varying concentrations of imatinib (1 and 10 μM). It was observed that following reduction of BCR-ABL tyrosine kinase activity, exemplified by decreased intensity of its downstream protein p-CRKL by imatinib, the miR-150 expression was considerably upregulated (Machová Poláková et al. 2011). It is also established that several miRNAs misrepresented in leukemia (miR-150, miR-146a, miR-142-3p, miR-199b-5p) are rapidly recapitulated under imatinib treatment (Flamant et al. 2010).

However, a paradigm in parallax also exists with reference to a set of miRNA which re-strengthens

BCR-ABL1-mediated signaling. Abrogation of BCR-ABL resulted in remarkable suppression of miR-130a, miR-130b, miR-148a, miR-212 and miR-425-5p. The re-introduction of mature sequences of miR-130a and miR-130b individually into BCR-ABL deficient HL60 cells suppressed CCN3 mRNA and protein. It seems intriguing that miR-130 is triggered by BCR-ABL and represses expression of CCN3 (Suresh et al. 2011). Recently, a research group documented BCR-ABL- and c-MYC-dependent regulation of miRNA expression using microarray analysis (miCHIP) and miRNA-specific quantitative real-time reverse transcriptase-polymerase chain reaction (miR-qRT-PCR). In a series of experiments, it was observed in various bcr-abl-positive cell lines that expression of miRNAs was encoded within the polycistronic miR-17-92 cluster. Additionally, there was an outstanding down-regulation of polycistronic miR-17-92 cluster after treatment with imatinib treatment or targeted inhibition of BCR-ABL (Venturini et al. 2007). Concordantly, concomitant expression of miR-125b and the BCR-ABL fusion gene in transplanted cells escalated leukemia in mice, compared with control mice expressing only BCR-ABL, signifying the fact that miR-125b worked with effective synergy with BCR-ABL (Bousquet et al. 2010).

It is believed that some miRNAs are aberrantly expressed in $CD34^{+}$ population and total leukocytes of CML patients (Agirre et al. 2008). It is clear that miRNA positively and negatively regulates BCR-ABL1-mediated signaling. It is comprehensible that tumor suppressor miRNAs undergo suppression that might be because of miRNA mutations or mis-expression that strongly correlates with hyperactive BCR-ABL1mediated signaling. Therefore, it is necessary to identify various sets of tumor suppressor miRNA which can effectively repress the expression of important cancer-related genes and might prove useful in the diagnosis and treatment of cancer.

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MiRNA and TRAIL: Every Cloud Has a Silver Lining

Emerging body of evidence suggested that miR-25 antagonized TRAIL-induced apoptosis. Thorough mechanistic insights indicated that TRAIL Death Receptor-4 (DR4) acted as a potential novel miR-25 target, and these findings were further confirmed by immunoblot, cell staining, and reporter assays (Razumilava et al. 2012).

Likewise, circumstantial studies indicate that some miRNAs (miR-221&222) impair TRAIL-mediated apoptosis. An overexpression of miR-221&222 results in abrogation of TRAIL-mediated signaling along with suppression of PTEN (Garofalo et al. 2008, 2009).

Additionally, miR-221 silencing predisposed T24 cells to undergo cell death induced by TRAIL and resulted in an upregulation of cyclin-dependent kinase inhibitor p27Kip1 (Lu et al. 2010). Similarly, relationship between p27(kip1) and or miR-221/222 was explored in U251 human glioblastoma cells. Therefore, miR-221/222 antisense oligonucleotides were transfected into U251 human glioblastoma cells that resulted in upregulation of p27(kip1) in the anti-miR-221/222 group (Zhang et al. 2009).

miR-185 is also documented to be involved in overcoming refractoriness against TRAIL by repressing Six1 (homeobox protein) by binding to its 3′-untranslated region (3′-UTR) (Imam et al. 2010). Emergent advanced information revealed the presence of two putative E-box (Myc-binding) sites, a Gli-binding site, and four NF-κB-binding sites in the region encompassing the transcriptional start site of miR-29 promoter. Confluence of findings suggested that various effectors of signal transduction cascades dampened TRAIL-mediated apoptosis via repressing miR-29b expression (Mott et al. 2010). Lately, role for miR-26a, miR-145 and miR-26a in TRAIL-induced apoptosis is demonstrated by Sudbery et al. (2010). Likewise, introduction of the miRNAs let-7c, miR-10a, miR-144, miR-150, miR-155, and miR-193 influenced the activation of the caspase cascade (Ovcharenko et al. 2007). Cells reconstructed for miR-15b displayed remarkable escalation in TRAIL-mediated apoptosis (Chung et al. 2010). However importantly, single miRNAs can have multiple target sites in the 3′-UTRs of a specific mRNA, thereby increasing repression efficiency. It can be assumed that suppression of tumor suppressor miRNA severely compromises the multi-site inhibitory effect on an oncogene. In addition, cancer gene mRNAs are predicted to be targets of many distinct miRNAs, signifying that different miRNAs might act in a synchronized manner to regulate mRNA translation and turnover. Therefore, it is perceptible that oncogenes escape translational repression or mRNA decay by suppressing a set of target specific miRNA and simultaneously facilitating overexpression of set of oncomirs which can trigger enhanced degradation or translational repression of tumor suppressors. As discussed above, various sets of miRNA have also been revealed to impinge on multiple targets in linear pathways or interconnected nodes in regulatory networks, thereby exerting a larger cumulative effect. miRNAs are also recurrently documented to act

in feedforward and feedback regulations that can intensify or attenuate signal output.

Regulation occurs in all horizontal layers of signalling networks and these transduction cascades temporally control stability, biogenesis and abundance of miRNAs, by regulating layers of the miRNA biogenesis pathway. In the upcoming section, we use an example to illustrate the extensive interdependence between miRNAs and signalling networks.

It is becoming progressively more understandable that MET triggers the expression of miRNAs and likewise there is posttranscriptional control of the *MET* mRNA by miRNAs. In line with this concept, it is obvious that exogenous expression of miR-34b, miR-34c, and miR-199a* remarkably decreased the level of MET mRNA and protein in tumor cells (Dong and Lou 2012). Investigational studies with a luciferase reporter fused to the 3′-untranslated region of the MET gene presented miR-199a*-mediated down-regulation of luciferase activity through a binding site of miR-199a*. Interestingly, extracellular signal-regulated kinase 2 was also considerably down-regulated by miR-199a*, thus depicting a multipronged approach in targeted inhibition of MET signaling (Kim et al. 2008).

Furthermore, miRNAs were also efficient in restricting the invasive growth ability of "MET-addicted" cancer cells that express high levels of a constitutively activated receptor. In the same line of opinion, it is believed that expression of miR-34b, miR-34c, and miR-199a* if suppressed could be instrumental to increased MET expression.

It has recently been reported that miR-30b, miR-30c, miR-221 and miR-222 are modulated by both epidermal growth factor (EGF) and MET receptors (Garofalo et al. 2011). Cells reconstituted for miR-130a repressed the expression of MET and overcome TRAIL resistance through the c-Jun-mediated downregulation of miR-221 and miR-222 (Acunzo et al. 2012). In a similar manner miR-340, miR-198 also repressed the expression of MET (Tan et al. 2011; Wu et al. 2011).

PED/PEA-15 (PED) is an anti-apoptotic protein, frequently over-expressed in various cancers and is negatively regulated by miR-212. Cells having overexpressed PED and downregulated miR-212 are resistant to TRAIL-mediated cell death (Incoronato et al. 2010). Reporter gene assays also verified miR-212 to target the 3′-UTR region of ABCG2. It was observed in K-562 cells that overexpression of ABC efflux transporters resulted in imatinib resistance (Turrini et al. 2012). Although no correlation between miR-212 and BCR-ABL has been extensively explored; however, it will be attractive to note miRNA expression in BCR-ABL-positive cells and how suppression of tumor suppressor miRNA in turn desensitizes cells to chemotherapeutic drugs and TRAIL-mediated apoptosis. As some hints have appeared in a finding addressing the fact that miR-212 also negatively regulates heparin-binding EGF-like growth factor and thus re-sensitizes cells to cetuximab (Hatakeyama et al. 2010).

Therefore, targeted inhibition of miR-221 is significant in de-repressing the attenuated sensitivity of cells to TRAIL (Lu et al. 2010).

Currently research is heading towards multipronged approach and consequently lesser off target effects. It has recently been shown that combinatorial drug design consisting of TRAIL and Bcl2 downregulation was better than the mono-therapy of TRAIL or synthetic Bcl2 miRNA alone (Zhang et al. 2010).

It is appealing to observe that TRAIL itself is mediated by broad range of signaling cascades which often are dysregulated or respective cascades are dampened by negative effectors. Recently, we have shown that TRAIL expression is triggered by transforming growth factor signaling (Farooqi et al. 2011). However, SMAD4 deficient cells displayed attenuated expression of TRAIL. miR-146a negatively modulated expression of SMAD4 (Geraldo et al. 2011; Liu et al. 2012; Xiao et al. 2012; Zhong et al. 2010). On a similar note, miR-483-3p and SMAD4 expression levels are inversely correlated (Hao et al. 2011). Collectively, we have observed that enforced expression of miR-146a and miR-483 repressed the expression of TRAIL alongwith notable suppression of SMAD4 (unpublished observations). In future, it will be important to outline role of miRNAs in BCR-ABL1-positive leukemic cells using mouse models to understand the roles of miRNAs in cancer and the potential for manipulating miRNAs for cancer therapy as these molecules make their way towards clinical trials.

BCR-ABL and Genomic Instability

SIRT1 is an important regulator of the sirtuin family that inactivates p53 by deacetylating a critical lysine residue. SIRT1triggers activity of error-prone DNA damage repair, in association with acquirement of genetic mutations (Wang et al. 2012). When leukemic cells are simultaneously exposed to nicotinamide and etoposide, there is a significant increase in miR-34a levels with a concomitant inhibition of SIRT1. Nicotinamide has the dual property of inhibiting SIRT1 and simultaneously inducing miR-34a (Audrito et al. 2011).

Targeted inhibition of oncoprotein Bcr-Abl by imatinib could cause a down-regulation in the expression of key DNA repair genes (Benito et al. 2012).

Prominently, BCR-ABL1 (nonmutated and T315I mutant) facilitated RAD51 recombinase-mediated unfaithful homeologous recombination repair (HomeoRR) in a

dosage-dependent mode. In this particular mechanism, BCR-ABL1 SH3 domain interacted with RAD51 proline-rich regions, resulting in direct phosphorylation of RAD51 on Y315 (pY315). RAD51 (pY315) facilitated uncoupling from the complex with BCR-ABL1 kinase, migrated to the nucleus, and enhanced formation of the nuclear foci indicative of recombination sites. Cells reconstituted for RAD51(Y315F) displayed reduction in HomeoRR and RAD51 nuclear foci (Slupianek et al. 2011a).

Leukemia cells expressing BCR-ABL1 kinase showcased robust histone acetylase activity and reduced histone deacetylase activity (HDAC), which was connected with abundant expression of acetylated histones 3 and 4 (Ac-H3 and Ac-H4, respectively). Besides, Ac-H3 and Ac-H4 instantaneously co-localized with the spontaneous and mitomycin C-induced DSBs in BCR-ABL1-positive leukemia cells signifying that histone acetylation and chromatin re-modeling are central for proficient repair of numerous double-strand breaks (DSBs) (Falinski et al. 2012). Confluence of information suggests that BCR-ABL-positive cells have downregulation of tumor suppressor genes. It is evident that BCR-ABL facilitates the tumor suppressor gene silencing primarily by histone H3 trimethylation at lysine 9 and histone H3 (lysine 14) deacetylation. Therefore, it is essential to reshape the landscape of tumor suppressors. It is vital to detect that ten-eleven-translocation (TET2) interacts with the Bcr-Abl protein and results in TET2 cytoplasmatic compartmentalization in a complex tethered by the fusion protein tyrosine kinase and encompassing the Forkhead box O3a (FoxO3a) transcription factor. However, tyrosine kinase inhibition results in TET2 release from Bcr-Abl protein and triggers a series of events including TET2 nuclear translocation, recruitment at the BIM promoter followed by BIM transcriptional induction (Mancini et al. 2012b). On a similar note, treatment of cells with Aurora kinase inhibitors MK-0457 induces the growth arrest DNA damage-inducible (GADD 45a) through recruitment of octamer-binding-1 transcription factor at a critical promoter region for gene transcription and covalent modifications of histone H3 (lysine 14 acetylation, lysine 9 de-methylation) (Mancini et al. 2012a). Therefore, such epigenetic chromatin modifications may illustrate a general mechanism promoting the de-repression of tumor suppressor genes silenced in Bcr-Abl-positive leukemic cells.

CML cells have a low BRIT1/MCPH1 level and explain a defective G2/M arrest, hence verifying that these cells have a constitutive genomic instability (Giallongo et al. 2011).

It is a well-appreciated fact that Fanconi D2 (FANCD2) is monoubiquitinated on K561 (FANCD2-Ub) in response to DNA DSBs to stimulate repair of these potentially lethal DNA lesions. Studies indicated that FANCD2-Ub was upregulated in $CD34^+$ CML cells and in BCR-ABL1 kinase-positive cell lines in response to elevated levels of reactive oxygen species (ROS) and DNA cross-linking agent mitomycin C. As a result, BCR-ABL1 positive $FANCD2^{-/-}$ cells represented greater accumulation of ROS-induced DSBs as compared to BCR-ABL1 positive $FANCD2^{+/+}$ cells. It is notable that decline in proliferation of BCR-ABL1 positive $FANCD2^{-/-}$ leukemia cells could be reversed by enforced FANCD2 expression. FANCD2-Ub has an essential role in BCR-ABL1 leukemogenesis because of its capability to make possible the repair of numerous ROS-induced DSBs (Koptyra et al. 2011). CML cells have a malfunctioning and inability to generate FANCD2 nuclear foci, either in dividing cells or after DNA damage. Similarly, human cord blood $CD34^+$ cells transduced with BCR/ABL retroviral vectors demonstrated severely compromised FANCD2 foci formation. Appreciably, both the impaired formation of FANCD2 nuclear foci, and also the predisposition of BCR/ABL cells to develop centrosomal and chromosomal translocations and rearrangements were reverted by the ectopic expression of BRCA1 (Valeri et al. 2010).

BCR/ABL is also documented to upregulate the expression and increase nuclear localization of WRN (mutated in Werner syndrome), which is vital for processing DSB ends during the repair. BCR/ABL induced WRN mRNA and protein expression to a certain extent by c-MYC-mediated activation of transcription and Bcl-xL-dependent inhibition of caspase-dependent cleavage, respectively. WRN coupled with BCR/ABL and was resultantly phosphorylated. WRN tyrosine phosphorylation, in turn, stimulated its helicase and exonuclease activities (Slupianek et al. 2011b).

BCR-ABL also upregulated error-prone DSB repair pathways single-strand annealing (SSA) by remarkably enhancing CtIP accumulation. Treatment with the BCR-ABL kinase inhibitor, attenuated CtIP accumulation or targeted inhibition of CtIP resulted in suppression of BCR-ABL-mediated genomic instability (Salles et al. 2011). In addition, BCR/ABL-mediated stimulation of SSA was accompanied by an increase in nuclear colocalization of RAD52 and ERCC1, which played a dominant role in the repair (Cramer et al. 2008).

It is becoming ever more perceptible that BCR/ABL switch on B-NHEJ which is more error-prone than D-NHEJ and contribute to the increase of the genomic instability of leukemic cells. BCR/ABL expressing cell line BV173 was investigated for different context-dependent NHEJ repair mechanisms. DNA damage induced by sobuzoxane was repaired by a NHEJ pathway which is reliant on the catalytic subunit of protein kinase dependent on DNA (DNA-PK(CS); D-NHEJ), while DNA damage mediated by etoposide was repaired by two discrete NHEJ

pathways, dependent on or independent of DNA-PK(CS) (backup NHEJ, B-NHEJ). Cells incubated with STI571, a highly specific inhibitor of BCR/ABL, showed refractoriness against these agents associated with an accelerated kinetics of DSBs repair. Collectively, it was observed that BCR/ABL downregulated DNA-PK(CS)-dependent and upregulated backup non-homologous end joining in leukemic cells (Poplawski and Blasiak 2010).

STI571 is used widely as a therapeutic intervention of CML and inhibits activity of the BCR/ABL oncogenic tyrosine kinase. Experimental evidence substantiated the fact that STI571 decreased the efficacy of nucleotide excision repair (NER) in leukemic cells expressing BCR/ABL. For that reason, STI571 is effective in overcoming the drug resistance associated with increased DNA repair in BCR/ABL-positive leukemias (Sliwinski et al. 2008).

Dysregulation of DNA damage repair may constitute an early step in leukemogenesis and genomic instability in leukemia is validated by related experimental data and functional analysis. Leukemia exhibited combined or mixed features at the clinical, pathological and molecular levels and subversion of this core protein network is associated with cancer progression.

Some multifunctional proteins in the major NHEJ pathway, Artemis and DNA ligase IV, are repressed, while DNA ligase IIIα, and the protein deleted in WRN are overexpressed. DNA ligase IIIα and WRN form a complex that is tethered to DSBs in CML cells. Targeted inhibition of either DNA ligase IIIα or WRN resulted in an increased accumulation of unrepaired DSBs, representing that they are contributory to the repair of DSBs. Therefore, it is conceivable that altered DSB repair in CML cells is caused by the increased activity of an alternative NHEJ repair pathway, involving DNA ligase IIIα and WRN (Sallmyr et al. 2008). BCR-ABL1-positive cells exploit an alternative non-homologous end-joining pathway (ALT NHEJ) to repair DNA DSBs. ALT NHEJ proteins, poly-(ADP-ribose) polymerase 1 (PARP1) and DNA ligase IIIα, were overexpressed in the BCR-ABL1-positive CML cell line K562 and, to a greater extent, in its imatinib-resistant derivative. Targeted inhibition of DNA ligase and co-treatment with PARP inhibitors inhibited ALT NHEJ and selectively decreased cell survival (Tobin et al. 2012).

Various lines of evidence unravel the implications of intra-tumor heterogeneity in diagnostics and the development of therapeutic resistance. DNA repair proteins are tightly interconnected with BCR-ABL signaling and desensitize BCR/ABL-positive cells to cytotoxic drugs. DNA repair mechanisms are exceptionally enhanced in BCR-ABL expressing cells and expression of various proteins was triggered by BCR-ABL. Particularly, Nbs1 expression was mediated and enhanced ATM kinase-dependent phosphorylation of Nbs1 on serine 343 (S343) in response to genotoxic treatment was detected in leukemia cells expressing BCR/ABL (Rink et al. 2007).

Higher levels of drug-mediated DSBs in leukemia cells were related with higher activity of ATR kinase, and remarkably augmented phosphorylation of histone H2AX on serine 139 (γ-H2AX). Additionally, the expression pattern and ATR-mediated phosphorylation of Chk1 kinase on serine 345 were more notable in BCR/ABL-positive leukemia cells than normal counterparts after genotoxic treatment. Therefore, ATR-Chk1 axis was robustly activated in BCR/ABL-positive cells and predisposed cells to resistance against DNA cross-linking agents causing numerous replication-dependent DSBs (Nieborowska-Skorska et al. 2006). Cells reconstituted for miR-373 displayed a decline in the NER protein, RAD23B and RAD52 that resulted in higher genome instability (Crosby et al. 2009).

There is sufficient evidence that indicates that treatment of CML-derived BCR/ABL expressing leukemia K562 cells with STI571 results in the inhibition of DNA repair and re-sensitization of these cells to doxorubicin (Majsterek et al. 2006).

Wingless and SHH Signaling Machineries in BCR-ABL Leukemic Cells

Wingless (Wnt) signal transduction pathway operates within a homeostatic range. It delicately triggers cell fate, proliferation and self-renewal of stem and progenitor cells. The importance of staying in such a gradient is emphasized by the evidence that links hyperactive or hypo active signalling to leukemogenesis. Detailed investigation into Wnt signalling pathways is accelerating as we learn how these pathways are linked to leukemia and as we begin to comprehend how modulation of these pathways might have therapeutic applications. Here, we try to take an overview of this aspect, highlighting recent work that describes the early steps leading to the synergism generated because of Wnt signals and BCR-ABL.

In a mouse model of CML, BCR/ABL^{+} let-7 complementary sites (LSCs) and leukemia progenitor cells were found to display cell surface expression of CD27. In addition, interaction of CD27 to its ligand, CD70, increased expression of Wnt target genes in LSCs by increasing nuclear shuttling of active β-catenin and TRAF2- and NCK-interacting kinase. This trafficking of proteins into nucleus resulted in an increased proliferation and differentiation of LSCs. Strikingly, targeted inhibition of CD27 signaling in LSCs delayed disease progression and prolonged survival. It is important to observe that expression of CD70 is limited to activated lymphocytes and dendritic cells, as a result adaptive immunity contributes significantly to leukemia progression. Therefore, abrogation of CD27 on

LSCs may symbolize an attractive therapeutic attempt to blocking the Wnt/β-catenin pathway in CML (Schürch et al. 2012). Furthermore, Wnt/β-catenin-signaling inhibitor (AV65) counteracted the uncontrolled proliferation of different CML cell lines including T315I mutation-harboring cells. AV65 also repressed the expression of β-catenin in CML cells, thus predisposing cells to apoptosis (Nagao et al. 2011).

Various lines of evidence suggested that numerous components of a Wnt/Ca^{2+}/NFAT signaling pathway are instrumental in driving carcinogenesis synchronously with BCR-ABL. Investigational strategies indicated that NFAT inhibition with cyclosporin A increased leukemia cell elimination by the Bcr-Abl inhibitor dasatinib and distinctly enhanced survival in a mouse model of Bcr-Abl^{+} acute lymphoblastic leukemia (ALL) (Gregory et al. 2010).

Contrary to positive regulars of Wnt signaling pathway, secreted frizzled-related protein 1 (SFRP1) is an important extracellular antagonist of the Wnt signalling pathway that plays an important role in the pathogenesis of haematopoietic malignancies. SFRP1 has been documented to be transcriptionally down-regulated due to hypermethylation in leukemia and is interconnected with an overexpression of the Wnt receptor Frizzled 3 (Fzd3) (Reins et al. 2010). Wnt signaling pathway triggers expression of various target genes which block cellular differentiation. Electrophoretic mobility shift assay, chromatin immunoprecipitation assay, and mass spectrometry assays indicated that Wnt signaling mediators (Wnt5A and Wnt11) were found to be attached to the SUZ12 promoter. Targeted inhibition of Wnts 1, 5A, and 11 demonstrated that Wnt members are competent in activating SUZ12 transcription with varying promoter affinities (Pizzatti et al. 2010).

It has recently been explored that CML progresses from a chronic phase in hematopoietic stem cells that harbor the BCR-ABL rearrangement, to blast crisis (BC), identified by irregular activation of β-catenin within granulocyte–macrophage progenitors. This irregular activation of β-catenin is as a consequence of its continuous escape from degradation. BC CML progenitors with misspliced GSK3β have a robust β-catenin expression, however, cells reconstituted for normal GSK3β displayed tumor retardation (Abrahamsson et al. 2009). β-Catenin is indispensible for survival of leukemic stem cells showing refractoriness to kinase inhibition in mice with BCR-ABL-induced CML (Hu et al. 2009). The association between the up-regulated expression of non-canonical Wnt genes and apoptotic stimulation is a new possibility suggesting the likely involvement of the non-canonical Wnt pathway in executing programmed cell death in BCR-ABL-positive cells (Sercan et al. 2007). Besides, administration of the Hh inhibitor GDC-0449 inhibits BCR-ABL-positive uncontrolled cellular proliferation and enhances the cytotoxic effects of dasatinib (Okabe et al. 2012).

BCR-ABL Interaction with mTORC

It is a well-appreciated piece of information that malignant phenotype of CML is due to the abnormal tyrosine kinase activity of the BCR-ABL oncoprotein, which signals several downstream cell survival pathways, including phosphoinositide 3-kinase/AKT, signal transducer and activator of transcription 5 and extracellular signal-regulated kinase 1/2. Unfortunately, because of tumor heterogeneity, refractoriness against tyrosine kinase inhibitors is progressively increasing. Emerging studies demonstrated that Ph^{+} BCR-ABL expressing cells were resistant to imatinib and dasatinib resistance because of hyperactive mammalian target of rapamycin complex-2 (mTORC2)/AKT signaling pathway. However, it has lately been shown that glucocorticoid-induced leucine zipper protein (GILZ) dampened imatinib and dasatinib resistance and suppressed tumor growth by inactivating the mTORC2/AKT signaling pathway. Additional studies suggested that GILZ attached to mTORC2, but not to mTORC1, repressing phosphorylation of AKT (at Ser473) and activating FoxO3a-mediated transcription of the pro-apoptotic protein Bim emphasizing the concept that GILZ is a key inhibitor of the mTORC2 pathway (Joha et al. 2012).

Double targeting of mTORC1 and mTORC2 signalosome using a catalytic mTOR inhibitor, OSI-027, resulted in generation of potent antileukemic effects against BCR-ABL transformed cells. Comparable results were seen in cells expressing the T315I mutation, which is resistant to all currently approved BCR-ABL kinase inhibitors (Vakana et al. 2010). Considerable verification indicated presence of mTORC2 complexes in BCR-ABL-transformed cells and identified phosphorylation of 4E-BP1 on Thr37/46 and Ser65 as RI-mTORC1 signals in primary CML cells. It is also important to note that autophagy abrogates apoptotic response in leukemic cells upon inhibition of mTORC2/mTORC1. Induction of apoptosis by OSI-027 is antagonized by induction of autophagy in some types of BCR-ABL transformed cells and can be overcome by concomitant inhibition of such autophagy (Carayol et al. 2010).

In an effort to translate laboratory findings into effective therapeutics, tremendous researches are in various phases of trials. Imatinib although is effective in inhibiting BCR-ABL expressing cells; however, it has other off target effects like activation of mTOR. It is, therefore, necessary to counterbalance the parallel leukemia promoting proteins and pathways. A recent study presented that rapamycin derivative RAD 001 (everolimus) inhibited mTOR and prominently hampered mTOR late re-activation in response to IM. RAD 001 interfered with the assemblage of mTORC1/mTORC2complexes. The impairment of

mTORC2 resulted in the de-phosphorylation of Akt at Ser(473) in the hydrophobic motif of C-terminal tail required for Akt full activation and inhibited Akt re-phosphorylation in response to IM. In addition, RAD 001-mediated inhibition of Akt resulted in the de-phosphorylation of tuberous sclerosis tumor suppressor protein TSC2 at 14-3-3 binding sites. Uncoupling of TSC2 from 14-3-3 sigma resulted in restoration of its inhibitory function on mTORC1 (Mancini et al. 2010). More significantly, Bcr-Abl and the rapamycin-sensitive mTORC1 complex work synchronously and contribute to the phosphorylation (inactivation) of 4E-BP1, an inhibitor of the eIF4E translation initiation factor. Experimental methodologies indicated that Bcr-Abl and mTORC1 induced formation of the translation initiation complex, eIF4F, by decline in 4E-BP1 binding and an enhanced eIF4G binding to eIF4E, two events that lead to the assembly of eIF4F (Prabhu et al. 2007).

mTORC1 is effectively triggered by various upstream signals, including those transduced by growth factors, nutrients, and energy, among which the PI3K–Akt signaling pathway is the most well-known. In recent times, a research group reported that inhibition of GSK3β induced by Wnt signaling triggered the mTORC1 signaling through TSC2 inhibition. Likewise another documentation addressing the same question further elucidated that the regulation of the mTOR level through Wnt signaling played a crucial role in the activation of mTORC1. Thus, Wnt signaling stimulated the expression of mTOR, leading to the mTORC1 activation (Fujishita et al. 2008; Inoki et al. 2006). It is undeniable that BCR-ABL re-enforces translational mechanisms related to over-production of oncoproteins. Moreover, in this particular cancer micro-enviroenment checkpoints related to post-transcriptional processing of oncogenes are inactive or repressed and need further investigation.

Corresponding findings indicate the fact that miR-520c and miR-373 directly target the 3′-UTR of mRNAs of mTOR and SIRT1. Experimental data presented the aspect that mTOR and SIRT1negatively regulated expression of MMP9 via inactivating the Ras/Raf/MEK/Erk signaling pathway and NF-κB activity. Laboratory techniques like western blots for these proteins substantiated the fact that miR-520c and miR-373 activated this particular signaling pathway and NF-κB. Therefore, miR-520c and miR-373 increased the expression of MMP9 by post-transcriptional processing of 3′-UTRs of mRNAs of mTOR and SIRT1 (Fig. 1) (Liu and Wilson 2012).

miR-99a is a tumor suppressor miRNA and targets mTOR and FGFR3. As cells re-established for miR-99a suppressed tumor growth of c-Src-transformed cells; however, this effect was reversed upon transient transfection of mTOR in the cells. Considerable downregulation of miR-99a was also observed in EGF- and Ras-transformed

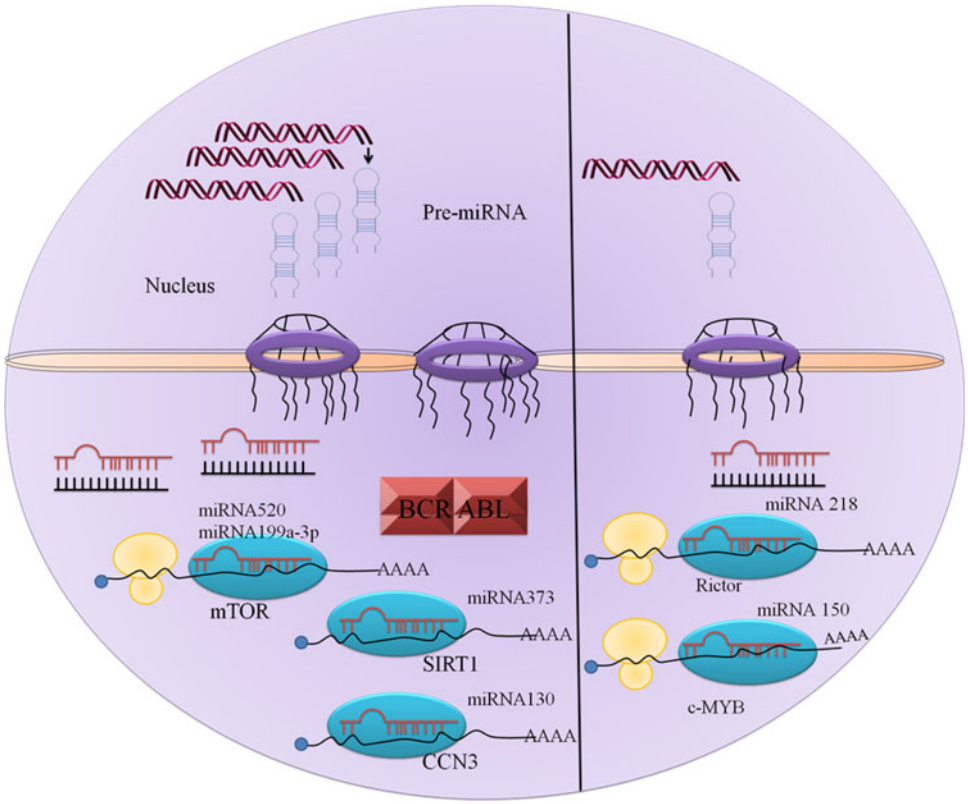

Fig. 1 Regulation of target genes by miRNA signature in leukemic cell

cells; however, it was reversed upon targeted inhibition of the MAPK pathway (Oneyama et al. 2011). Cells reconstructed for miR-218 and miR-585 displayed reduced cell growth in part through caspase-mediated apoptosis. In addition, it was observed that miR-218 remarkably suppressed levels of the rapamycin-insensitive component of mTOR, Rictor that correlated with a suppression of Akt S473 phosphorylation (Uesugi et al. 2011). Re-introduction of miR-199a-3p in cells resulted in a decrease in mTOR and Stat3 expression (Duan et al. 2011). Another research group performed an in vitro functional analysis by either restoring or silencing miR-199a-3p. For a better understanding of the mechanism, HepG2 and Huh-7 cell lines were opted for the reconstruction experiments of miR-199a-3p, because they exhibit the lowest constitutive levels of this miRNA and a high mTOR protein expression (Fig. 1) (Fornari et al. 2010). It appears that we have just begun to understand the true extent of the role miRNA has in regulation of its target suppressor and oncogenic transcriptome. However, rapid breakthroughs in biology of miRNA have formed the foundation for the latest leap in knowledge. Momentously widening landscape of the role of miRNA in physiological processes and disease is being scrutinized equally quickly for its potential use in the clinical setting. Although most of the original research focused on cancer, misrepresentations of miRNA in the BCR-ABL-positive cells are now being studied extensively and at an ever increasing rate.

miRNA Signatures in Oncogenic Fusions Carrying Cells

Various oncogenic fusions have been extensively studied in mouse models and MLLT3-MLL gene fusion has been shown to disrupt proliferation and survival of some myeloid progenitors, thereby indirectly driving monocyte outgrowth, but the fusion appears insufficient in these models to directly induce leukemia. In accordance with this approach, Ras mutations have been shown to synergize with MLLT3-MLL in vitro by suppressing apoptosis and blocking differentiation (Chandra et al. 2010). There is a well-established mechanism of inhibition of let-7 in carcinogenesis that relieves the expression of its target genes. 3′-UTRs of the RAS genes contain multiple LCSs, allowing let-7 to post-transcriptionally regulate RAS (Johnson et al. 2005). It is, therefore, worthwhile to describe that misrepresentations of miRNA signature in a fusion positive cell underlie overexpression of oncogenes.

Growing sophisticated information indicated that several tumors containing HMGA2 3′-UTR rearrangements demonstrated breakpoints after let-7 miRNA CBS 1. However, elimination of binding sites in HMGA2 UTR neither gives a clue of transcriptional upregulation of the gene nor a surrogate to genomic HMGA2 rearrangements (Bianchini et al. 2011; Wang et al. 2009). On a similar note, the loss of seven 3′-UTR binding sites has recently been reported (Martin et al. 2012). miR-34b, miR-326, miR-432, miR-548c-3p, miR-570, and miR-603 negatively regulate HMGA1 and HMGA2 (D'Angelo et al. 2012).

EWS-FLI-1 alters tumor suppressor miRNA expression and miRNA145 and let-7a are direct EWS-FLI-1 targets (De Vito et al. 2011). However, interestingly miRNA145 also regulates FLI-1, as three highly conserved putative miRNA145-binding sites are identified within the wild-type FLI-1 3′-UTR. miRNA145 also negatively regulates EWS-FLI-1 and is convincingly verified by animals model studies as subcutaneous injection of A673 cells overexpressing miRNA145 into NOD-SCID mice results in a momentous decrease in tumor-forming capacity (Riggi et al. 2010).

It is reasonable to mention that cell lines derived from thyroid adenomas with 19q13.4 rearrangements and primary adenomas with this particular rearrangement presented overexpressed C19MC and miR-371-3 cluster compared to chromosome abnormality deficient cells (Rippe et al. 2010). However, it has currently been shown in brain tumor cells carrying chromosome 19q13.42 amplification that C19MC rather than miR-371-373 was amplified (Nobusawa et al. 2012). There is a documented relationship between miR-371-373 cluster and Wnt/β-catenin-signaling pathway. Laboratory investigations have indicated three TCF/LEF1-binding elements (TBEs) in the promoter region and reported to be required for Wnt-dependent activation of miR-371-373. Few protein genes related to the Wnt/β-catenin-signal transduction cascade were characterized as direct targets of miR-372 and -373, including Dickkopf-1, a negative regulator of Wnt/β-catenin cascade (Zhou et al. 2012). Synergism of subset of miRNA with signal transduction cascades facilitates overexpression of target genes of a cascade and tactfully negative regulators of signaling pathways are targeted by oncomirs that disrupts spatio-temporal behavior and underpins carcinogenesis.

miR-196b has been shown to be upregulated in CALM-AF10, SET-NUP214 carrying cells (Schotte et al. 2010). Several miRNAs are also downregulated because of high degree of methylation of the miR-152 CpG island in t(4;11)-positive infant ALL. The proof of the concept was provided after exposure of fusion positive cells to the demethylating agent Zebularine that derepressed miR-152 (Stumpel et al. 2011). Further research in the domain of miRNA signature in fusion positive cancer cells will undoubtedly bring a heightened understanding of the molecular mechanisms underlying post-fusion cellular physiology. Later on delivery of tumor suppressor miRNA mimicking oligonucleotides to the fusion positive cells will

enable us in exploring effects on cell proliferation and apoptosis.

BCR-ABL: Troublemonger in Cytoplasm and Troubleshooter in Nucleus

Earlier it was thought that BCR-ABL is involved in leukemogenesis and targeted inhibition of BCR-ABL is necessary as an important facet in the clinical management of leukemia. However, paradigm in parallax has recently been uncovered. It is now perceptible that altering the location of ectopically expressed Bcr-Abl can kill leukemia cells. Multiple nuclear localization signal (NLSs) are required to counteract Bcr-Abl binding to actin, thus catapulting it into the nucleus and causing apoptosis. Tactfully, ectopically expressed Bcr-Abl could predispose K562 cells to apoptosis when specifically directed to the nucleus via strong NLSs (Dixon et al. 2009). Additional mutations of three specific tyrosines (Y232, Y253, Y257) in the kinase domain inhibited the NLS function of kinase-proficient and kinase-defective BCR-ABL. Structural studies indicated that C-terminal region of ABL contained an F-actin binding domain (FABD) that was necessary for the activated kinase to inhibit the NLS function (Preyer et al. 2011). Disentangling the structural interactions among the kinase domain, the NLS region and the FABD may hence provide insights into the design of next generation BCR-ABL inhibitors for the clinical management of leukemia.

Furthermore, to enhance nuclear translocation, discrete binding domains for capture of Bcr-Abl have recently been designed and attached to proteins with signals destined for the nucleus. Multi-site targeting of Bcr-Abl by construction of high affinity intracellular antibody domains to regions of Bcr-Abl known to enhance cytoplasmic retention, through its coiled-coil domain (CC), and via protein–protein interaction domain (RIN1) was also evaluated for shuttling into the nucleus (Dixon et al. 2011). It has also been documented that inhibition of the BCR-ABL tyrosine kinase, either by mutation or by the drug STI571, stimulated its nuclear entry. More effectively, combining STI571 with leptomycin B (LMB) blocked nuclear export and consequently entrapped BCR-ABL in the nucleus and the nuclear BCR-ABL tyrosine kinase induced apoptosis (Vigneri and Wang 2001). It is also encouraging that coiled-coil designs exhibit disruption of Bcr-Abl oligomeric complexes and result in decreased proliferation of leukemic cells and induction of apoptosis (Dixon et al. 2012).

Ph-Positive Leukemia: a Transgenic Mouse Model

It has recently been shown that CML Model can be established via introducing K562 cells into the Murine Caudal Vein and later can be comfirmed by histopathologic examination and detection of BCR-ABL fusion gene using RT-PCR (Zhang et al. 2012a).

It has lately been demonstrated that the −364 to +22 domain of TEC promoter can effectively and proficiently modulate particular expression of related genes in hematopoietic tissue, which can be used for construction of P210(bcr/abl) transgene mice model (Zhu et al. 2012).

A further link between splicing and transformation has been obtained in functional studies in Philadelphia chromosome-positive CML and AML cells that express, besides the main BCR/ABL transcripts, novel BCR/ABL transcripts derived from alternative splicing between BCR exons with ABL exons. Translational products derived from ABL gene have an amino acid sequence at C-terminus derived from out-of-frame (OOF) reading. OOF ABL portion is immunogenic in HLA-A2.1 transgenic mice. HLA A2.1 mice were immunized with novel peptides designed in accordance with ABL OOF sequence, carrying epitopes with considerably increased affinity for HLA A2.1 molecule (Casnici et al. 2012).

Efforts are being made to predispose BCR-ABL cells to apoptosis by introduction of an exogenous SH2-DED (SD) fragment into the CML cells that may hamper the binding of BCR/ABL Tyr177 and Grb2, simultaneously activating caspase-8 induced apoptosis. High anti-proliferative activity of Ad5/F35-SD was additionally verified in nude mice and its leukemia-protective effect was evident in CML model mice injected with BCR/ABL(+) BaF3 cells (Peng et al. 2012).

Exosomes are microvesicles which are released by normal and tumor cells and play an important role in cell-to-cell communication and intriguingly these exosomes are released from K562 CML cells as well. CML cell-derived exosomes induced angiogenic activity in HUVEC cells as observed in set of laboratory experiments in which fluorescent-labeled exosomes were internalized by HUVECs during tubular differentiation on Matrigel. Activated Src family kinases (SFK) in leukemic cells can promote the expression and secretion of angiogenic growth factors and cytokines. These can interact with their receptors on endothelial cells that recruit and activate SFK. Treatment of K562 cells with clinically active tyrosine kinase inhibitors drastically decreased their total exosome release (Mineo et al. 2012).

It has currently been investigated that leukemic spleen cells are comparatively more efficient than bone marrow-derived cells in a transgenic mouse model of CML. Mice transplanted with pre-induced spleen cells displayed drastically enhanced neutrophilia and splenomegaly compared with mice receiving pre-uninduced spleen cells, suggestive of the notion that Bcr-Abl expression in the donors had increased splenic tumor burden. Astonishingly, splenic

cells are less responsive to imatinib than BM cells (Schemionek et al. 2012). A recent finding suggests that NK-cell proportions among lymphocytes are very low in CML before and on imatinib treatment and in BCR-ABL-positive mice (Chen et al. 2012).

There is an increasing evidence that p90 ribosomal S6 kinase 2 (p90RSK2) is commonly activated in different leukemia cell lines carrying hallmark oncogenic fusion proteins, including BCR-ABL and FMS-like tyrosine kinase 3-internal tandem duplication (FLT3-ITD). Targeted inhibition of RSK2 did not efficiently evoke apoptosis in BCR-ABL-expressing murine Ba/F3 cells, human K562 cells. Discordantly, inhibition of RSK2 induced momentous apoptosis not only in FLT3-ITD-positive Ba/F3 cells, Molm14 and Mv(4;11) leukemia cells, but also in primary tissue samples from AML patients (Elf et al. 2011).

In vivo studies co-administering HDACIs and dual Bcr/Abl-Aurora kinase inhibitor (KW2449) have recently been investigated for a synergy using a systemic IM-resistant ALL xenograft model. Multipronged approach resulted in inhibition of Bcr/Abl and downstream targets (e.g., STAT5 and CRKL), alongwith an increased ROS generation and DNA damage (γH2A.X) (Nguyen et al. 2011).

Crude extract of the mushroom Daedalea gibbosa outstandingly abrogated BCR-ABL kinase activity in BCR/ABL-positive mouse model (Khamaisie et al. 2011).

Findings derived from studies in Philadelphia chromosome-positive (Ph^+) ALL animal model indicated that prolonged drug exposure resulted in relapse accompanied by appearance of leukemic clones harboring BCR-ABL mutations. Kinase domain P-loop mutations predominated in mice receiving less intensive therapy, while high-dose treatment resulted in the emergence of T315I "gatekeeper" mutations resistant to all BCR-ABL kinase inhibitors (Boulos et al. 2011).

It is also worth unfolding that coiled-coil (CC) domain located in BCR(1-72) mediates BCR-ABL tetramerization, which is critical for the activation of tyrosine kinase and transformation potential of BCR-ABL. Researchers purified a TAT-CC protein to notably abrogate oligomerization of BCR-ABL. TAT-CC co-located and interacted with BCR-ABL in Ba/F3-p210 and K562 cells that resulted in induction of apoptosis and significantly escalated the sensitivity of these cells to imatinib (Huang et al. 2012).

CDK 7/9 inhibitor (SNS-032) extraordinarily repressed both the mRNA and protein levels of FIP1L1-PDGFRα and Bcr-Abl and counteracted uncontrolled proliferation of xenografted malignant cells expressing FIP1L1-PDGFRα or Bcr-Abl in nude mouse models (Wu et al. 2012).

Conclusions

It is noteworthy that whole-genome approaches to categorize and characterize genetic and epigenetic alterations of tumor suppressors and oncogenes in tumor microenvironment and cancer genomes have begun to present new insights into the array of molecular events that occur in leukemia. Although in some cases, this knowledge opens new and exciting avenues towards diagnostic or therapeutic implementation, however, novel mutations in BCR-ABL offer major stumbling blocks in standardization of therapy. It is necessary to use current range of methods, assays and approaches for genome-scale interrogation of BCR-ABL and TRAIL in leukemia. Consistent with the same concept, integration of functional-genomics approaches with the outputs from cancer genome sequencing cannot be overlooked. The signaling pathways in BCR-ABL1-positive cancer cells are key players in tumorigenesis and cancer progression. BCR-ABL1 signaling has been reported to play critical roles in tumorigenesis and cellular proliferation. It will also be important to explore using genome-wide analysis, the binding sites of oncogenes and tumor suppressors in BCR-ABL1 positive leukemic cells that will lead to important discoveries of their cell type-specific and context-dependent functions. Furthermore, application of genome-wide techniques to experimental models and human samples derived from cancer patients will facilitate progressively in elucidation of their multifaceted mechanisms during leukemogenesis, and may present potential prognostic biomarkers for future cancer therapy. Moreover, there is a gradual increase in the number of mutations in different domains of BCR-ABL and effective therapies must be designed. Recently, a novel mutation is reported to be responsible for imatinib resistance, K356dup, which is one of the first observed functional insertions in BCR-ABL oncogene. It is relevant to mention that mutation lowers the autoinhibition of the protein and facilitates active conformation of the protein (Gniot et al. 2012).

It is undeniable that miRNAs predominantly mediate the post-transcriptional processing of tumor suppressor genes and oncogenes and this realization has resulted in a quest to understand the pathways that are regulated by these miRNAs using in vivo model systems and remarkable hints have emerged. Future research must converge upon comprehending the practicability of targeting oncogenic miRNAs and restoring tumor-suppressive miRNAs for cancer therapy.

Conflict of interest The authors declare that they have no conflict of interest.

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