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Review

Targeting of BCR-ABL: Lessons learned from BCR-ABL inhibition

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Abstract: In 1960 researchers reported that balanced translocation between chromosomes 22 and 9 resulted in the generation of Philadelphia chromosome. This breakthrough revolutionized our knowledge related to leukemia biology and contemporary studies revealed that chromosomal translocation resulted in the fusion between the 5′ segment of BCR gene and 3′ segment of the ABL gene to form BCR/ABL fusion gene. Research over the years has progressively and systematically improved our understanding of the genetic and proteomic basis of Leukemia. Genome-wide profiling studies, including genome sequencing and microarray analysis, have helped us in identification of different intracellular signaling cascades that are frequently mutated in Leukemia. We partition this multi-component review into different sections related to biochemical characteristics of BCR-ABL+ cells, underlying mechanism of generation of mutations and crosstalk of BCR-ABL with various intracellular signaling cascades. We also summarize how BCR-ABL encoding mRNA is negatively regulated by different miRNAs and the strategies which are currently being used to effectively target BCR-ABL protein. We also provide an overview of the natural products which have been used for targeting of BCR-ABL protein. Better understanding of the protein network of Philadelphia positive leukemic cells will prove to be helpful in getting a step closer to personalized medicine.

Key words: BCR-ABL, Cancer, Apoptosis, Therapeutics, Signaling.

Introduction

Data obtained through high-throughput technologies has considerably improved our understanding of the underlying causes of Chromosomal reciprocal translocations and it is now clear that these translocation are contributory in genesis of oncogenes encoding fusion tyrosine kinases (FTKs) such as: BCR/ABL, TEL/ABL, BCR/FGFR1, TEL/JAK2, TEL/TRKC(L), TEL/PDGFbetaR, PCM1/JAK2, NPM/ALK, ZNF198/FGFR1 and others. Research over decades has provided sharper knowledge of structural variability of fused oncogenic proteins and different strategies have been used to target these proteins (1,2). Increasingly it is being realized that ABL (Protein kinase) has evolved highly organized mechanisms and pendulously swings between inactive and active states. Crystal structures of inactive kinases have shown kinase domain plasticity, which facilitates characteristically unique structural reorganizations. Substantial fraction of information has been added into the existing pool of knowledge and researchers have identified point mutations in BCR–ABL1 kinase domain. BCR–ABL1 mutations have been frequently reported in imatinib-resistant patients depending on the CML phase, detection techniques and definition of resistance (1,2,3).

Imatinib mesylate (Gleevec; Novartis) was the first drug to target BCR-ABL and gained considerable appreciation because of its efficacy. However, most patients who received imatinib still had molecular residual disease and some developed resistance against these drugs. To overcome the stumbling block, second-generation inhibitors (dasatinib and nilotinib) were designed to target seemingly undruggable BCR-ABL (3). However, confluence of information suggested that even these drugs were negligibly effective against BCR–ABL T315I mutation and clinical outcome was modest in CML advanced phases. It has previously been reported that gain-of-fitness correlated significantly with notably enhanced kinase activity and increased potential of transformation. However, T315I mutation has reduced kinase activity and higher oncogenicity. Information harnessed from mass-spectrometry-based phosphotyrosine profiling revealed that T315I had characteristically unique pattern of phosphorylation in the phosphate binding loop of BCR–ABL (4). In the upcoming sections we will discuss biochemical and molecular features of BCR-ABL+ cells.

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Biochemical characteristics of BCR-ABL$^+$ cells

Even under normoxic conditions, BCR-ABL has been shown to tactfully induce hypoxia-like conditions to trigger changes in the transcriptome in CD34$^+$ cells. BCR-ABL$^+$ cells are clonogenically potent, however down-regulated expression of HIF1 (Hypoxia Inducing Factor-1) or HIF2 considerably impaired proliferation of BCR-ABL stem/progenitor cells (5). There was a significant increase in the glycolysis and lactate production in BCR-ABL$^+$ cells under normoxic condition which increased further under hypoxic condition. Biochemical and physiological analysis of BCR-ABL+ cells indicated that there was an increase in glutamine uptake for generation of glutamate and finally into α-ketoglutarate for maintenance of Krebs cycle activity in glycolytic cells (5). There is an exciting piece of evidence suggesting that imatinib resistant KBM5-T315I cells show decreased lactate production, cell proliferation, fatty acid synthesis and ROS production (6). ROS scavengers particularly, SOD2 and catalase were also down regulated. Data clearly suggested that the lower growth ability of KBM5-T315I cells might be related to the decreased ROS levels and reduced expression levels of glycolysis-related genes (6).

Tyrosine Kinase inhibitors have been shown to efficiently induce autophagy in CML cells. ATG7, an important modulator of autophagy is an E1-like enzyme required in both an ATG12 and ubiquitin-like LC3 conjugation systems (7). ATG7 inhibition individually inhibited expansion of CML CD34$^+$ cells. Moreover, inhibition of autophagy resulted in a metabolic shift from glycolysis to oxidative phosphorylation in CML cells that promoted ROS (Reactive Oxygen species) formation followed by differentiation (7).

Underlying mechanism of generation of mutations

KCL-22 cells acquire T315I mutation and developed resistance against imatinib.SIRT1, LSD1 and KU70 worked synchronously and were noted to be contributory in the acquisition of T315I mutation by occupying ABL exon 6 (8). LSD1 and SIRT1 were shown to occupy ABL exon 6 in untreated KCL-22 cells. There was a significant reduction in exon 6-bound LSD1 but loading of SIRT1 was further enhanced in imatinib treated cells. Surprisingly, increase in LSD1 level and simultaneous reduction in SIRT1 was noted in KU812 cells. These stoichiometrically different ratios of SIRT1 and LSD1 on ABL locus in different cells indicated that these proteins interacted oppositely with KU70 in response to imatinib (8). There was a direct piece of evidence suggesting reduction in BCR-ABL mutant formation in KU70 3'UTR knockdown cells. However BCR-ABL mutant formation was significant in WT KU70 expressing cells. BCR-ABL mutation frequency was rescued in cells that ectopically expressed mutant LSD1 (K661A). SIRT1-KU70 interaction facilitated repair and opened the chromatin that consequently increased the chances of mutation acquisition and development of resistance. LSD1-KU70 interaction reduced repair with more "repressive" chromatin that resulted in DNA damage accumulation. ΔSAP KU70 mutant was not found to be significantly capable to restore BCR-ABL mutations (8).

Certain hints have emerged suggesting generation of alternatively spliced variant derived from BCR-ABL transcript in which BCR (exons 1-13 or 14) fused with ABL (exon 4) (9). This alternatively spliced version induced a change in ABL reading frame and generated an early STOP codon in 5th exon of c-ABL. Phospho-Akt and p38 levels were considerably higher in BV173 Philadelphia positive hematopoietic cells transfected with BCR-ABL-OOF (9). BCR/ABL-OOF level was analyzed in 16 imatinib treated patients after 3 months. 3 patients had moderately reduced BCR/ABL-OOF transcript levels after treatment with imatinib for 3 months (9).

BCR ABL crosstalk with Wnt mediated signaling modulators

WNT transduced the signals intracellularly upon binding to frizzled (FZD) and co-receptors (LRP5 or LRP6) that resulted in the activation of the Dishevelled (Dvl) protein. Functionally active Dvl exerted inhibitory effects on formation of destruction complex and rescued β-catenin from degradation. Consequently, there are increasing levels of nuclear β-catenin, which facilitate TCF/LEF-mediated transcriptional activation of target genes (10, 11, 12).

Scientists have now developed a very clear understanding of the crosstalk between BCR-ABL and β-catenin in leukemia. Degradation of β-Catenin is triggered by the UPS (ubiquitin-proteasome system). However, because of increase in cellular stresses, UPR is unable to process excess of protein turnover (13). Consequently, β-catenin gets stored within densely structured assemblies named as aggresomes and sorted towards degradation by autophagosome–lysosome associated machinery (13). Cytoplasmic retention of β-catenin by its antagonist induced autophagy in BCR–ABL1 expressing leukemic cells. Autophagic flux induced Calpain modulated proteolytic processing of β-catenin into a 75 kDa fragment of β- catenin that still retained transcriptional activity, thus behaving like a component of autophagy-induced leukemic cell survival. Autophagy protected leukemic cells against effects of Tyrosine kinase inhibitors (dasatinib and nilotinib) (13). However further investigations are necessary to clarify role of aggresomes as holding stations for transcriptionally active β-catenin.

Both BCR and p210 BCR-ABL contained a UBD (ubiquitin-binding domain) within their NH2-terminal. Structural association between ubiquitin-binding mutant BCR-ABL and β-catenin was significantly hampered (14). Phosphorylated β-catenin (Tyr654) levels were notably enhanced in cytoplasmic and nuclear fractions in p210 BCR-ABL expressing cells (14).

Levels of BCR-ABL were considerably reduced in imatinib-resistant K562 leukemia cells treated with Tetrandrine citrate. Tetrandrine citrate also dose- and time-dependently decreased β-catenin protein levels in leukemia cells (15).

Researchers were also interested in observing the effects of deletion of β-catenin in Bone marrow (BM) cells after the engraftment of BCR-ABL-transduced stem cells in primary recipient mice. BM from mice with the genotypes Ctnnb1fl/fl knockout, Ctnnb1+/

fl, and Ctnnb1+/+ were transduced with BCR-ABL expressing retrovirus and injected into wild-type syngeneic recipient mice (16). Establishment of disease in recipient mice was verified by detection of GFP^+ cells in the peripheral blood after BM transplantation. Upon detection of GFP^+ cells in peripheral blood, tamoxifen (TAM) was intraperitoneally injected. Survival rate of recipient mice of Ctnnb1fl/fl knockout BM was similar to either treated wild-type or untreated controls. All mice succumbed to a CML-like disease between 21 and 35 days after BM transplantation (16). To clarify whether inhibition or activation of Wnt pathway could be effective in improving outcomes of imatinib, experiments were conducted on constitutive activation or loss-of-function models of the Wnt induced signaling cascade. CML was induced by transformation of murine stem and progenitor cells with the BCR-ABL oncogene (16). IM treatment was initiated after engraftment of BCR-ABL expressing cells and confirmed with either genetic deletion (by administration of tamoxifen in Ctnnb1fl/fl Esr1-Cre+ BMC) or activation of Ctnnb1 (16). Results revealed that β-catenin activation seemingly did not affect disease-initiating population, however, β-catenin deletion reduced leukemic stem cells (LSCs) as evidenced by reduction in disease recurrence. $Ctnnb1^{-/-}$ bone marrow cells were unable to maintain disease when used to subsequently generate recipients in a serial BM transplantation assay. IM treatment was noted to work effectively when used in combination with genetic depletion and abrogated LSCs in secondary (treated) recipients and delayed disease kinetics in tertiary recipient mice (16).

AV65, a Wnt/β-catenin signaling inhibitor considerably reduced total β-catenin and phosphorylated β-catenin levels in treated leukemic cells. AV65 also promoted β-catenin degradation via ubiquitin–proteasome pathway (17). β-catenin level was significantly higher in KCL22/HA and K562/HA cells and AV65 showed remarkable activity against these cells. AV65 was effective against mutant BCR-ABL expressing Ba/F3 cells. Growth of Ba/F3 cells was considerably inhibited by AV65 with IC50 values ranging from 21.6 to 46.5 nM (17).

$CD34^+/CD38^-$ leukemic stem cells had markedly higher expression of β-catenin. Sulforaphane (SFN), an isothiocyanate substantially improved response of $CD34^+/CD38^-$ leukemic stem cells to imatinib (18).

SHH Signaling and BCR-ABL

In the absence of SHH induced signaling, smoothened (SMO) is inhibited by patched (PTCH1) that allows protein kinase A directed phosphorylation and truncation of GLI2 and GLI3. GLI2rep and GLI3rep accumulated in the nucleus for transcriptional repression of target genes (19, 20).

Smo, Gli1 and Shh mRNA levels were significantly higher in CML group as compared to control group.

Changes in Smo, Gli1 and Shh mRNA levels were statistically insignificant in chronic phase CML patients in IM treated and untreated groups (21). Proliferation rate of OM9;22, a Philadelphia positive cell line, increased significantly after treatment with 10 ng mL^{-1} of Shh for 24 hours. GDC-0449, a hedgehog inhibitor notably reduced Gli1 expression in OM9;22 cells (22). Colony forming ability and cell proliferation were remarkably reduced in the Gli1 and Gli2 silenced K562 cells (22). Recombinant Shh N-terminal peptide not only increased Gli-1 and Shh but also up-regulated BCR-ABL expression in imatinib sensitive and resistant K562 cells. Similarly, BCR-ABL expression was reduced in Gli-1 silenced cells (23).

Mice with CML-like syndrome were used for the study to analyze role of SHH signaling in leukemia. BM from these mice consisted of approximately 50% regular BM cells and 50% BCR-ABL/GFP^+ cells (24). It was observed that treating mixed BM cultures with Cyclopamine for 3 days dose-dependently reduced BCR-ABL/GFP^+ population. There was almost 40% increase in spleen weight in all mice models inoculated with $Ptch1^{+/-}$, Smo WT, Ptch1 WT or $Smo^{+/-}$/ BCR-ABL/GFP fetal liver cells (24). However, weight of the spleen was normal (ranging from 90 to 110 mg) in mice models inoculated with Smo//BCR-ABL/GFP embryonic liver cells, which indicated that Smo was contributory in the expansion of BCR-ABL^+ cells (24).

Notch Signaling

Notch signaling is triggered by interaction between ligand-expressing cell and NOTCH-expressing cell. Ligand receptor interaction induced proteolytic processing of NOTCH to form NOTCH intracellular domain (NICD). Proteolytically processed NICD worked synchronously with co-activators, mastermind-like 1 (MAML1) and other proteins, to convert CSL-repressor complex into a transcriptionally active complex to trigger expression of the target genes (25, 26, 27).

NUMB, a NOTCH inhibitor has been shown to be frequently downregulated in different cancers. K562 cells that constitutively expressed dominant negative NUMB had higher proliferation rate. These cells were also partially resistant to imatinib (28). Hes1, a target gene of NOTCH was considerably enhanced in K562 cells at 48h post-treatment with 10 μM of imatinib. Gamma secretase inhibitors (GSI) notably reduced expression level of Hes1, however BCR-ABL activity was dramatically enhanced (29). BCR-ABL and NOTCH1ΔC expressing transgenic mice developed ALL in a shorter timespan with STAT5 activation. Results clearly suggested that synchronized activity of Notch1ΔC/NICDΔC with BCR-ABL and STAT5 played instrumental role in oncogenicity (30).

Regulation of Bcr-Abl by miRNA

Bcr-Abl mRNA levels were noted to be reduced dose dependently in K562 cells treated with curcumin. Detailed mechanistic insights revealed that curcumin induced an increase in the expression of miR-196b. miR-196b negatively regulated Bcr-Abl in curcumin treated LAMA84 and K562 cells (31). Shown in figure 1. miRNA-30e negatively regulated 3'- UTR of the abl gene and it has been shown that K562 cells reconstituted with miRNA-30e had notably reduced Bcr-abl levels and response of the K562 cells to imatinib treatment was significantly enhanced (63). 20% reduction in Abl and 50% reduction in Bcr–abl mRNA levels were noted in miR-

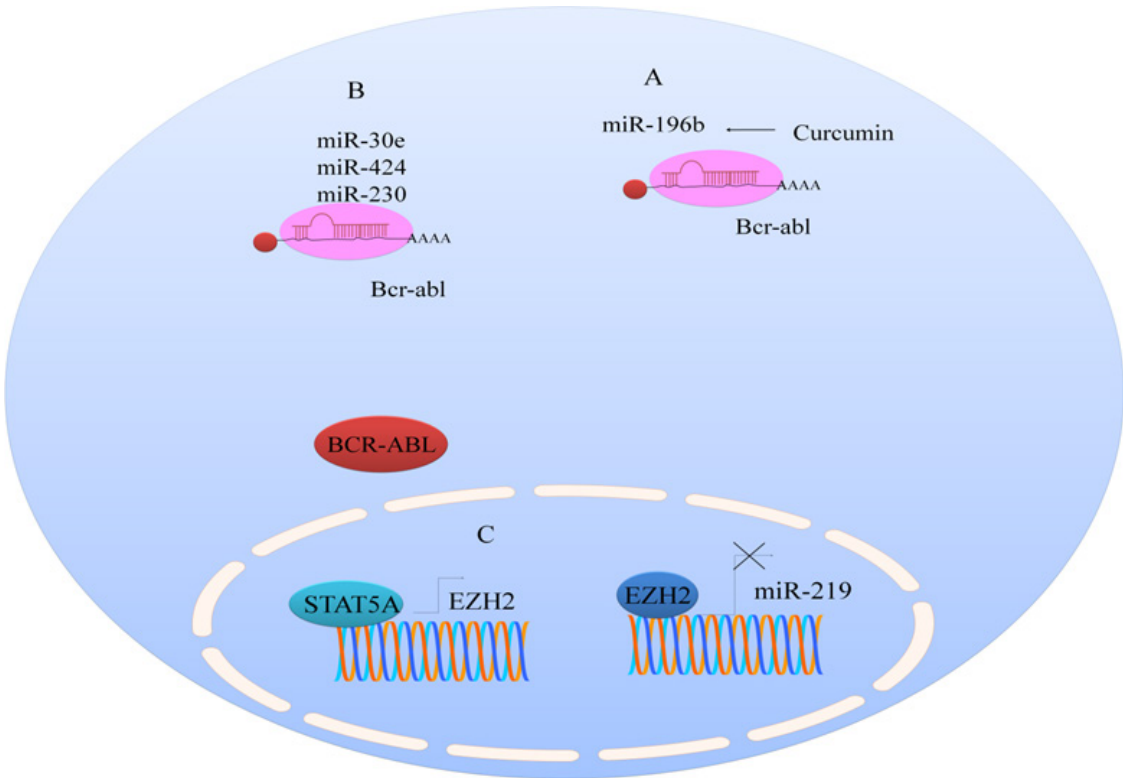

Figure 1. Shows (A) Curcumin induced upregulation of miR-196b and downregulation of Bcr-abl transcript. (B) Targeting of Bcr-abl by different miRNAs. (C) BCR-ABL facilitated loading of STAT5A on promoter region of EZH2. EZH2 consequently epigenetically repressed miR-219.

424 over-expressing K562 cells. Shown in figure 1. It was noted that IC50 concentration of imatinib calculated for the samples having upregulated expression of miR-424 was approximately 0.05 μM. Whereas, IC50 concentration of imatinib was between 0.17 and 0.63 μM for the samples having lower expression of miR-424 (32). Bcr-abl mRNA level was notably reduced in miR-23a expressing K562, SKMES1 and H157 cells. Transfection of miR-23a in the cells previously transfected with miR-23a recognition sequence containing 3′-UTR of Bcr-abl luciferase reporter constructs displayed significantly reduced Bcr-abl 3′-UTR reporter activity (33).

BGL3 (Beta Globin Locus 3), a noncoding RNA has been shown to regulate γ-globin expression during developmental stage. LncRNA-BGL3 level was notably enhanced in BCR-ABL silenced and imatinib treated K562 cells. Tumor growth was markedly reduced in mice inoculated with K562 cells that ectopically expressed lncRNA-BGL3. BCR-ABL has been shown to repress expression of lncRNA-BGL3 synchronously with c-Myc. lncRNA-BGL3 level was noted be significantly enhanced in c-Myc silenced K562 cells (34).

PcG protein enhancer of zeste homolog 2 (EZH2), a histone methyltransferase catalyzed trimethylation at 27th lysine of histone H3 (H3K27me3). BCR-ABL-induced increase in EZH2 in Ba-F3 cells was notably reduced upon treatment with dasatinib (35). BCR-ABL promoted loading of active STAT5A to promoter region of the EZH2 gene. EZH2 was noted to epigenetically silence miRNA-219 in leukemic cells and gene silencing of EZH2 restored expression of miRNA-219 (35).

Transcriptional regulation of BCR-ABL

β-arrestin1 structurally interacted with EZH2 to form a protein complex to mediate histone H4 acetylation. EZH2 catalyzed methyl groups addition to histone (H3) at 27th Lys (H3K27) to form H3K27me3 (36). There was a decrease in loading of EZH2 at the promoter regions of BCR-ABL and consequent reduction in H3K27me3 levels in β-arrestin1 silenced cells. EZH2 and β-arrestin1 interaction was considerably reduced in β-arrestin1 silenced K562 cells (36).

Treatment strategies to target BCR-ABL

Copanlisib (BAY80-6946) is a potent drug that selectively and reversibly inhibits PI3Kα and –δ. Copanlisib notably reduced phosphorylated level of BCR-ABL in BaF3/T315I and K562 cells. Shown in figure 2. Copanlisib and ponatinib synergistically reduced tumor growth in mice intravenously injected with BaF3/T315I cells (37). Specificity protein 1 (Sp1) is reportedly involved in transcriptional upregulation of Bcr-abl in K562 and KU812 cells. Transferrin (Tf)-targeted liposomal formulation for bortezomib delivery has recently been shown to be effective against BCR-ABL expressing leukemic cells. It considerably downregulated Bcr-abl mRNA level in treated leukemic cells. Nanotechnologically delivered bortezomib inhibited Bcr-abl level via by inhibiting the expression of Sp1 in leukemic cells (38).

SH2-U-box, a chimeric ubiquitin ligase harbored U-box domain of CHIP and SH2 domain of Grb2 to target both the mutant and wild type BCR-ABL (39). CHIP U-box domain of SH2-U-box had E3 activity and transferred E2-loaded ubiquitin to target protein bound via SH2 domain. SH2-U-box significantly increased wild type and mutant BCR-ABL ubiquitination. SH2-U-box significantly reduced total phosphorylated levels of BCR-ABL (39). Moreover, phosphorylated level of

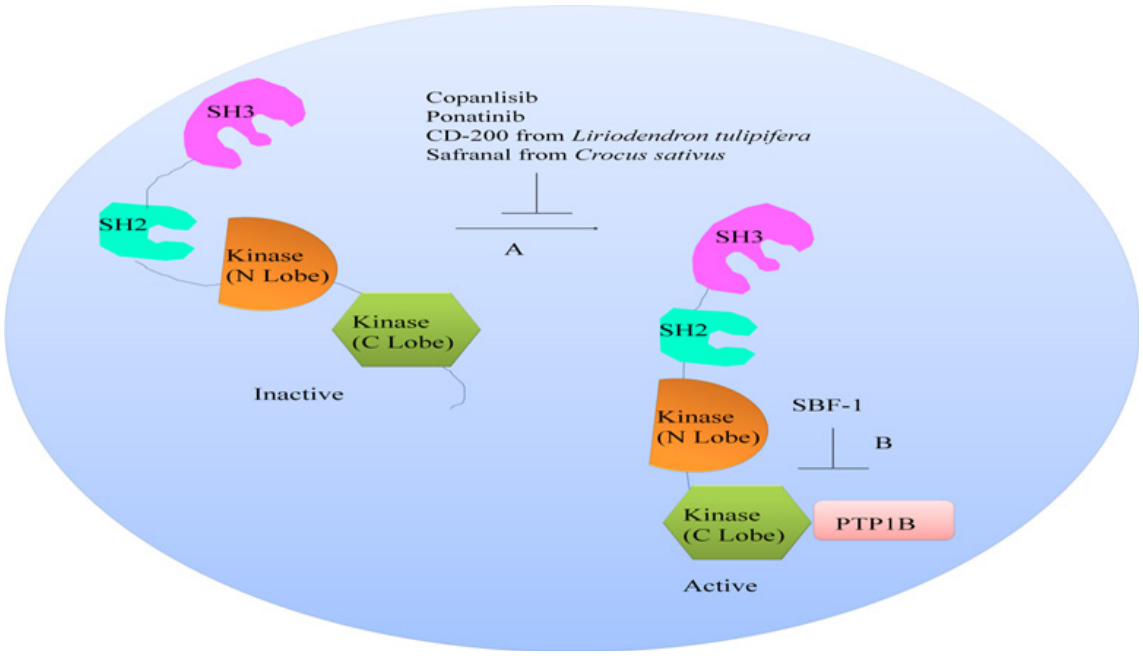

Figure 2. shows (A) conversion of BCR-ABL from inactive to active state. Different chemicals have been shown to inhibit the conversion of BCR-ABL into functionally active state. (B) BCR-ABL interacted with PTP1B and different strategies are being used to disrupt the interaction of BCR-ABL and PTP1B.

STAT5 was also reduced by SH2-U-box in both K562R and K562 cells. Tumor bearing mice were intra-tumorally injected with pLenti-SH2 at one side and pLenti-SH2-U-box at the other side. Expectedly, growth of the tumor at the SH2-U-box- treated sides was much slower as compared to tumor growth at the sides injected with SH2- expressing lentiviral vectors (39).

Interestingly, during a comparison between changes in the proteomic landscape of derived imatinib-resistant cells (CML-T1/IR) and imatinib-sensitive cells (CML-T1), NHERF1 (Na+ /H+ exchange regulatory factor) was observed to be most evidently changed regulator in the CML-T1/IR cells. Calcium signaling inhibitors and calcium transport blockers may prove to be effective in selective inhibition of the growth of imatinib-resistant cells (64). MPT0B169, an anti-tubulin agent effectively downregulated Bcr-Abl mRNA by approximately 65%–75% in IMR2, IMR3 and K562 cells (40).

SBF-1, a synthetic steroidal glycoside, time-dependently degraded BCR-ABL when used at a concentration of 40 nM (41). BCR-ABL and PTP1B were pulled down from total extracts of K562/G and K562 cells. BCR-ABL and PTP1B (Protein-tyrosine phosphatase 1B) were noted to structurally interact with each other. SBF-1 significantly inhibited structural interaction between PTP1B and BCR-ABL by competitively binding to the site where PTP1B and BCR-ABL interacted with each other (41), shown in figure 2.

PEITC, a natural isothiocyanate worked synergistically with imatinib and depleted protein levels of BCR-ABL (42). Data clearly suggested that PEITC inhibited both BCR-ABL and PKCs (Protein kinase C) independently at protein level that consequently resulted in inhibition of Raf1 and ERK1/2 proteins (42). Multi-targeted approaches have also shown encouraging results. Isoprenylcysteine carboxyl methyltransferase (ICMT) is involved in prenylation of different oncogenic proteins. Combinatorial inhibition of ICMT and BCR-ABL markedly reduced growth of tumors in xenograft CML mouse model (43).

Cyclin Dependent Kinase (CDK4) and Cyclin D2 played role in cell cycle progression of BCR-ABL positive leukemia. SU/SR, an imatinib-resistant subline of SU-Ph2 did not show response to imatinib (44). Levels of cyclin D2 and CDK4 were not downregulated in imatinib treated SU/SR cells. PD0332991, an inhibitor of CDK4/6 was noted to be effective against SU-Ph2 and SU/SR cells. Oral administration of PD0332991 for 14 days blocked rapid dissemination of SU/SR as well as SU-Ph2 into peripheral blood and bone marrow of xenografted mice (44).

CM363, a novel naphthoquinone derivative, time dependently increased apoptosis as evidenced by enhanced cytosolic accumulation of cytochrome C. Constitutive activation of both $pTyr^{177}$-BCRL-ABL and $pTyr^{694}$-Stat5 were considerably reduced after exposure of K562 cells to 5 µM CM363 (45).

Bisindolylmaleimide derivative, IX, has been shown to effectively target wild type and mutant BCR-ABL. Results revealed that response of BaF3 cells transduced with BCR-ABL was notable upon treatment with Bisindolylmaleimide IX (46). MEF cells that ectopically expressed BCR-ABL were also sensitive to Bisindolylmaleimide IX mediated killing effects. BCR-ABL expressing BaF3 cells had notably reduced Topoisomerase IIa levels which were further repressed in Bisindolylmaleimide IX treated cells (46).

PBA2, an inhibitor of BCR-ABL (imatinib-resistant) reduced phosphorylated BCR-ABL (Tyr177) levels in both BaF3/T315I and BaF3/WT cells at a concentration of 3 uM (47). Emodin, an anthraquinone isolated from Rheum palmatum L. exerted inhibitory effects on CML cells through different mechanisms. Emodin not only dose dependently inhibited Bcr-abl levels but also reduced mRNA levels of PI3k and Akt in treated CML cells. PTEN, a negative regulator of PI3K/AKT signaling axis was also notably enhanced in treated CML cells (48).

Protein knockdown technologies, which are particularly aimed at degradation of target proteins via the ubi-

quitin proteasome system (UPS) are also gaining considerable appreciation. SNIPER (Specific and Non-genetic inhibitors of apoptosis proteins [IAP]-dependent Protein Erasers) has recently been tested for efficacy against BCR-ABL (49). SNIPER(ABL)-2 was designed using a hexyl linker and it dose-dependently reduced BCR-ABL levels at concentrations of >30 uM after 8 hours of incubation. SNIPER(ABL)-3 contained a decyl linker and it reduced BCR-ABL levels after 24 hours of incubation (49).

Natural products mediated targeting of BCR-ABL

CD-200 isolated from *Liriodendron tulipifera* L. dose dependently inhibited BCR-ABL induced signal transduction in Imatinib resistant BaF3/T315I cells. Phosphorylated levels of BCR-ABL (Tyr177) were notably reduced in treated cell. Shown in figure 2. CD-200 also significantly inhibited tumor growth in mice xenografted with BaF3/T315I cells (50).

Safranal isolated from *Crocus sativus* L (saffron) interacted with BCR-ABL protein and was noted to reside inside drug binding cavity of the protein at similar place with the imatinib mesylate. The predicted hydrophobic contacts and polar interactions that consequently form a hydrophobic cavity inside the active site may be the underlying mechanism of phytochemical mediated inhibition (51).

Virosecurinine, isolated from *Securinega suffruticosa* considerably reduced expression level of BCR/ABL in K562 cells. Different concentrations were tested and it was noted that BCR/ABL levels reduced dose dependently and significantly reduced expression level was observed at 50 μmol/l of Virosecurinine (52). Kalpaamruthaa (KA), a modified Siddha preparation induced regression of tumor in 6-10-week-old female BALB/c mice injected with 12B1 cells (53). Denbinobin (5-hydroxy-3,7-dimethoxy-1,4-phenanthraquinone) notably inhibited expression level of BCR-ABL. Phosphorylated levels of CrkL were also reduced in Denbinobin treated K562 leukemia cells cells (54). Nemorosone, isolated from *Clusia rosea* has been shown to effectively target BCR/ABL in leukemia cells (55). Triptolide dose-dependently transcriptionally reduced BCR-ABL in KBM5-T315I cells when used at nanomolar concentrations. Triptolide also inhibited phosphorylated level of BCR-ABL in treated cells. Moreover, phosphorylated levels of CrkL and STAT3 were also potently inhibited by triptolide in wild-type or T315I BCR-ABL expressing BaF3 cells (56). 100 μg/mL Korean Red Ginseng Extract worked synergistically with 0.1 μM of Imatinib and considerably induced apoptosis (57). β-Hydroxyisovalerylshikonin worked synergistically with STI571 and reduced protein tyrosine kinase (PTK) activity of BCR-ABL (58). Pro-EGCG, a pre-drug version of green tea polyphenol EGCG has been shown to be effective against leukemic cells. Celastrol, isolated from the 'Thunder God Vine' in combination with Daunorubicin induced a 70% decrease in BCR-ABL protein levels in K-562 cells (59). Oleic acid isolated from *Daedalea gibbosa* significantly reduced autophosphorylation of BCR-ABL in BaF3 cells. Tumor growth was notably reduced in mice intraperitoneally injected with 10 mg/kg imatinib and 450 mg/kg of active fraction of *Daedalea gibbosa* (60). NPB001-05, isolated from of Piper betle leafs remarkably reduced proliferation of BCR-ABL expressing BaF3 cells and targeted 12 different clinically relevant mutations that induced resistance against imatinib treatment. NPB001-05 was noted to be effective against kinase activity of mutant and wild type BCR-ABL and exerted inhibitory effects on autophosphorylating enzyme time- and dose-dependently (61).

Nuclear Accumulation of BCR-ABL: Putting BCR-ABL Behind the Bars

Inward and outward trafficking of cargo between cytoplasm and nucleus occurs through nuclear pore complex (NPC). It is a channel across the nuclear membrane for modulation of bi-directionally transported cargo (62). Export of proteins from nucleus requires is a well co-ordinated process in which nuclear protein complexes with RanGTP and passes through the NPC. Hydrolysis of Ran-GTP to Ran-GDP is triggered by RanGTPase activating protein 1 (RanGAP1) that results in the release of nuclear protein in the cytoplasm (62). RanGAP1 levels are frequently overexpressed in K562 and KU812 cells. Imatinib efficacy was notably enhanced in K562 cells transfected with RanGAP1 shRNA. Accumulation of RanGAP1 at the nuclear membranes was significantly higher in the scramble group as compared to the RanGAP1 shRNA transfected cells. BCR-ABL protein is present predominantly in cytoplasm because of masking of its kinase domain by NLS (nuclear localization sequence). Imatinib can release the NLS domain to induce nuclear accumulation of BCR-ABL. miR-1301 quantitatively controlled expression of RanGAP1 in CML cells, however miR-1301 was noted to be frequently downregulated in CML cells (62). Rate of apoptosis was notably enhanced in miR-1301 plasmid transfected K562 cells upon treatment with imatinib. Mechanistically, miR-1301 mediated decrease in RanGAP1 level severely impaired trafficking of BCR-ABL to the cytoplasm and there was an increase in the nuclear distribution of BCR-ABL (62).

Conclusion

Data obtained from massively parallel sequencing technologies has helped us to comprehend cancer genomes at a finer resolution. Increasingly it is being realized that different cancers have different kinase fusions, however they share common hallmark characteristic of 'oncogene addiction'. It is an increased dependency on the activated kinase for uncontrolled proliferation of the cells and their survival. There are different tools which will be helpful for investigators for improvement of the procedures of development and optimization of clinically effective BCR–ABL inhibitors. LC–MS, to screen libraries of inhibitors having binding affinities for various functionally active state/s of protein kinases (target), high-throughput medicinal chemistry methodologies including, multicomponent reactions, diversity oriented synthesis and solid-phase parallel synthesis) coupled with structures of protein kinases for identification of lead compounds. Better comprehension of intracellular signaling cascades which are dysregulated in leukemia

and use of global phosphoproteome strategies will help us to identify 'phospho-signatures' and 'phospho-partners' associated with target tyrosine kinase. Strategies targeting BCR-ABL will be critically important for improving outcomes of patients, because as mentioned at the outset, mutant BCR-ABL is instrumental in rewiring intracellular signaling cascades.

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