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News and Commentary

BCR/ABL, mRNA translation and apoptosis

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Altered mRNA translation has been recently associated with malignant transformation of several cell types, including hematopoietic cells. The deregulated kinase activity of the BCR/ABL oncoproteins, hallmark of chronic myelogenous leukemia (CML), enhances proliferation and survival, and arrests differentiation of hematopoietic progenitors by aberrantly modulating the activity of signaling molecules that control gene transcription, induce post-translational modifications and regulate translation of mRNAs into functional proteins. In this review, we discuss the mechanisms whereby BCR/ABL, by altering the expression/function of specific RNA-binding proteins and the activity of the translational machinery, impairs maturation and decreases susceptibility to apoptosis of myeloid progenitors.

CML, a myeloproliferative disorder of the pluripotent bone marrow stem cell, involves the progression from an indolent 'chronic phase' (CML-CP) to the aggressive and fatal 'blast crisis' (CML-BC) marked by the clonal expansion of an immature population of differentiation-arrested myeloid or lymphoid blasts.[1,2] Pathognomonic for CML is the oncoprotein BCR/ABL,[3,4] the product of the translocation t(9;22)(q34;q11) designated as the Philadelphia chromosome (Ph[1]).[5,6] The chronic phase, which lasts several years, is characterized by accumulation in the bone marrow and peripheral blood of myeloid precursors, showing markedly enhanced proliferative potential and reduced susceptibility to drug-induced apoptosis, while retaining the ability to terminally differentiate into mature cells. The terminal and fatal 'blast crisis' phase lasts only a few months and is characterized by the rapid expansion and accumulation of myeloid or lymphoid precursors that exhibit enhanced proliferation and survival, increased genomic instability, altered motility and trafficking, while being completely unable to terminally differentiate.[7]

BCR/ABL expression is responsible for inducing and sustaining the leukemic phenotype through its deregulated tyrosine kinase activity, which is essential for the recruitment and activation of multiple pathways that transduce oncogenic signals leading to growth factor-independent proliferation, increased survival and altered differentiation of myeloid precursors (*reviewed* calabretta and Perrotti,[2] Sawyers[8]). Among the features of BCR/ABL-expressing cells, reduced susceptibility to apoptosis is a characteristic shared between hematopoietic cell lines ectopically expressing BCR/ABL, CML blast crisis and chronic-phase progenitor cells that exhibit prolonged survival in serum-free cultures.[9–12] By contrast, growth factor-independent proliferation and suppression of granulocytic differentiation are characteristics of BCR/ABL-expressing hematopoietic cell lines and CML blast crisis cells, but not of CML-chronic phase progenitors.[2,7,8,13]

The mechanism underlying progression from chronic phase to blast crisis, although still largely unclear, also appears to depend on BCR/ABL expression. In fact, levels of BCR/ABL often increase during disease progression[14–16] and sustained BCR/ABL expression in myeloid progenitor cells induces phenotypic changes characteristic of CML-BC.[2] Accordingly, inhibition of BCR/ABL kinase activity with imatinib mesylate (Gleevec, formerly STI571) is effective not only in the therapy of CML-CP[17] but also, albeit temporarily, of CML-BC,[18] in which imatinib resistance and relapses are contingent upon BCR/ABL reactivation.[19] However, there is still no evidence of a causal relation between BCR/ABL expression and blastic transformation. Similarly, it is not clear whether the secondary molecular (i.e. p53 genetic inactivation) and chromosomal (eg double Ph[1] chromosome, trisomy 8 or isochromosome 17) abnormalities, frequently detected in blast crisis CML,[2] occur as a consequence of increased BCR/ABL expression/activity. In this regard, although the recently reported inability of BCR/ABL-transduced committed myeloid progenitors to confer self-renewal ability *in vitro* and induce an acute leukemia-like process *in vivo*[20] apparently argues against the possibility that enhanced BCR/ABL expression may contribute to CML disease progression, it does not exclude it.

While the requirement for leukemogenesis of certain BCR/ABL downstream effectors, like those involved in the RAS/MAPK, PI-3K/Akt and STATs pathways,[2,8] is understood in some detail, considerably less is known about the mechanisms by which other *bona fide* BCR/ABL targets, including those controlling mRNA metabolism and, specifically, mRNA translation contribute to the phenotype of BCR/ABL-transformed cells. The findings discussed herein indicate that aberrant protein synthesis can be considered one of the mechanisms by which BCR/ABL transforms and sustains the leukemic phenotype of the hematopoietic progenitor cells.

Global Effect of BCR/ABL on mRNA Translation

Role of the PI-3K/Akt/mTOR pathway

One of the mechanisms whereby BCR/ABL regulates mRNA translation is the activation of the PI-3 K signaling

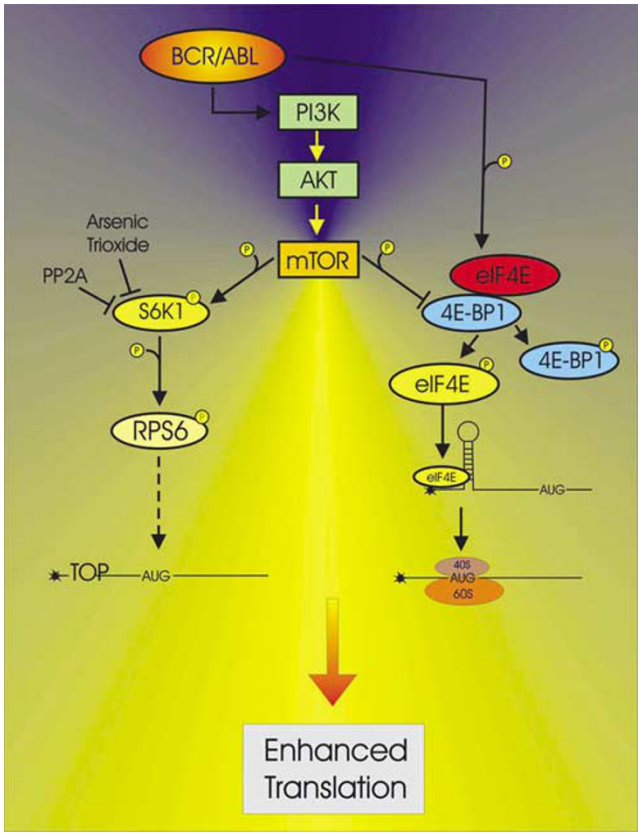

Figure 1 Effect of BCR/ABL on the translational machinery. The activation of PI-3K/Akt, the major anti apoptotic pathway in CML, by the oncogenic tyrosine kinase activity of the BCR/ABL oncogene is also responsible for activation of mTOR, which leads to inhibition of 4EBP1 and activation of S6K1 and eIF4E. BCR/ABL may also post-translationally control eIF4E activity by modulating eIF4E phosphorylation. Thus, the oncogenic activity of BCR/ABL has a global positive effect on the activity of the translational machinery

pathway[21–23] that, in turn, controls the efficiency of the translational machinery[24,25] (Figure 1). In CML, the PI-3K/Akt-generated signals not only enhance survival of hematopoietic progenitors[26–30] but also activate the *mammalian target of rapamycin* (mTOR).[31–33] The sustained activation of the mTOR pathway affects the function of two important regulators of the translation machinery, S6 kinase (S6K1) and 4E-BP1, which become constitutively phosphorylated in a BCR/ABL-dependent manner.[34] Specifically, S6K1 is considered to take part in the control of cell growth by enhancing mRNA translation.[35] Activated S6K1 enhances translation of 5′TOP (terminal oligopyrimidine tract) mRNAs, which contain a short polypyrimidine stretch (4–14 nucleotides) immediately adjacent to the 5′ cap site.[36] Conversely, 4E-BP1 is a negative regulator of translation that upon phosphorylation dissociates from the eucaryotic translation initiation factor 4E (eIF4E), which, in turn, binds the mRNA 5′ methyl cap structure and contributes, as part of the eIF4F complex, to the unwinding of the mRNA 5′-proximal secondary structure which facilitate the interaction with the 40S ribosomal subunit.[37,38] The function of eIF4E is particularly important for a subset of genes that are poorly translated in resting cells but recruited to ribosomes after a proliferative signal.[37] The importance of increased eIF4E activity for CML emergence and, perhaps, progression into blast crisis rests also on the evidence showing that overexpression of eIF4E transforms cells[39] most probably by increasing the translation of specific mRNAs (i.e. c-myc and cyclin D1).[40–42] Furthermore, it has been shown that the mTOR-regulated *c-myc* and *cyclin D1*[43,44] are required for BCR/ABL-dependent leukemogenesis.[45,46] Thus, the constitutive activation of the PI3K/Akt/mTOR pathway indicates that BCR/ABL may exert its leukemogenic potential also by altering the rate of translation of mRNAs encoding factors regulating survival, proliferation and differentiation of hematopoietic progenitors.

Although it is unclear whether phosphorylation of eIF4E increases cap-affinity, and is associated with increased rates of translation,[47–50] Dr Ong's group has recently demonstrated that eIF4E is phosphorylated at serine 209 in a BCR/ABL kinase-dependent manner.[51] Interestingly, it has been shown that phosphorylation of eIF4E favors its transforming activity[52] and leads to increased cyclin D1 protein levels which, in blast crisis CML cells, results from eIF4E-dependent cyclin D1 mRNA nucleocytoplasmic transport.[53] Cyclin D1 is not the only gene whose expression is regulated in BCR/ABL-transformed cells in an eIF4E-dependent manner; Ong's group has also found that specific genes, including cyclin D3, are regulated at the level of mRNA translation by BCR/ABL and mTOR kinase activity in the Ba/F3 system (Tiong S Ong, personal communication).

Possible translational autoregulation of BCR/ABL expression

There is evidence supporting the potential translational autoregulation of BCR/ABL expression. It has recently been reported that the BCR/ABL transcript itself can be found among the TOP mRNAs whose translation is enhanced by the activation of S6K1.[54] Indeed, treatment with Arsenic Trioxide markedly reduces the intracellular levels of BCR/ABL and induces apoptosis of BCR/ABL-expressing cells[55–57] through a mechanism that involves suppression of S6K1 activity and, therefore, translational inhibition of BCR/ABL expression.[54]

Role of PP2A

In BCR/ABL-transformed cells, translational control of gene expression might also result from inhibition of PP2A phosphatase activity. It has been shown that PP2A, a phosphatase with tumor suppressor activity,[58] regulates cell proliferation survival and differentiation[59,60] by inhibiting at post translational levels the function of mitogenic, anti- and/or proapoptotic and differentiation factors, including the BCR/ABL-activated Akt and S6 kinases.[61–64] Since in CD34$^+$ CML blast crisis cells PP2A activity is impaired by increased levels of BCR/ABL oncogenic kinase (Neviani *et al*., 2005; manuscript submitted), inhibition of PP2A activity may represent another mechanism by which BCR/ABL sustains and enhances the PI-3K/Akt/mTOR/S6K-dependent efficiency of the translation machinery.

Role of p53

In blast crisis CML, p53 is genetically inactivated in 30% of cases[2, 65–71] and, in the remaining 70% of cases, it is possible that increased MDM2 expression[72] may lead to p53 proteasome degradation. It has been shown that wild-type p53 regulates the activity of RNA polymerases I and III, which control the transcription of rRNA and tRNA, respectively. Thus, p53 may negatively affect the overall efficiency of the translational apparatus.[73,74] Furthermore, p53 can (a) associate with ribosomes[75] and modulate the translation of several mRNAs; (b) induce dephosphorylation of 4E-BP1 and its association with eIF4E;[76] and (c) modulate S6K1kinase activity.[76] Although it is not clear yet whether the effect of p53 on the regulation of mRNA translation is relevant for the leukemic phenotype of BCR/ABL-transformed cells, these observations strongly support the idea that, in blast crisis CML, loss of p53 expression and/or function not only decreases susceptibility of myeloid progenitors to chemotherapeutic drug-induced apoptosis but also could play a role in the translation regulation of mRNAs encoding factors important for the aggressive phenotype of blast crisis CML progenitor cells.

Effect of Imatinib mesylate on mRNA translation

It has been widely reported that imatinib mesylate induces *in vitro* and *in vivo* apoptosis of BCR/ABL-transformed hematopoietic cell lines and patient-derived CML bone marrow cells.[77] In BCR/ABL-transformed cells, inhibition of BCR/ABL kinase activity by imatinib not only alters at post-translational level the activity of many signal transducers or induces changes in gene transcription but also modifies the polysome loading and, therefore, the translation rate of several mRNAs, which encode proteins involved in the regulation of cell proliferation, survival and differentiation. For example, treatment of BCR/ABL-expressing cells with imatinib mesylate modifies the polysome/monosome distribution of several mRNAs (Perrotti and Calabretta, unpublished results). Interestingly, sequence analysis of these mRNAs revealed that almost 90% of them include in their 5′UTR certain elements (i.e. uORFs, multiple AUGs), which serve as targets for translational regulation. For example, c/ebpβ and p53 mRNAs, which reportedly undergo translational regulation[78–82] and are downregulated in BCR/ABL-transformed cells,[72,83] were less abundant (by oligonucleotidearray hybridization) in the polysome-associated mRNA fractions of untreated BCR/ABL-expressing cells, in which mdm2 mRNA levels were, instead, markedly increased.[72] Accordingly, Northern blot hybridization of polysome- and monosome-associated RNA separated by linear sucrose gradient centrifugation revealed that mdm2 mRNA was predominantly in the polysome-associated fractions of BCR/ABL-expressing cells, whereas it was clearly shifted toward the monosome fractions after STI571 treatment.[72] Further analysis revealed that increased mdm2 mRNA translation in BCR/ABL cells was dependent on the integrity of a 27-base nucleotide sequence of mdm2 mRNA (located between the second uORF and 36 nucleotide upstream of the main AUG) which specifically interacts with the La antigen.[72]

BCR/ABL, RNA Binding Proteins and Translational Regulation of Specific mRNAs

In BCR/ABL-expressing myeloid progenitor cell lines, high levels of BCR/ABL, as those observed in CML-BC, suppress differentiation, enhance survival and increase resistance to drug-induced apoptosis in part by enhancing the expression and activity of specific RNA binding proteins[72,84–87] (Figure 2). Such an increase in the expression of these mRNA-binding proteins correlates with the levels of BCR/ABL and is sensitive to the treatment with imatinib mesylate.[72,84,87,88] In BCR/ABL-expressing hematopoietic cells, expression of these RNA-binding proteins results from enhanced gene transcription (eg hnRNP A2/B1, hnRNP K, JKTBP1, hnRNP D1, Tra2β, RNPS1, EWSh, SC-35, Pabp2 and hnRNP H1) (Notari and Perrotti, manuscript in preparation), or increased protein stability (ie TLS/FUS, hnRNP A1, hnRNP E2 and La/SSB).[72,85–88]

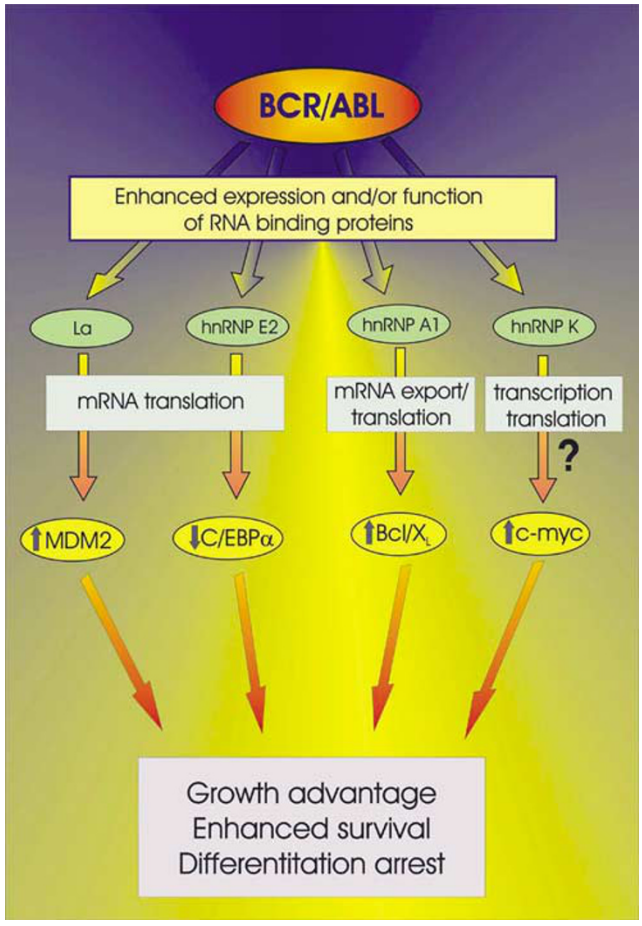

Figure 2 RNA binding protein-mediated effect of BCR/ABL on the translation of specific mRNAs. High levels of BCR/ABL tyrosine kinase activity, as those observed in blast crisis CML progenitors, are responsible for enhanced expression/function of specific RNA-binding proteins with translation regulatory function. For example, hnRNP E2 and La regulate the translation of *cebpa* and mdm2 mRNA, respectively, upon binding, in a sequence-specific manner, regulatory elements contained in the 5′UTR of those mRNAs. Similarly, the translation regulatory activity of other RNA-binding proteins, like hnRNP K and hnRNP A1, may be relevant for the phenotype of CML cells

BCR/ABL-dependent translational regulation by hnRNP K and hnRNP E2

In p210 BCR/ABL-expressing myeloid progenitor cell lines and in mononuclear marrow cells from patients with CML in blast crisis, there is an increased expression and function of two KH domain-containing hnRNPs, hnRNP E2[85] and hnRNP K[89] (Notari and Perrotti, manuscript in preparation).

These shuttling RNA-binding proteins together with hnRNP E1 form the hnRNP subfamily of the K homology (KH)-domain containing proteins[90]. hnRNP E2 is primarily localized in the cytoplasm, binds C-rich regions in the 5′ or 3′ UTR of cellular and viral mRNAs,[91–95] and functions primarily as a negative regulator of translation (but a positive effect on translation of viral mRNAs has been reported).[96] Likewise, hnRNP K is also a poly(rC)-binding hnRNP that reportedly regulates mRNA transcription, trafficking and translation.[97] It binds RNA through three repeats of an evolutionary conserved motif termed KH (K homology) domain, and its nucleocytoplasmic shuttling activity depends on the integrity of a 67 amino-acid motif, the KNS (hnRNP K-nuclear shuttling) domain.[98] Nevertheless, hnRNP K also binds DNA in a sequence-specific manner consistent with its role in regulation of gene transcription.[99,100] hnRNP K enhances c-myc transcription upon binding to a *cis* element in the c-myc promoter.[101–103] By contrast, it inhibits C/EBPβ-dependent transactivation by directly interacting with C/EBPβ itself.[104] hnRNP K also functions as a docking platform in proto-oncogene(s)-mediated signaling. For example, hnRNP K through a proline-rich element interacts with the SH3 domain of p95Vav[105] and c-Src.[105,106] In addition, PKCδ[107] and MAPK/ERK[108] phosphorylate hnRNP K on serine 302 and on serines 284/353, respectively. While hnRNP K-Vav interaction may be relevant for Vav-transforming activity,[109] the importance of hnRNP K phosphorylation by PKCδ for hnRNP K function as regulator of mRNA metabolism is still unclear.

By contrast, Src- and ERK-dependent hnRNP K phosphorylation seems to regulate the translation of those mRNAs that, like the 15-lipoxygenase (LOX) mRNA, contain in their 3′ UTR the CT-rich DICE (differentiation control element) motif.[108,110] Indeed, mutation at the ERK-phosphoacceptor site in hnRNP K abolishes the ability of hnRNP K to accumulate in the cytoplasm and impairs its ability to function as a silencer of LOX mRNA translation in immature erythroid precursor cells.[108,111] More recently, Willis's group reported that hnRNP K and hnRNP E2 or E1 regulates c-myc translation in a cap-independent mechanism which involves the binding of these hnRNPs to the c-myc IRES element located in the 5′UTR of myc mRNA.[112]

Thus, it is possible that BCR/ABL may alter the normal myeloid differentiation program by increasing hnRNP K transcriptional and/or translational activity. Interestingly, transcriptional increase of c-Myc expression and activation of the MAPK/ERK-dependent signal transduction pathway are features of BCR/ABL-transformed cells.[46,113–116] As c-Myc transcription and translation are, in part, dependent on hnRNP K[103,112,117–119] whose translation-regulatory activity and cellular localization are regulated by MAPK/ERK,[108, 120] it is possible that in BCR/ABL cells increased Myc expression results from increased expression/activity of hnRNP K. Indeed, by using hnRNP K mutants defective either in the transcription[102] or in the translation[108] regulatory function, we have evidence suggesting that hnRNP K translational activity is required *in vitro* for BCR/ABL leukemogenic potential.[89] Thus, the employment of a recently described microarray-based approach, termed Ribonomics,[121,122] would be useful for the identification of those mRNAs that interact with hnRNP K through a DICE element and whose translation is suppressed. This approach was originally developed by Dr Keene's laboratory where mRNAs interacting with the HuB RNA-binding protein where identified.[123] The best illustration of the utility of this approach rests in the recent identification of the mRNAs associated with the FMRP RNA-binding protein of the fragile X syndrome[124] and in the isolation of the hnRNP E2-associated mRNAs in the Ph[1] K562 cell line.[125]

As levels of hnRNP K increase during CML disease progression,[89] it is likely that BCR/ABL might require the translational regulatory activity of hnRNP K for inhibiting or enhancing the translation of DICE- or IRES-containing mRNAs, respectively, that may encode factors regulating differentiation, proliferation and/or survival of myeloid cells. This is, indeed, the case of the hnRNP E2-dependent regulation of c/ebpα mRNA translation.[85]

C/EBPα, whose expression is essential for granulocytic differentiation of multipotent progenitor cells,[126,127] is down-modulated in established BCR/ABL-expressing 32Dcl3 lines, in Ph[1] myeloid CML blast crisis cell lines and in primary bone marrow cells from CML blast crisis patients.[85] The down-regulation of C/EBPα expression correlates with the levels of BCR/ABL, suggesting that the effects are dose-dependent. However, the presence of detectable c/ebpα mRNA but not protein and the decreased neo-synthesis of C/EBPα in BCR-ABL-transformed cells indicate that inhibition of C/EBPα expression results from defective translation.[85]

C/EBPα expression is regulated by an evolutionarily conserved short uORF that acts in *cis* as a translational repressor.[79] However, a stalling mechanism caused by enhanced translation of the uORF that would prevent reinitiation from the c/ebpα AUG does not seem to be responsible for decreased C/EBPα synthesis in BCR/ABL-expressing hematopoietic cells. This conclusion is supported by the observation that mutation of the upstream AUG does not restore C/EBPα expression in BCR/ABL cells.

By contrast, decreased C/EBPα synthesis in BCR-ABL-expressing cells appears to depend on the integrity of the intercistronic region since mutations in this segment allowed more efficient translation from the main AUG.[85] Despite the possibility that the seven-nucleotide spacer element controls ribosomes release after termination of the uORF translation,[79] our data suggest that translation from the main c/ebpα AUG is inhibited by the physical interaction of hnRNP E2 with the intercistronic region of c/ebpα mRNA. The c/ebpα uORF and spacer region has the potential to form a stem-loop secondary structure that, if stabilized by interaction with hnRNP E2, may physically impede the assembly of the initiation complex and consequently the translation from the main AUG.[128] However, this seems not to be the case because only the C-rich intercistronic region, but not the integrity of the potential stem-loop structure, was required for interaction of hnRNP E2 with

the c/ebpα mRNA-binding protein and suppression of c/ebpα translation. Together with hnRNP E1 and hnRNP K, hnRNP E2 has been implicated in regulation of translation upon binding to cytosine-rich regions contained within RNA secondary structures.[129] In particular, hnRNP E1, hnRNP E2 and hnRNP K bound the 3′ untranslated region of 15-lipoxygenase mRNA and suppressed translation;[94,111,130] they also inhibited translation of human papillomavirus type 16 L2 mRNA upon interaction with a regulatory element in the 3′ of L2 mRNA coding sequence.[93] Moreover, these proteins have been implicated in regulation of poliovirus and hepatitis A RNA translation upon interaction with the 5′-untranslated region.[90] Despite its similarity to hnRNP E2 in sequence and RNA-binding characteristics, hnRNP E1 did not form a complex with the c/ebpα mRNA and its expression was essentially identical in normal and BCR-ABL-expressing cells. The reason for these differences is not known, but it should be noted that hnRNP E2, but not hnRNP E1, bound to the stem-loop IV of the poliovirus RNA 5′ noncoding region and regulated viral RNA translation in HeLa cells.[91]

hnRNP E2 expression inversely correlated with that of C/EBPα; in fact, hnRNP E2 levels were abundant in CML blast crisis cells, but virtually undetectable in mononuclear marrow cell from CML chronic-phase samples. Moreover, hnRNP E2 levels were upregulated by BCR-ABL in a dose- and kinase-dependent manner. Ectopic expression of hnRNP E2 in myeloid progenitor 32Dcl3 cells led to downmodulation of C/EBPα and G-CSFR, inhibited G-CSF-induced granulocytic differentiation[85] and caused apoptotic cell death most probably due to insufficient G-CSFR-dependent survival and proliferation signals.[85]

Potential role of hnRNP A1 as post-transcriptional and translational regulator in BCR/ABL-transformed myeloid cells

The expression of the ubiquitously expressed hnRNP A1 is higher in proliferating and/or transformed cells than in differentiated tissues.[131] This pattern has been linked to enhanced transcription associated with the presence of several regulatory elements in the hnRNP A1 promoter that may respond to proliferative signals.[131] hnRNP A1 is a nucleocytoplasmic shuttling protein that controls pre-mRNA and mRNA metabolism at different levels.[132] Expression of hnRNP A1 is increased in BCR/ABL-expressing cells.[87] Upon transformation of 32Dcl3 myeloid precursor cells by BCR/ABL, hnRNP A1 expression is markedly upregulated.[87] Increased hnRNP A1 expression is also detected in CML-BC samples compared to CML-CP samples and correlates with BCR/ABL levels. In 32D-BCR/ABL cells, increased expression of hnRNP A1 depends on enhanced protein stability and decreased ubiquitin/proteasome-dependent hnRNP A1 turnover.[87] The nucleocytoplasmic shuttling and RNA-binding activities of hnRNP A1 are activated by the PI3 K and BCR/ABL-regulated PKCζ[133,134] that phosphorilates A1 on Ser 199.[135] In BCR/ABL-transformed cells, PI-3K-dependent activation of PKCζ increases hnRNP A1 stability and enhances its nucleocytoplasmic trafficking (Perrotti, unpublished observation). Furthermore, expression of a nucleus-localized dominant negative A1, which lacks shuttling activity, impairs not only survival and differentiation of normal myeloid precursors but also growth factor-independent proliferation, colony formation and tumorigenic potential of BCR/ABL-expressing 32Dcl3 cells and primary $CD34^+$ CML-AP cells.[87] Moreover, cells expressing this mutant showed reduced levels of cytoplasmic Bcl-X_L mRNA,[87] suggesting that increased hnRNP A1 expression and, consequently, hnRNP A1-dependent mRNA export, may enhance Bcl-X_L mRNA nuclear export in BCR/ABL-expressing cells. Indeed, hnRNP A1 is able to directly bind to Bcl-X_L mRNA in immunoprecipitation assays from the Ph^1 K562 cells (Perrotti, unpublished observation). Bcl-X_L transcription is an event that follows the constitutive activation of the STAT5 antiapoptotic pathway in BCR/ABL-transformed cells.[136–141] Since the nuclear export of Bcl-X_L mRNA most likely depends on the nucleocytoplasmic shuttling activity of hnRNP A1, it is possible that, in BCR/ABL-expressing cells, expression of antiapoptotic factors like Bcl-X_L is not only controlled at a transcriptional but also at post-transcriptional level, through the activity of specific RNA-binding proteins. Interestingly, because hnRNP A1 was also recently described as a regulator of cap-independent IRES-mediated mRNA translation,[142] it is conceivable that the translation regulatory function of hnRNP A1 might contribute to the leukemic phenotype of BCR/ABL-expressing cells.

Role of La/SSB antigen as translational regulator in BCR/ABL leukemogenesis

In BCR/ABL-expressing cells, the La antigen was identified as the protein that upon binding to the intercistronic region (exon 2) of mdm2 mRNA enhances its translation.[72] La expression is markedly enhanced by BCR/ABL and correlated with that of MDM2.[72] Although La antigen is primarily localized in the nucleus, it is also present in the cytoplasm, and increases there under certain conditions.[143,144] For example, in poliovirus-infected cells, La is redirected to the cytoplasm, where it is believed to interact with the 5′ UTR of poliovirus mRNA to positively influence its translation.[144, 145] In addition to multiple virus-derived mRNAs, La has also been reported to interact with cellular mRNAs. Recently, the La antigen was reported to activate IRES-dependent translation of the immunoglobulin heavy chain binding protein (BiP) mRNA upon interaction with its 5′ UTR,[146] and of the X-linked inhibitor of apoptosis (XIAP) mRNA.[147] Interestingly, the only significant homology between mdm2 and BiP mRNA 5′ UTRs is in a short core sequence conserved in human and mouse mdm2 5′ UTR which corresponds to the region of interaction of BiP mRNA with La.

In BCR/ABL-transformed cells, the segment of the mdm2 5′UTR which contains the region involved in binding with the La protein can enhance GFP expression when placed in front of the GFP coding sequence (Calabretta B, personal communication). However, this segment did not function as IRES when driving GFP expression in BCR/ABL-expressing cells transduced with a bicistronic retrovirus.[72] This suggests that the La protein has a general role in the regulation of mRNA translation rather than controlling IRES-dependent translation only.[144] Indeed, two recent reports indicate that

La can regulate translation of a specific subset of mRNAs, the TOP mRNAs, containing a terminal oligopyrimidine tract sequence in their 5′UTR, in a casein kinase 2 (CK2)-dependent manner.[148–150] Note that CK2 plays an important role in the proliferation of BCR/ABL-expressing cells,[151] thus it is also possible that CK2-dependent regulation of La might lead to translation modulation of the expression of factors important for the growth advantage of CML cells.

In support of a more global role of La as regulator of mRNA translation, expression of XIAP was downmodulated in STI571-treated BCR/ABL-expressing cells,[152] suggesting that, in these cells, MDM2 and XIAP levels might be in part coregulated by La. Consistent with this, La is abundant in CML blast crisis primary samples and its levels appear to correlate with BCR/ABL levels and tyrosine kinase activity.[72] Thus, La is a *bona fide* positive regulator of mdm2 translation because: (a) it recognizes a specific conserved sequence tract in mdm2 mRNA that is required for efficient MDM2 expression *in vitro* and *in vivo*; (b) a dominant-negative La mutant inhibited mdm2 mRNA translation *in vitro* and suppressed MDM2 levels in BCR/ABL-expressing cells; (c) downregulation of La expression by siRNAs led to a marked decrease in MDM2 levels and (d) overexpression of wild-type La led to an increase in MDM2 expression.[72]

That La-mediated effect on MDM2 expression is functionally relevant for BCR/ABL leukemogenesis is indicated by the changes in susceptibility of BCR/ABL-expressing cells to adriamycin-induced apoptosis, as wild-type La-overexpressing cells were more resistant than parental cells, whereas cells expressing dominant negative La were more sensitive.[72] Although MDM2 levels were markedly downmodulated in BCR/ABL cells expressing the dominant-negative La, these cells neither exhibited spontaneous apoptosis nor altered cell cycle activity, consistent with the primary role of MDM2 as a regulator of p53 and with the fact that the proapoptotic activity of p53 is enabled by stress-inducing stimuli (e.g. DNA damage). Since activation of La RNA-binding activity to the mdm2 5′ UTR was also observed in v-Src-transformed 32Dcl3 myeloid precursor cells (Trotta R, unpublished observation); altogether these data suggest that the La-dependent translational stimulation of MDM2 expression might be relevant in the enhanced survival of cancer cells expressing constitutively active tyrosine kinases and might contribute to progression of CML into blast crisis.

Conclusions

Translational regulation by the BCR/ABL oncoproteins has not yet been investigated as extensively as other mechanisms regulating expression and function of factors important for the emergence, maintenance and blastic transformation of CML. As discussed here, some of the changes in gene expression might depend on altered activity of the basal transcription machinery or on aberrant expression of RNA-binding proteins with translation-regulatory function. For example, the block in differentiation associated with downregulation of C/EBPα protein levels and the increased survival associated with MDM2-dependent inhibition of the p53 proapoptotic effects are essential features of the highly malignant cell clones of CML blast crisis. It seems likely that aberrant translation regulation in cancer cells is not limited to BCR/ABL-dependent leukemogenesis, and that there are many more mRNA in addition to *CEBPA* and *mdm2* that undergo translation regulation in CML. However, an obvious question raised by the recent discoveries in the emerging field of translation regulation in cancer is whether the current methodology (e.g. ribonomics) is sufficiently sensitive and specific for the identification of translationally regulated mRNAs, and whether the interaction of RNA-binding proteins with specific mRNAs can provide targets for therapeutic intervention. As an example, we can envision the use of small molecules that, by interfering with the La/mdm2 mRNA interaction, may inhibit MDM2 expression which, in turn, may render leukemic cells more susceptible to chemotherapeutic drugs-induced p53-dependent apoptosis. A similar approach can be potentially used to restore C/EBPα expression and, therefore, granulocytic differentiation of CML blast crisis myeloid progenitors, in which block of differentiation depends, in part, on the translation inhibitory activity of hnRNP E2. Thus, although the mechanisms regulating mRNA are enormously complex, we may safely predict that the continuous improvement in the specificity and sensitivity of the available genomic and proteomic high-throughput platforms will lead to the identification of translationally regulated key-players that may serve as target for the rational development of new anticancer molecular therapies.

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