Ubiquitination is not omnipresent in myeloid leukemia

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doi:10.3324/haematol.2019.224162

Chronic myelogenous leukemia (CML) is a clonal biphasic hematopoietic disorder most frequently caused by the expression of the BCR-ABL fusion protein. The expression of BCR-ABL fusion protein with constitutive and elevated tyrosine kinase activity is sufficient to induce transformation of hematopoietic stem cells (HSC) and the development of CML.[1] Despite the introduction of tyrosine kinase inhibitors (TKI), the disease may progress from a manageable chronic phase to a clinically challenging blast crisis phase with a poor prognosis,[2] in which myeloid or lymphoid blasts fail to differentiate. Progression of BCR-ABL-positive ($^+$) leukemia from the chronic phase to the acute blast crisis phase is accompanied by increased BCR-ABL expression and genomic instability leading to the acquisition of secondary genetic lesions including +8, +Ph, +19, mutations in *P53*, *Runx1*, *ASXL1*, *WT1TET2*, *IDH1*, deletion of *INK4A/ARF*, and/or *PAX5*, *IKZF1*, *EBF1* resulting in myeloid or B-lymphoid blast crises.[3,4] However, our understanding of the mechanisms of transformation in blastic crisis remains incomplete.

In this issue of the Journal, Magistroni *et al.*[5] identified the presence of mutations in three different amino acids (D144, I33, M34) impairing the expression and/or activity of one of the alleles of the ubiquitin conjugating enzyme E2A (UBE2A, also called RAD6A) in the blastic phase, but not in the chronic phase, of two out of ten CML patients. Analysis of an unmatched, larger cohort of 24 blast crisis, 41 chronic phase, 40 acute myeloid leukemia (AML), and 38 BCR-ABL-negative CML specimens confirmed the presence of these mutations in 16.7% of blastic phase CML patients but not in any of the other groups analyzed. Mechanistically, the silencing of UBE2A or overexpression of one of the UBE2A mutants (I33M) in BCR-ABL$^+$ leukemic cells results in myeloid differentiation blockade *in vitro* with upregulation of *ITGB4*, *RDH10* and *CLEC11A,* and downregulation of *CFS3R/CSF3R* and *RAP1GAP*. The fact that UBE2A mutations were exclusively found in the blast crisis CML patients, and these mutations control the process of differentiation arrest indicates that mutant UBE2A is a potential target for intervention in blastic phase CML.

Ubiquitin conjugating enzymes in inflammation and cancer

UBE2A is an E2 ubiquitin conjugating enzyme. Ubiquitination is a highly conserved post-translational modification process affecting the proteasome-mediated degradation as well as activity of target proteins. The process occurs in three sequential steps mediated by ubiquitin-activating enzyme (E1), ubiquitin conjugating enzyme (E2), and ubiquitin ligase (E3).[6] In humans, nearly 40 E2 ubiquitin conjugating enzymes regulate ubiquitination of target proteins through their cognate E3 ubiquitin ligases belonging to three different families (RING, HERCT, RING-between-RING or RBR type E3).[7]

The ubiquitin conjugating enzymes including UBE2N (UBC13) and UBE2C are over-expressed in a myriad of tumors such as breast, pancreas, colon, prostate, lymphoma, and ovarian carcinomas.[8] Higher expression of UBE2A is associated with poor prognosis of hepatocellular cancer.[9] In leukemia, bone marrow (BM) cells from pediatric acute lymphoblastic patients show higher levels of UBE2Q2 expression in comparison to normal counterparts.[10] Ubiquitin conjugating enzyme E2E1 (UBE2E1) expression is adversely correlated with AML survival.[11] However, in this report, the inactivating mutation of UBE2A seems to facilitate CML progression, and therefore UBE2A seems to act as a tumor suppressor.

Based on our understanding of mechanisms controlled by UBE2A, four different signaling pathways may be involved in blast crisis transformation (Figure 1).

Inflammatory myeloid differentiation is mediated by ubiquitination

First, the abundance of pro-inflammatory cytokines including interleukin-1 (IL-1) and tumor necrosis factor alpha (TNF-α) in the leukemic microenvironment regulates myeloid differentiation through the activation of NFκB and MAPK signaling pathways.[12] These activities are mediated by TNF-α receptor-associated factor (TRAF) family E3 ligases.[13] The activation of IL-1 and TNF-α receptor induces the recruitment of MyD88, IL-1 receptor-associated kinase (IRAK4, IRAK2) to the myddosome complex resulting in the activation of TRAF6. UBE2A might act as upstream ubiquitin conjugating enzymes for TRAF6 E3 ligase in CML myeloid blasts, and loss-of-function of UBE2A may attenuate the TRAF E3 ligase-mediated activation of NFκB and MAPK signaling pathways, leading to the impaired myeloid differentiation (Figure 1, signaling path A). This is a signaling mechanism involved in myeloid differentiation with unclear significance in the context of UBE2A loss-of-function mutations.

Ubiquitination regulates BCR-ABL and MYC expression in myeloid leukemia

The transformation to blast crisis phase is associated with selection of clones with high BCR-ABL1 expression. However, the mechanism of enhanced BCR-ABL1 expression remains poorly understood. It has been shown that arseniate, a curative agent in acute promyelocytic leukemia, induced cell apoptosis and degradation of BCR-ABL in CML cells. The ubiquitination and degradation of BCR-ABL was mediated by c-CBL, a RING-type E3 ligase.[14] Although speculative, it is possible that c-CBL acts as a cognate E3 ligase for UBE2A for the ubiquitina-

tion and subsequent degradation of BCR-ABL. Furthermore, expression of transcriptional factor MYC plays a critical role in the proliferation and self-renewal of leukemic stem cells. Reavie *et al.* demonstrated that the E3 ubiquitin ligase FBW7 is required for the survival and maintenance of BCR-ABL$^+$ leukemia initiating cells (LIC) by modifying the expression of MYC through FBW7-mediated ubiquitination and degradation.[15] Deletion of Fbw7 leads to c-Myc overexpression, p53-dependent LIC-specific apoptosis, and the eventual inhibition of tumor progression. A decrease in either c-Myc protein levels or attenuation of the p53 response rescues LIC activity and disease progression. UBE2A acts as E2 conjugating enzyme for FBW7, and mutations in UBE2A attenuate FBW7 activity and maintain the basal expression level of MYC required for survival and propagation of leukemic blast (Figure 1, signaling path B).

UBE2A activity maintains genomic integrity

Myeloid blastic transformation in CML requires genomic instability which may originate from imatinib-refractory CML stem cells.[16] Genomic instability is mediated by loss-of-function of DNA repair process. The UBE2A described in this report is the human homolog of yeast Rad6, and has been demonstrated to play a critical role in DNA repair and genome integrity.[17] UBE2A and UBE2B regulate DNA damage through post-translational modification of proliferating cell nuclear antigen (PCNA).[18] The ubiquitination of PCNA at Lys 164 in response to genotoxic stress recruits DNA polymerase

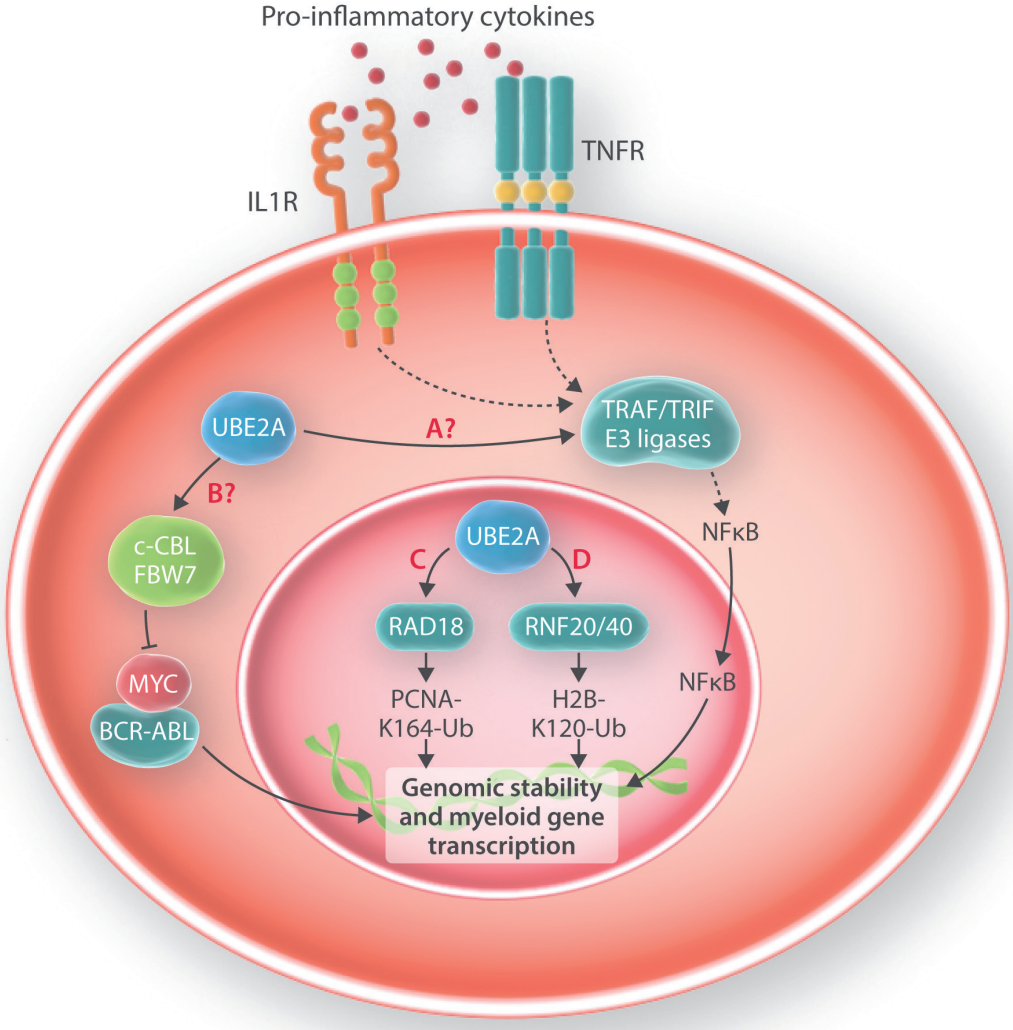

Figure 1. Schematic representation of possible UBE2A-mediated mechanisms controlling myeloid blastic transformation in BCR-ABL leukemias. Possible targets of UBE2A relevant to leukemic myeloid transformation. (A and B) Cytosolic functions. UBE2A is an E2 ubiquitin ligase important in emergency myelopoiesis induced by inflammatory cytokines, abundant in the leukemic microenvironment, through the TRAF/TRIF E3 ligases, regulators of the transcriptional factor NFkB. Loss-of-function of UBE2A may associate with impaired myeloid differentiation. (B) UBE2A regulates the activity of E3 ligases c-CBL and FBW7, which are tumor suppressors with known activity to induce degradation of BCR-ABL and MYC, whose expression in turn is required for leukemic acceleration. Question marks denote that these pathways of activity of UBE2A are speculative and not supported by direct experimental designs. (C and D) Nuclear functions. (C) UBE2A is a well known regulator of DNA repair through its cognate E3 ligase RAD18, which monoubiquitinates the proliferating cell nuclear antigen (PCNA), a modification that recruits translesion DNA polymerases to stalled replication forks. (D) Active UBE2A (phosphorylated by CDK9) regulates H2Bmonoubiquitination through recruitment of the E3 ligase RNF20/40, a major step in regulation of RNA polymerase II and gene transcription.

and activates translesion synthesis DNA repair pathway.[19] Furthermore, cell cycle dependent kinase-9 (CDK9) regulates UBE2A activity by phosphorylating at serine 120.[20]

UBE2A regulates the ubiquitination of histone H2B and proliferating cell nuclear antigen (PCNA) through the cognate E3 ubiquitin ligase RNF20/40 and RAD18, respectively. In addition to its role in transcriptional elongation, histone H2B K120 monoubiquitination plays a crucial role in DNA double strand break (DSB) repairs.[21] Both these processes describe the role of UBE2A in DNA repair and maintenance of genome integrity. The loss-of-function mutations of UBE2A in advanced phase CML patients may be associated with impaired ubiquitination of H2B and PCNA, and hence increased genome instability resulting in the acquisition of additional mutations (Figure 1, signaling paths C and D). The work by Magistroni *et al.*[5] focuses on the latter signaling paths as possibly being at the root of the myeloid transformation. While the mechanisms that control the blastic transformation of CML by UBE2A mutations remain unclear, mutation studies like that of Magistroni *et al.* do generate hypotheses that should be tested in further studies into BCR-ABL leukemia initiation and propagation.

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Six-packed antibodies punch better

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doi:10.3324/haematol.2019.224196

In this issue of the Journal, Oostindie *et al.* investigate CD37-specific monoclonal antibodies (mAb) engineered to undergo hexamerization.[1] Efficient hexamer formation is induced by a single amino acid substitution, E430G, in the IgG1 constant domain previously described by the same group.[2] The modification potentiates complement-dependent cytotoxicity (CDC) against chronic lymphocytic leukemia (CLL) cells *in vitro*. Next, the authors show that combinations of hexamerization-enhanced mAb against CD20 and CD37 provide synergistic activity. Intriguingly, the CD20- and CD37-targeting mAb formed mixed hexameric complexes on the cell surface with increased anti-tumor activity.

The anti-CD20 mAb rituximab is a critical component of treatment regimens for many B-cell malignancies.[3] In combination with chemotherapy, rituximab has been shown to increase response rates, response duration, and overall survival. Single-agent rituximab is quite commonly used in follicular lymphoma and as maintenance therapy in several types of B-cell non-Hodgkin lymphoma (B-