AU-rich RNA binding proteins in hematopoiesis and leukemogenesis

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Running Title: AUBPs in normal and malignant hematopoiesis
Abstract

Post-transcriptional mechanisms are now widely acknowledged to play a central role in orchestrating gene regulatory networks in hematopoietic cell growth, differentiation and tumourigenesis. While much attention has focussed on microRNAs as regulators of mRNA stability/translation, recent data has highlighted the role of several diverse classes of AU-rich RNA binding protein in the regulation of mRNA decay/stabilisation. AU-rich elements are found in the 3’ untranslated region of many mRNAs that encode regulators of cell growth and survival such as cytokines and onco/tumour-suppressor proteins. These are targeted by a burgeoning number of different RNA-binding proteins. Three distinct types of AU-rich RNA binding protein (ARE poly-U-binding degradation factor-1/AUF1, Hu antigen/HuR/HuA/ELAVL1 and the tristetraprolin/ZFP36 family of proteins) are essential for normal hematopoiesis. Together with two further AU-rich RNA binding proteins, nucleolin and KHSRP/KSRP, the functions of these proteins are intimately associated with pathways that are dysregulated in various hematopoietic malignancies. Significantly, all of these AU-rich RNA binding proteins function via an interconnected network that is integrated with microRNA functions. Studies of these diverse types of RNA binding protein are providing novel insight into gene regulatory mechanisms in hematopoiesis in addition to offering new opportunities for developing mechanism-based targeted therapeutics in leukaemia and lymphoma.
Introduction

Around two-thirds of protein abundance variation of mammalian cells can be accounted for by post-transcriptional mechanisms. Although the role of micro-RNAs (miRNA) in regulating mRNA stability and translation is well-established, an expanding number of proteins that bind AU-rich elements (ARE) in the 3' untranslated regions (UTR) of mRNA have been discovered to mediate mRNA decay/stabilisation or translational control. Of the dozen or so AU-rich RNA binding proteins (AUBP) that have been characterised in detail, most bind a multimeric pentamer sequence AUUUA, typically located within a 50-150 nucleotide adenine- and uridine-rich 3' UTR element (and references therein). An estimated 8% of mammalian mRNA transcripts are potentially targeted by AUBPs and many well-characterised AUBP mRNA targets encode key regulatory proteins such as growth factors, cytokines, chemokines and oncoproteins (and references therein). The mechanisms through which AUBPs mediate post-transcriptional regulation, particularly in the context of immune regulation and the inflammatory response, have been reviewed extensively elsewhere. Most function as accessory proteins to recruit mRNAs and to regulate their fate in various sub-cellular compartments, such as the exosome mediating 3'-5' decay, processing bodies for 5'-3' decay and stress granules for translational arrest. Some function cooperatively or antagonistically with each other or with argonaut (AGO) endoribonucleases within RNA-induced silencing complexes (RISC) mediating miRNA-dependent decay. In addition, most AUBPs display some degree of molecular promiscuity in their ability to modulate gene expression through ARE-independent mechanisms involving either DNA-binding or protein interaction.

Accumulating data implicates five AUBP types as key regulators of normal and malignant hematopoiesis. These are: ARE poly-U-binding degradation factor-1 (AUF1), HuR (ELAV-like family 1), KH-type splicing regulatory protein (KSRP/KHSRP), nucleolin, and the members of the ZFP36 (Tis11) family, ZFP36, ZFP36L1, ZFP36L2. In this review, we summarise the relevant experimental evidence for this and discuss the complex regulatory interactions that exist between these AUBPs and their mRNA targets and how their functions are integrated with miRNA gene regulatory pathways in hematopoietic cells.
AUF1

The AUF1 protein occurs in four different isoforms (p37, p40, p42, p45) and regulates gene expression through multiple mechanisms. It directly regulates a number of mRNAs that play key roles in hematopoietic cells including Bcl-2, TNF-α, IL-3, IL-6, COX-2, Cyclin D1, IL-8, c-Fos, c-Jun9-14. It can also modulate mRNA translation and gene transcription. For example, while AUF1 mediates decay of c-Myc proto-oncogene mRNA in cell-free systems15, it induces Myc translation in an ARE-dependent manner in K562 erythroleukemia and THP-1 myelomonocytic cell line models10. AUF1 associates with the AUBP, nucleolin, to form LR116, a heterodimeric protein complex, that binds to the GGNCNAG(G/C)CTG(G/A) consensus sequence present in the c-Myc P1 promoter17 to induce transcription. LR1 binds a similar consensus sequence present in the EBV EBNA-1 promoter and immunoglobulin heavy chain S regions (G-rich regions) involved in class switch recombination16,18. By contrast, AUF1 (p42, and p45 isoforms) exhibits phosphorylation-dependent sequence-specific binding to and transcriptional repression of the CD21 promoter in several B cell lines and in primary B cells19.

AUF1 function is essential for normal lymphopoiesis (Fig 1). Knock-out mice that are deficient in all four AUF1 isoforms display decreased numbers of splenic T and B cells (especially Follicular B cells) together with a modest expansion of the splenic Marginal Zone B cell compartment20 (Fig 1). The reduced numbers of Follicular B cells is attributable to increased turn-over and apoptosis, most likely arising as a consequence of decreased levels of pro-survival proteins, Bcl-2, Bcl-Xl and Bfl-1/A120. AUF1-deficient mice also display reduced IgG serum levels in response to T-independent antigen despite no impairment in germinal centre formation and class switch recombination20.

AUF1 associates with the Nucleophosmin-Anaplastic Lymphoma Kinase (NMP-ALK) fusion protein that results from the most common translocation, t(2;5)(p23;q35), found in anaplastic large cell lymphoma (ALCL) and some other lymphomas21. It co-localises with NMP-ALK-containing granules where it is targeted for phosphorylation. In its phosphorylated state, AUF1 has been suggested to result in stabilisation of mRNA targets that would otherwise be targeted for decay. The expression of several AUF1 targets such as cyclin A, cyclin D, c-Myc21 and c-Jun22 correlates with ALCL disease (Table 1).
HuR/ELAV1

The ubiquitously expressed HuR/ELAV1 member of the ELAV family of AUBP acts to both stabilise and to modulate the translational efficiency of various mRNAs involved in normal and malignant hematopoiesis. It stabilises mRNAs encoding Bcl-2, Mcl-1, Cyclin A, cyclin B1, cyclin D1, lymphotoxin α, GM-CSF, IL-4, VEGF, CD3ζ, CD95L, GATA-3, XIAP, survivin\textsuperscript{23-30}. HuR has also been reported to destabilise mRNAs for AML1/Runx1, CD2, Vav1, NF-κBIE, CD3ε, TNF-α and Stat3\textsuperscript{27}. HuR enhances the translational efficiency of mRNAs encoding p53, cytochrome c, XIAP and Bcl-2 while suppressing translation of p27, c-Myc and Wnt5α\textsuperscript{26,29,31}. The mechanism of HuR-mediated translational repression of c-Myc has been studied in some detail and requires recruitment of let-7 miRNA in an Ago2-dependent manner\textsuperscript{32}. Let-7 miRNA functions as translational repressor through recognition of the m\textsuperscript{7}G cap on c-Myc mRNA leading to either impaired recruitment of eIF4E or to inhibition of eIF4E-eIF4G association\textsuperscript{33}. HuR itself is subject to miRNA-mediated regulation through translational repression by several miRNAs (see Fig 2) including miR-16 and miR-125α\textsuperscript{34,35}, miR-519\textsuperscript{36} and miR-34α\textsuperscript{37}.

Recent transcriptome-wide screening has identified many thousands of additional mRNAs that are direct and functional targets of HuR in human cells\textsuperscript{38,39}. HuR binding sites that overlap with or are adjacent to miRNA binding sites facilitate combinatorial regulation by HuR and miRNAs, most likely through alleviation of miRNA-mediated repression by HuR\textsuperscript{39}. Significantly, many HuR binding sites occur in introns and are often associated with splice sites to regulate alternative splicing. RNA processing mechanisms also account for HuR-mediated repression of the miR-7 miRNA that functions as a potent tumour suppressor in many human cancer types\textsuperscript{38}.

HuR is required for normal mouse embryonic development\textsuperscript{40}. The postnatal function of HuR was investigated in a tamoxifen-dependent cre recombinase deletion mouse model\textsuperscript{40}. Following tamoxifen-induced HuR deletion, mice die within 10 days. An analysis of the hematopoietic system within the first four days of tamoxifen treatment revealed that these mice are characterized by possession of atrophic spleens, lymph nodes and thymus accompanied by reduced numbers of lymphoid, myeloid and erythroid progenitor cells in the bone marrow\textsuperscript{40} (see Fig 1). The peripheral blood lymphocyte count is markedly diminished while granulocyte numbers are modestly increased; red blood cell and platelet counts are unaffected\textsuperscript{40}. Pro-B and pre-B cells, but not mature, late pre-B cells, are characterised by
widespread apoptosis and necrosis in HuR-deficient mice that can be explained by increased levels of pro-apoptotic p53, NOXA, PUMA, p21, caspase-9 mRNA levels and decreased levels of pro-survival Bcl-2 and Bcl-Xl mRNA levels in bone marrow of HuR-deficient mice. The disparity found between immature and mature B cells may be due to the fact that HuR is normally expressed at higher levels in immature-B compared with mature-B cells. There is also a shift in sub-cellular distribution of HuR protein from both nuclear and cytoplasmic in immature pre-B cells to exclusively cytoplasmic in mature B cells.

The same HuR-deficient mice also display decreased numbers of double positive (CD4+ CD8+, DP) T cells but with an increase in the number of double negative (CD4'CD8', DN) and single positive cells (CD4' or CD8', SP) thymic T cells. However when deletion of HuR was specifically targeted to thymocytes, there were increased numbers of thymic DN, SP and DP cells in thymus (Fig 1). In the latter study, HuR was found to affect the proliferation of double negative cells, the process of positive selection and the egress of mature T cells to the periphery. Increased proliferation of the DN cells was attributed to decreased p53 protein levels. Decreased positive selection was attributed to decreased phosphorylation of ZAP-70, Lck and PKCθ while reduced egress of T cells was attributed to reduced chemotaxis in response to the CCR7 ligand, CCL21 and the CXCR4 ligand, SDF-1.

Finally, when deletion of HuR was targeted to B cells (CD19-Cre), the mice were characterised by reduced numbers of marginal zone B cells in the spleen, reduced number of B1 cells in the peritoneal cavity while follicular B cells remained unchanged (Fig 1). Additionally these mice displayed an impaired germinal centre formation and IgG1 secretion in response to a T-cell-dependent antigen.

In common with AUFI, HuR co-localises with NMP-ALK in NMP-ALK granules in ALCL and is a substrate for tyrosine phosphorylation by this kinase. The level of cytoplasmic HuR is not affected by the presence of NMP-ALK which instead promotes its localisation to polysomes and binding to cEBPβ mRNA. This results in stabilisation and increased translation of cEBPβ mRNA. Increased expression of c-EBPβ is a unique feature of ALCL compared with several other leukemias and lymphomas. The c-EBPβ protein regulates apoptosis in ALK-positive cell lines as well as tumour growth in mice in vivo.
HuR may also play a wider role in leukemogenesis (Table 1). It is over-expressed in M4 Acute Myeloid Leukemia (AML) and correlated with high levels of eIF4E. It is also over-expressed in acute phase and blast crisis in Chronic Myeloid Leukemia (CML) compared with chronic phase disease with expression increasing progressively during transit from the chronic phase to the blast crisis. In chronic lymphocytic leukaemia (BCLL), HuR mRNA is differentially expressed between cases with high and low levels of miR-16/miR-15 consistent with its mRNA being targeting by miR-16.

One intriguing mRNA target for HuR in leukemogenesis is the mRNA encoding the pro-survival protein, survivin (Table 1). Over-expression of HuR results in inhibition of p53-dependent transcription and down-regulation of expression of the survivin protein. HuR stabilises p53 mRNA leading to increased levels of p53 protein which in turn negatively regulates survivin gene transcription. However, when p53 is silenced, HuR over-expression actually increases levels of survivin by stabilising its mRNA. This suggests that the outcome of HuR expression on survivin levels may depend on the p53 mutational status of leukemic cells.

Finally, HuR have been implicated in post-transcriptional genotoxic/oxidative stress pathways in mammalian cells and recent data has shown that it functions as a key mediator of the checkpoint kinase, ATM (ataxia telangiectasia mutated) in B lymphocyte cell lines. In response to double-stranded DNA breaks, the ATM kinase phosphorylates a cascade of downstream effectors including the checkpoint kinase, Chk2, which in turn phosphorylates HuR to modulate its mRNA-binding and translational control functions. Ionising radiation elicits a dramatic change in the profile of mRNAs that are associated with HuR in an ATM-dependent manner. Of these, the mRNAs encoding cancer-associated proteins p21, FOXO3, MEK1, MEK2, DUSP10 and (interestingly) the AUBP, ZFP36L1 were experimentally validated as ATM-regulated HuR targets. In each case, radiation-responsive up-regulation of their encoded proteins was mediated by translational control rather than by changes in mRNA levels.

Individuals with the autosomal recessive ataxia telangiectasia (AT) condition who inherit defective ATM function are predisposed to develop lymphomas and lymphoid leukemias at high frequency. The ATM gene is also commonly mutated in sporadic lymphoid malignancies. Thus HuR likely plays a key role in mediating the effects of loss of function of
the ATM checkpoint kinase tumour suppressor in sporadic lymphoid malignancies as well as in AT patients\(^48\) (Table 1).

**KSRP**
KSRP is a multi-functional protein involved in various processes including transcription, alternative pre-mRNA splicing, and mRNA localization in addition to its role as an AUBP mediating mRNA decay. Some key targets of KSRP that are relevant to hematopoiesis include ZFP36, NOXA, PRDM1 variant 1 (Blimp-1 variant 1), BACH2, cIAP-2, BMP2, BMP6, CCL20, Cyclin D3, CCR1, CCR3, CCR7, CXCL2, CXCL3, CXCL10, CXCL11, Id2, IL-6, SOCS2, E Selectin and TLR4\(^49\). In addition, KSRP is involved in the maturation of several miRNAs that are dysregulated in leukemias and lymphomas as summarized in Table 1. KSRP has been found to immunoprecipitate with Drosha and DGCR8 proteins and to regulate the maturation of let7a, miR-15b, miR-16, miR-20, miR-21, miR-26b, miR-27b, miR-98, miR-106a, miR-125b, miR-196a, miR-199a, miR-301, miR-595\(^50,51\). KSRP seems to promote the association of Drosha with pri-miRNA and of Dicer with pre-miRNA at least for let-7a and miR-21\(^51\) and as thus to promote their maturation. Another report found that KSRP is involved in the maturation of miR-155 from pri- and pre-miR-155 after LPS stimulation in macrophages\(^52\).

The KSRP protein also targets Id3 inhibition of E2A-regulated transcription of the Notch1 gene\(^53\). Deregulated Notch1 expression arising either through mutational mechanisms or the t(7;9) translocation has been causally associated with the development of human T-acute lymphoblastic leukemia (T-ALL)\(^53\). Although one published study has reported high expression of KSRP in CML acute phase/blast crisis compared with chronic phase disease\(^45\) (Table 1), it remains to be determined whether altered expression/function of KSRP plays a causal role in leukemogenesis via miRNA maturation or other pathways.

**Nucleolin**
Nucleolin is found in the nucleolus, nucleoplasm, and the cell membrane\(^54\) and possesses a diverse set of functions such as regulation of PolI- and PolII-mediated transcription, nucleocytoplasmic transport, the formation of nucleosomes and chromatin remodeling, in addition to its role in the stabilization of certain mRNAs through binding to AREs\(^54\). Amongst the mRNAs that are stabilised by nucleolin are several that encode proteins with established roles in normal and malignant hematopoiesis including IL-2, Bcl-2, Bcl-Xl and CD40L (CD154)\(^55\).
Independently of its ability to bind Bcl-2 mRNA, nucleolin can also interact with phosphorylated Bcl-2 protein in the nucleus during the transition from prophase to anaphase in mitosis. This may affect spindle formation as well as chromosome segregation. In hematopoietic cells, nucleolin regulates transcription of the CD34 gene by direct binding to the CD34 promoter, a process that is negatively regulated by hypophosphorylated Rb. As mentioned previously, nucleolin, together with AUFI forms the transcriptional regulatory LR1 complex that regulates multiple genes in lymphocytes. Additionally, nucleolin binds to and stabilises G-quadruplex DNA in the c-Myc promoter to inhibit c-Myc transcription in vitro. Since nucleolin transcription is itself activated by c-Myc, it may function as part of a negative regulatory feedback loop with c-Myc. The functions of nucleolin are also integrated with p53-dependent pathways. It binds to the 5′ UTR of p53 mRNA and causes inhibition of translation under basal conditions and following irradiation. Down-regulation of nucleolin results in induction of apoptosis and inhibition of proliferation through induction of p53. However, nucleolin can also interact with the Hdm-2 protein resulting in stabilization of p53.

A unique feature of nucleolin compared with the other AUBPs is that it can undergo N-glycosylation-dependent localization to the plasma membrane to modulate key signalling pathways via protein interaction mechanisms. Membrane bound nucleolin interacts with and induces accumulation of the K-ras proto-oncogene and subsequent activation of the MAPK/ERK pathway. In response to CD21 activation, it also becomes tyrosine phosphorylated and associates with the unphosphorylated p85 subunit of the PI3K pathway. Finally, through RNA-dependent association with DGCR8, nucleolin may serve an adaptor function in transferring the pri-miRNAs to the nucleolus and in the processing-cleavage of pri-miRNAs to pre-miRNA within the nucleolus by a Drosha-DGCR8-nucleolin complex.

As summarised in Table 1, deregulated expression of nucleolin is a consistent feature of several types of leukemia. In BCLL, nucleolin expression is correlated with high levels of Bcl-2 protein, reflecting the function of nucleolin in stabilising Bcl-2 mRNA. Similarly, in AML, high levels of nucleolin may act to maintain transcription of the CD34 gene, particularly since AML cells typically lack Rb function which acts to negatively regulate the transcriptional regulatory functions of nucleolin. Nucleolin is also over-expressed in paediatric ALL, irrespective of disease sub-type. In a more recent study, expression of nucleolin, together with nucleophosmin was found to be deregulated in de novo primary ALL.
and AML patients as well as in refractory and relapsed leukemia patients. Expression of these two proteins positively correlated with higher relapse rates and negatively correlated with overall survival and relapse-free survival (Table 1).

**ZFP36 family**

The three ZFP36 family members, ZFP36, ZFP6L1 and ZFP36L2, function primarily by targeting an extensive, overlapping repertoire of mRNAs for degradation via the exosome or via Xrn1 exonuclease. One exception to this is ZFP36 which has recently been shown to also target the NF-κB pathway via ARE-independent mechanisms involving recruitment of HDAC1 or HDAC3 to the NF-κB p65 subunit and through inhibition of p65 nuclear import.

Several studies in cell line models and primary cells have implicated all three members of the ZFP36 family of proteins in both pro-apoptotic functions and as regulators of cell differentiation through a variety of mechanisms (reviewed in). The ZFP36L1 protein for example is required for Rituximab-mediated apoptosis of BCLL cells. It also functions as a negative regulator of plasma cell differentiation in the mouse BCL1 cell line model by targeting mRNA encoding the plasma cell transcription factor, PRDM1/BLIMP1 (unpublished observations). Since PRDM1/BLIMP1 acts to repress transcription of the ZFP36L1 gene, these two regulatory proteins may function as a bi-stable switch mechanism during the transition from mature B to plasma cells. Over-expression of ZFP36L1 and ZFP36 in human CD34-positive cord blood cells inhibits erythroid differentiation and proliferation by targeting degradation of Stat5b mRNA which in turn leads to repression of STAT5b-dependent transcription of GATA-1.

Definitive insight into the distinctive role of each ZFP36 family member in hematopoiesis has been provided by gene targeting studies in mice (see Fig 1). Loss of ZFP36 function results in an increase in the numbers of granulocytes in the spleen and bone marrow accompanied by a decrease in the numbers of B cells in the bone marrow. This is attributable to increased proliferation of short term hematopoietic stem cells and multipotent progenitor cells arising from increased levels of G-CSF, IL-1β, IL-6, TNF-α found in the plasma of ZFP36 knockout mice and these effects are non-cell-autonomous. The defective hematopoiesis may also arise from dysregulation of the transcription factor, E47, the mRNA of which is normally targeted for degradation by ZFP36. The defective hematopoietic
phenotype seen in ZFP36L2-deficient mice (Fig 1) is associated with elevated Cxc11 expression and diminished levels of Cxcl4, Cxcl7, integrin a2b (CD41), integrin b3 (CD61), CD59α, SLAMF1, PBX1. Conditional knock-out mice in which loss of ZFP36L1/L2 function is targeted to lymphocytes (Fig 1) display widespread defects in lymphopoiesis. Notably, all ZFP36L1/L2 double knockout mice developed thymic tumours and the majority also displayed other abnormalities including splenomegaly and lymphadenopathy. Interestingly, in these same mice, there was a block in B cell development from the CD25+B220+ stage (Fig 1), although presumably this was not B cell intrinsic as deletion of ZFP36L1/L2 in these mice was under control of CD2-cre and CD2 is not expressed in B cells.

Consistent with their pro-apoptotic functions, members of the ZFP36 family have been implicated as tumour suppressors in solid tumours. A similar functional role for these proteins is emerging in leukemogenesis. Compelling evidence for this is provided by the recent finding that concomitant targeted deletion of ZFP36L1 and ZFP36L2 in mouse thymocytes results in T-ALL as early as 3 months of age while single deletion has no effect. The CD8+ tumours are oligoclonal in nature, and their development is attributable to deregulated expression of Notch1 mRNA, which is normally targeted for degradation by ZFP36L1. As mentioned previously, deregulated Notch1 expression has been causally associated with the development of human T-ALL.

At a mechanistic level, loss of function of different ZFP36 family members is very likely to contribute to leukemogenesis through multiple mechanisms (Table 1). In adult T cell lymphoma (ATL), ZFP36 but not ZFP36L1, has been found to physically associate with the Tax protein and to inhibit its transactivation function. The Tax-ZFP36 complex is targeted to the proteasome resulting in increased degradation of Tax. In addition, ZFP36 may recruit HDAC1 and HDAC3 to Tax with concomitant inhibition of p300/CBP recruitment as shown for the p65 NF-κB subunit. Indeed, Tax protein has been found to associate with p300/CBP and HDAC1 or HDAC3; the presence of HDAC1 inhibits the binding of p300/CBP to Tax that is required for Tax transactivation function. The physical association between ZFP36 and Tax also appears to titrate out ZFP36 function since Tax inhibits degradation of TNF-α mRNA by ZFP36 in ATL. A similar mechanism may contribute to the deregulated expression of other cytokines in ATL such as IL-2, IL-6, IL-10, GM-CSF that are induced by HTLV-1 virus and that are targets for ZFP36. Consistent with a role for loss of ZFP36
function in disease progression of ATL, ZFP36 mRNA is down-regulated in acute phase ATL compared with chronic phase disease \(^86\) (Table 1).

The transcription factor, NF-κB functions in one of the most frequently deregulated pathways in leukemias and lymphomas \(^87\), and is a key target for mRNA decay and transcriptional repression by the ZFP36 protein as mentioned previously \(^73,74\). ZFP36 together with ZFP36L1 also functions in miRNA pathways in leukemogenesis. mRNA targets for miR-16/miR-15 are enriched in AREs motifs \(^46\) and miR-16 requires the presence of ZFP36 and ZFP36L1 in order to bind to ARE-containing mRNA \(^88\). miR-16/miR-15 are commonly down-regulated in BCLL and indeed miR-16/miR-15 knockout mice develop BCLL \(^89\). One intriguing possibility is that in BCLL cases in which miR-16/miR-15 expression is not down-regulated, the absence of ZFP36 family members may promote the development of CLL indirectly by attenuating the function of miR-16/miR-15. ZFP36L1 is expressed at low levels in most BCLLs \(^75\). In some cases this may be attributable to an interstitial deletion of the ZFP36L1 locus at 14q24 \(^90\). Interestingly, ZFP36L2 mRNA is differentially expressed between BCLL cases with high and low levels of miR-16/miR-15 \(^46\) consistent with its mRNA being targeting by miR-16 \(^34\). Other genetic/epigenetic mechanisms are also likely to be involved in down-regulating expression of ZFP36 family members. In B cell lymphomas for example, the promoters of the ZFP36 and ZFP36L1 genes are targeted for transcriptional repression by the BCL-6 oncogene \(^91,92\).

In myeloid leukemias, the expression of ZFP36 family members is correlated with clinicopathological features of disease (Table 1). In two independent studies of CML, ZFP36 expression displays a progressive decrease during transit from chronic phase to blast crisis \(^35,93\). ZFP36 is known to target Stat5b mRNA for degradation in CD34-positive haematopoietic cells \(^77\). Since proliferation and survival of BCR-ABL-transformed cells requires Stat5 function \(^94\), loss of ZFP36 function would be expected to enhance the malignant properties of CML cells. ZFP36L1 by contrast, is reportedly over-expressed in M2 AML carrying the AML1-ETO translocation and this is correlated with induction of cell proliferation and inhibition of differentiation \(^95\). Similarly, ZFP36L2 mRNA was found to be over-expressed in resistant/relapsed AML patients \(^96\).

Most established tumour suppressor genes are found in mutant form in at least a sub-set of tumours in which they are involved. Recent data indicates that at least for ZFP36L1 and
ZFP36L2 this criterion may be fulfilled (Table 1). High throughput sequencing of primary multiple myeloma (MM) cases has identified deletions within a regulatory intron of the ZFP36L1 gene in a significant minority of cases\(^9^7\). Although the functional significance of this remains to be established, the same study also found that the Rrp44 gene (encoding exosome component 11) is mutated within its coding region in 11% of MM patients\(^9^7\). The resulting predicted loss of exosome function would likely lead to widespread impairment of cellular mRNA degradation machinery in MM. Mutations in the ZFP36L2 gene have also recently been reported in various sub-types of AML and ALL\(^9^8\); one of these mutants was shown to encode a protein with impaired anti-proliferative activity compared with wild type ZFP36L2\(^9^8\).

**Conclusions and Perspectives**

Given the multitude of important regulators of cell growth, differentiation and survival that are targeted at the post-transcriptional level by AUBPs, it is perhaps unsurprising that the phenotypes of mice that are deficient in their genes manifest in various defects in hematopoiesis. As discussed previously, multiple AUBPs often function in association within defined sub-cellular compartments and bind concomitantly to the same mRNA\(^3^,^6^,^7\). For example, ZFP36 physically interacts with KSRP, nucleolin, HuR and AUFI. AUFI and HuR both target several mRNAs through direct binding to non overlapping 3′ UTR sequences\(^9^9\). A similar phenomenon occurs for nucleolin with ZFP36 and PABP proteins\(^1^0^0\), and with ZFP36L1 and ZFP36L2\(^8^1\). The mRNAs of several AUBPs that contain 3′ ARE elements are themselves subject to auto- and, or, trans-regulation, as occurs with ZFP36 family members\(^1^0^1\). Examples of trans-regulation include the destabilisation of HuR by AUFI\(^1^0^2\) and the inhibition of translation of ZFP36L1 by HuR\(^4^8\). The AUBPs discussed in this review are also integrated with miRNA pathways at multiple levels in hematopoietic cells. For example, HuR represses miR-7 expression\(^3^8\). It is subject to translational repression by several miRNAs\(^3^4^-^3^7\) and also functions in AGO2-dependent Let-7 repression\(^3^2\), KSRP is involved in miRNA maturation\(^5^1^,^5^2\) while ZFP36 family members function in miRNA-mediated decay\(^4^6^,^8^8\). Finally, these AUBPs together with the miRNAs with which they functionally interact are intimately associated with some of the key oncogene/tumour suppressor pathways that are perturbed in malignant hematopoietic cells. The post-transcriptional ‘regulome’ of hematopoietic cells can therefore be viewed as a highly interconnected regulatory network in which AUBPs represent major hubs. In Fig 2 we have constructed a global functional
connectivity map of AUBPs in normal/malignant hematopoiesis based on the experimental data discussed in this review. This graphic view is not exhaustive but provides an appreciation of some of the major regulatory interactions that have been documented for AUBPs in hematopoietic cells. In particular, it highlights the close functional interconnectivity between AUBPs that in many cases mirrors their physical association and illustrates the critical intersection of AUBPs and miRNAs in the pathways that regulate oncogene/tumour suppressor functions in leukemogenesis.

Several lines of evidence implicate individual AUBPs in the pathogenesis of leukaemia/lymphoma through diverse mechanisms. However, only the ZFP36 family exhibit characteristics of tumour suppressors where malignancy is associated with loss-of-function. The use of mouse models of various hematopoietic malignancies should, in the future, prove particularly instructive in elucidating the precise function role and mechanisms of individual AUBPs in specific disease types. Finally, AUBPs and the mechanisms through which they function offer new potential therapeutic targets for leukaemia and lymphoma, as exemplified by a recent study in which restoration of ZFP36L1 function to solid tumour cells suppressed angiogenesis and tumourigenesis.¹⁰³

**Acknowledgements**

Work in the authors’ laboratories is supported by Cancer Research UK. We are grateful for helpful discussions amongst several colleagues and investigators in the field during the preparation of this manuscript.

**Author contributions**

M.B. conducted literature research and wrote the manuscript and designed Table 1 and Figure 1. J.D.N. conducted literature research, wrote the manuscript and designed Figure 1 and Figure 2. J.J.M. conducted literature research, contributed to drafting sections of the manuscript and checked the content of the final manuscript for accuracy.

**Conflict of interest disclosures**

The authors declare no competing financial interests.
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<th><strong>AUBP</strong></th>
<th><strong>LEUKEMIA</strong></th>
<th><strong>TARGETS</strong></th>
<th><strong>REFERENCE</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>AUF1 (hnRNP D)</td>
<td>Co-localises with NMP-ALK in ALCL, phosphorylated and inactivated</td>
<td>Myc, Cyclins A and D, Bcl-Xl, Bfl-1, c-Jun</td>
<td>21</td>
</tr>
<tr>
<td>HuR (ELAVL1)</td>
<td>Overexpressed in M4 AML and in CML blast crisis, co-localises with NPM-ALK in ALCL and is phosphorylated, functions downstream of ATM</td>
<td>elf4e, cEBlp, p21, FOXO3, MEK1, MEK2, DUSP10, ZFP36L1, c-Myc, p53, CD95L, XIAP, survivin, miR-7</td>
<td>38, 42, 44, 45, 48</td>
</tr>
<tr>
<td>KHSRP (KSRP)</td>
<td>Not shown yet but possibly in AML, ALL, CLL, MM</td>
<td>Let 7 family, miR-16, miR-15, miR-20, miR-106b, miR-26b, miR-155, etc Bcl-2, Bcl-Xl, CD40L, c-Myc</td>
<td>50, 51, 52</td>
</tr>
<tr>
<td>Nucleolin</td>
<td>Overexpressed in CLL, AML, pediatric ALL, refractory and relapsed AML and ALL.</td>
<td><strong>Interaction and inhibition of Tax transactivation,</strong> VEGF, several cytokines</td>
<td>55, 70, 71, 72</td>
</tr>
<tr>
<td>ZFP36 (Tis11)</td>
<td>Decreased in CML blast crisis, decreased in DLBCL, decrease in acute phase ATL</td>
<td></td>
<td>45, 84, 86, 92, 93</td>
</tr>
<tr>
<td>ZFP36L1 (Tis11b)</td>
<td>Decreased in CLL, decreased in DLBCL, deleted in CLL and NHLs, mutated in MM</td>
<td>c-IAP-2, VEGF, several cytokines</td>
<td>75, 90, 92, 97</td>
</tr>
<tr>
<td>ZFP36L2 (Tis11d)</td>
<td>Increased in AML, mutated in AML</td>
<td>several cytokines</td>
<td>96, 98</td>
</tr>
</tbody>
</table>

lymphoma, DLBCL: diffuse large B cell lymphoma, MM: multiple myeloma. Gene targets that are highlighted in bold have been experimentally validated for their direct role in AUBP-mediated leukemogenesis.

Legends to Figures

Figure 1: Schematic representation of phenotypic abnormalities in hematopoietic compartments of AUBP-deficient mice. A generalised scheme of hematopoietic cell differentiation is shown, onto which are ‘mapped’ the various compartments that are reported to be affected in AUF1, HuR, HuR-CD19cre, HuR-CD2cre, ZFP36, ZFP36L2 and ZFP36L1+L2-CD2cre knock-out mice, as described in the text. A blue colour of hematopoietic cell types indicates diminished cell numbers; a red colour indicates elevated cell numbers. Abbreviations – HSC: haematopoietic stem cells, MPP: multipotent progenitor cells, CLP: common lymphoid progenitor, CMP: common myeloid progenitor, DN: double negative (CD4-/CD8-) thymocytes, DP: double positive (CD4+/CD8+) thymocytes, SP: single positive (CD4+, CD8+) thymocytes, Im. B: immature B cells, FL B: Follicular B cells, MZ B: Marginal zone B cells, PB T: peripheral blood T cells, PB B: peripheral blood B cells, GMP: granulocyte-macrophage progenitor, CFU-G: colony factor unit granulocytes, CFU-M: colony forming unit macrophage, MEP: megakaryocyte erythroid progenitor, CFU-Meg: colony forming unit megakaryocytes, CFU-Eo: colony forming unit eosinophil, CFU-Ba: colony forming unit basophil, BFU-E: burst forming unit-erythroid.

Figure 2: Functional connectivity map of AUBPs in hematopoiesis and leukemogenesis. The major regulatory interactions between AUBPs (grey nodes), miRNAs (blue nodes) and onco/tumour suppressor-proteins (pink/orange nodes) are shown as a network graph in which edges represent experimentally-validated functional connections that have been manually curated from the literature and are described in the text. In most cases, the directionality of positive (→) or negative (---|) regulatory interactions is known. Solid line edges represent interactions documented in normal/malignant hematopoietic cells; dashed-line edges represent interactions identified in other cell types that are very likely to occur in hematopoietic cells. The network graph shows an organic layout view constructed using Cytoscape (version 2.8.0).
Figure 2
AU-rich RNA binding proteins in hematopoiesis and leukemogenesis

Maria Baou, John D Norton and John J Murphy

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