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Deubiquitinases at the interplay between hematopoietic stem cell aging and myelodysplastic transformation

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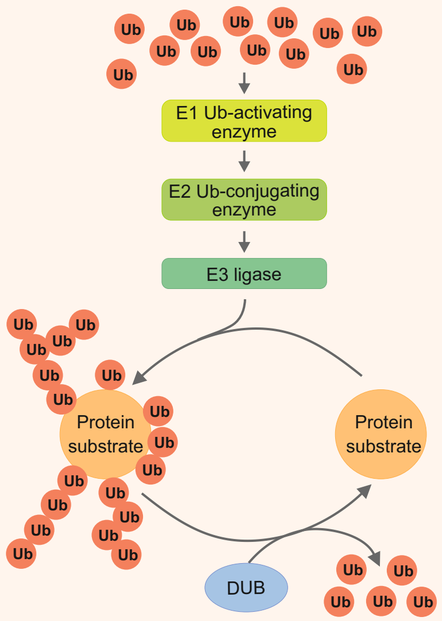
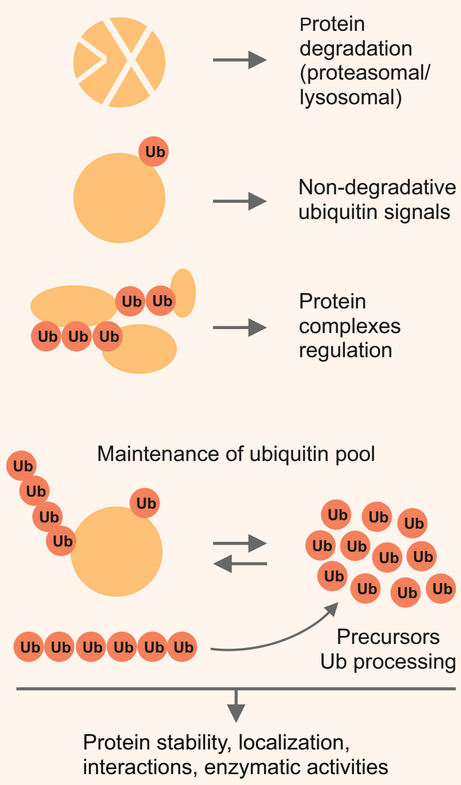
GRAPHICAL Review

THE UBIQUITIN SYSTEM

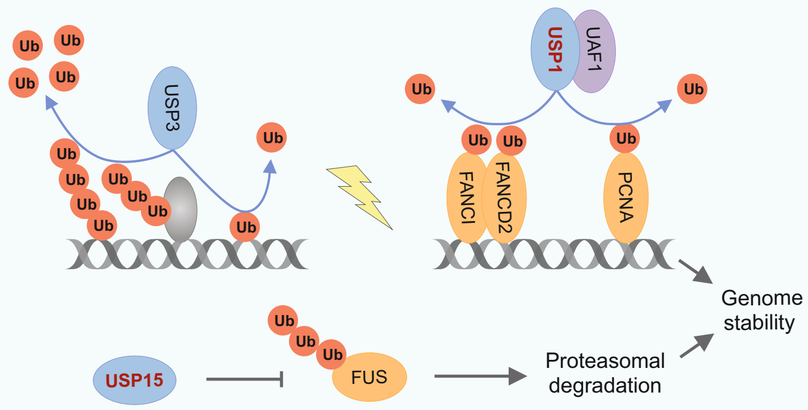


Major roles of DUBs

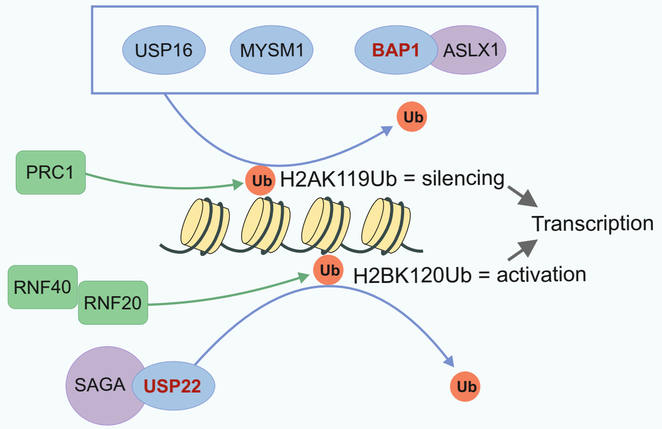


MAIN DUB TARGETS AND MECHANISMS OF REGULATION OF HSC ACTIVITY

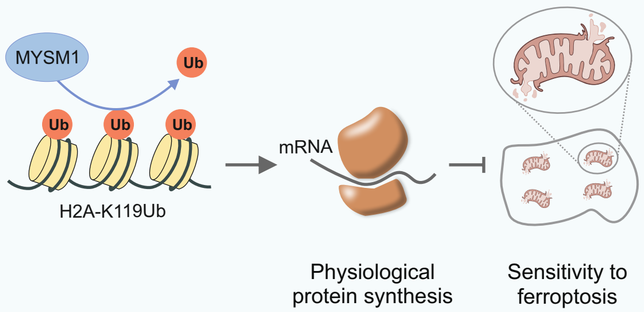
A DNA damage response



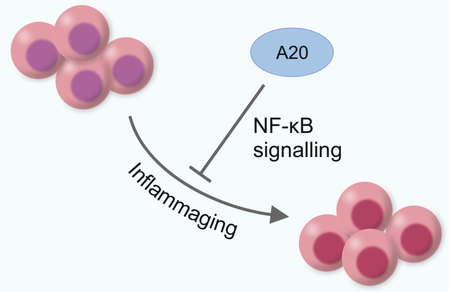
B Epigenetic regulation



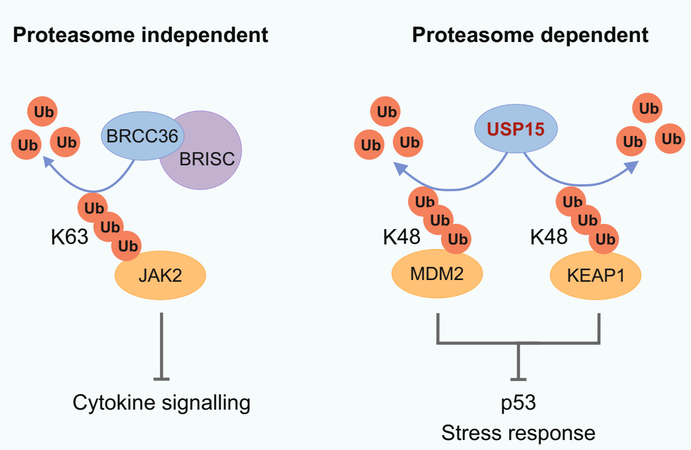
C Protein biosynthesis



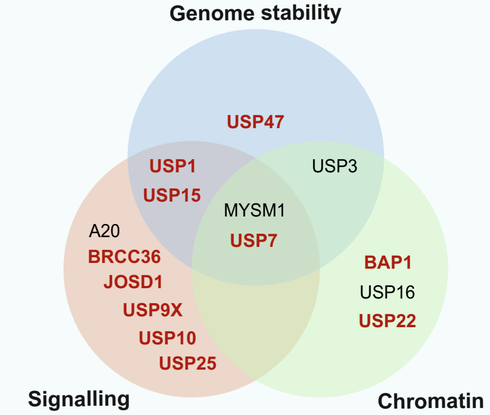
D Inflammation



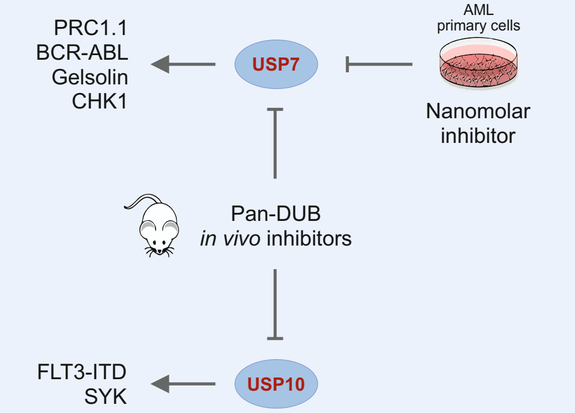
E Signalling



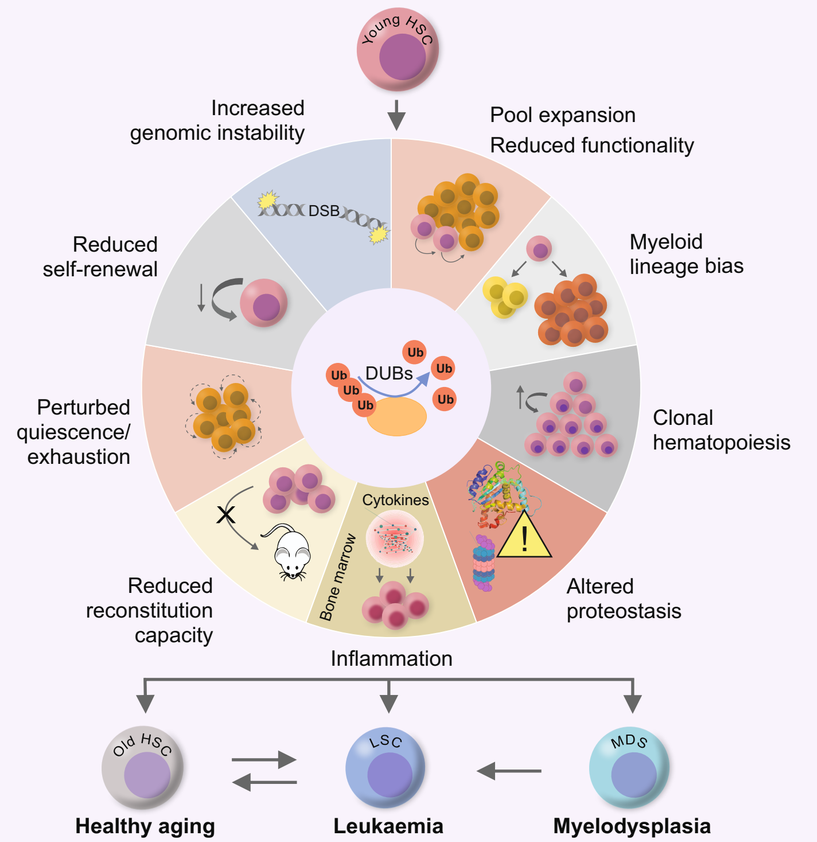
F Pleiotropic effects



SMALL MOLECULES TARGETING DUBs IN MYELOID LEUKAEMIA



IMPACT OF DUBs ON HALLMARKS OF HSC AGING



KEY: Ub Ubiquitin · E3 ligase · DUB · DUB Role in leukaemia · Self-renewal · DUB cofactor · DUB substrate · Unknown substrate

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Deubiquitinases at the interplay between hematopoietic stem cell aging and myelodysplastic transformation

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GRAPHICAL Review

Hematopoietic stem cells (HSC) maintain blood production throughout life. Nevertheless, HSC functionality deteriorates upon physiological aging leading to the increased prevalence of haematological diseases and hematopoietic malignancies in the elderly. Deubiquitinating enzymes (DUBs) by reverting protein ubiquitination ensure proper proteostasis, a key process in HSC maintenance and fitness.

The ubiquitin system

Ubiquitination is a reversible post-translational modification involved in the regulation of most cellular processes. Ubiquitin (Ub), a 76 amino acid polypeptide, is primarily conjugated to lysine residues of substrate proteins through the sequential activity of E1 Ub-activating, E2 Ub-conjugating and E3 Ub-ligating (Ub-ligase) enzymes, generating a complex code written on thousands of proteins [1].

Major roles of deubiquitinating enzymes (DUBs)

DUBs catalyse the removal of ubiquitin from substrates, maintaining the balance between ubiquitination and deubiquitination [2]. The 100 human DUBs have been categorized into seven structurally related superfamilies: the cysteine proteases ubiquitin-specific proteases (USP), ovarian tumor proteases (OTU), ubiquitin C-terminal hydrolases (UCH), Josephin/MDJ, MINDY and ZUFSP families as well as the JAMM/MPN+ family of metalloproteases [2]. Besides rescuing proteins from degradation, DUBs regulate protein function by cleaving and editing non-degradative ubiquitin signals. Through these mechanisms, DUBs control proteostasis and cellular signalling networks [2, 3].

Main DUB targets and mechanisms of regulation of HSC activity

In physiological aging as in Myelodysplastic Syndrome (MDS) and Acute Myeloid Leukaemia (AML), DUBs, including USP7, USP15, and A20, exhibit either increased or decreased expression [4-7]. Moreover, in leukaemia, USP10, USP9X, BRCC36, and BAP1 participate in chromosomal translocation or are inactivated by somatic mutations [8, 9] (Table 1). Substrate analysis, genetic deletion and/or mutation of DUBs in mouse models, and data from human samples confirm a wide role of DUBs in biological functions relevant to both normal and malignant HSC (leukemic stem cells, LSC) [8]. This is achieved through engagement in different molecular mechanisms: DNA damage response [5, 10-15], epigenetic drift [9, 15-19], protein biosynthesis [20], inflammation [21], and proteasome-independent or dependent signalling [6, 11, 22-29] (panels A-E and Table 1). These processes ultimately impact signal transduction, cell cycle and differentiation. Individual DUBs are often involved in multiple pathways such as maintenance of genome stability, chromatin modifications and intra/extracellular cytokine and stress signalling. Many DUBs act on the same mechanism with pleiotropic effect (F).

DUBs in hallmarks of HSC aging and myelodysplastic transformation

The same biological processes mentioned earlier contribute to key features observed in old HSC which are shared by LSC in age-related pathologies such as MDS and AML [30, 31]. DUB knockout mice display features of premature aging HSC including perturbed quiescence (i.e. A20 [21]), reduced self-renewal and reconstitution capacity (i.e. USP3 [12], USP15 [5], USP16 [17]), pool expansion and myeloid lineage bias (i.e. A20 [7], USP22 [18], BAP1 [9]), inflammation (A20 [21]), increased genomic instability (i.e. USP1 [10], USP3 [12], USP15 [5]), epigenetic changes (i.e. USP16 [17], USP22 [18], BAP1 [9], MYSM1 [15, 19]) and clonal hematopoiesis (BRCC36 [28]). Given their mechanistic pleiotropic effect, DUBs often impact more than one aspect and have partially overlapping roles in HSC fitness (Table 1).

Small molecules targeting DUBs in myeloid leukaemia

Several DUB inhibitors small molecules have been developed and tested in *in vitro* and *in vivo* models of myeloid leukaemia, where their inhibition promotes proteasomal degradation of oncogenic proteins (such as FLT3-ITD upon USP10 inhibition [24]) [22, 23, 26, 27, 29] (Table 1). First-generation compounds, such as P22077, have largely non-specific pan-DUB inhibitory activity [3]. The new class of small molecules, like the USP7 inhibitor FT671, shows high specificity with efficacy in primary AML cells at nanomolar concentration [16].

Final remarks

DUBs dysfunction exacerbates HSC aging and predisposes them to leukaemia. As such, they are attractive therapeutic targets. New generation, structure-based selective inhibitors are currently under evaluation in pre-clinical models alone or in combinational therapies to overcome resistance and relapse [3, 32]. The translation of DUB inhibition into clinics will require meeting the parallel challenge of selecting key DUBs - and their substrates - that sustain aberrant cell proliferation in the specific leukaemia subtypes.

Abbreviations

AML, acute myeloid leukaemia; CML, chronic myelogenous leukaemia; DUB, deubiquitinating enzyme; H2AK119Ub, monoubiquitinated H2A; H2BK120Ub, monoubiquitinated H2B; HSC, hematopoietic stem cells; HSPC, hematopoietic stem and progenitor cells; KO, knockout; LSC, leukemic stem cells; MDS, myelodysplastic syndrome; MonoUb, monoubiquitinated; Ub, ubiquitin.

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Table 1: Mammalian DUBs with consolidated roles in hematopoietic stem cells and their implications in aging and myelodysplastic transformation.

DUB	Main target(s) and impact on HSC aging hallmarks and myelodysplastic transformation. *Role in MDS/myeloid leukemia ^Differential expression in aging (https://agingsignature.webhosting.rug.nl/)	Pan-DUB inhibitors tested in AML
	Ubiquitin specific proteases (USP)	
^USP1	-USP1 regulates monoUb FANCD2 Fanconi Anemia protein, promoting resistance to DNA cross-linking chemotherapeutic agents [10]. -USP1 deubiquitinates monoUb PCNA[10]. *USP1 inhibition promotes the degradation of ID1, suppressing the growth of AML cells[11].	C527; SJB2-043; pimozide [11]
^USP3	-*Usp3* KO mice display enhanced monoUb H2A and H2B, lymphopenia, reduced HSC compartment, and decline in HSC repopulation potential upon aging[12]. -USP3 regulates Ub-dependent DNA damage signalling to protect HSC from genotoxic stress[12].	
USP7	*USP7 inhibition: a) upregulates Gelsolin to induce differentiation of MDS cells[33]; b) reduces the viability of primary AML cells and tumour burden *in vivo* in PDX models[13, 16]; c) delays MLL-AF9-induced leukaemia *in vivo* in human leukaemia xenografts [16]. *USP7 deubiquitinates and stabilises CHK1 in AML cells[13]. *USP7 interacts with PRC1.1 complex and its catalytic activity is required for PRC1.1 stability[16]. *USP7 deubiquitinates and stabilises BCR-ABL to promote the survival of CML cells[22]. *USP7 is differential expressed in MDS[4].	P22077 [13, 16, 33] P5091 [22, 33] FT671 (nanomolar USP7 inhibitor) [16]
^USP9X	*USP9X binds to FLT3-ITD and its downregulation cooperates with WP1130 DUB inhibitor to promote FLT3-ITD degradation and apoptosis of AML cells [23]. *USP9X deubiquitinates and stabilises ALKBH5 and promotes cell survival in AML cellular and murine models [34].	Wp1130; G9 [23, 34].
^USP10	*USP10 stabilises oncogenic FLT3-ITD in AML[24]. *USP10 deubiquitinates and stabilises SKP2 enhancing the growth of CML xenografts *in vivo[25]*. *USP10 deubiquitinates and stabilises the tyrosine kinase SYK promoting the proliferation of SYK-driven AML cells[26].	HBX19818, P22077; [24, 26] Spautin[25]
USP15	-*Usp15* KO compromises mHSC maintenance and reconstitution potential *in vivo [5]*. *USP15 stabilises FUS and safeguards genome integrity in HSC and LSC [5]. *USP15 stabilises KEAP1 and MDM2 to modulate redox and p53 signalling in AML cell [6]. *USP15 is overexpressed in hAML[5, 6] and differentially expressed in MDS [4].	USP15-Inh [6]
USP16/ Ubp-M	-USP16 deubiquitinates H2AK119Ub and regulates transcriptional programs in HSPC [17, 19]. Its KO is embryonic lethal [35]. Conditional KO impairs HSC cell cycle and differentiation [17], whereas trisomy in a model for Down's syndrome associates with reduced HSC self-renewal [35].	
USP22	-USP22 deubiquitinates H2BK120Ub to regulate the expression of inflammatory and immune responsive genes. Its KO induces cell-intrinsic emergency haematopoiesis with increased HSC proliferation and extramedullary haematopoiesis[18]. *USP22 deubiquitinates and stabilises PU.1 and its KO facilitates transformation in Ras-driven AML[36]. *USP22 stabilises SIRT1 in FLTD-ITD AML cells [32].	
USP25	*USP25 deubiquitinates and stabilises BCR-ABL in Philadelphia+ CML [27].	AZ-1; AZ-2 [27]
USP47	* USP47 deubiquitinates and stabilises YB-1 to promote DNA repair in CML[14].	P22077 [14]
	Ovarian tumour proteases (OTU)	
^A20	-A20 expression is reduced in hHSC upon aging [7]. -A20 hematopoietic KO enhances NF-kB activation and IFN-γ signalling resulting in loss of HSC quiescence [21]. -A20 heterozygous deletion results in HSC pool expansion, decreased regenerative potential and myeloid-biased haematopoiesis in mice[7]. *A20 confers proliferative advantage to TLR-TRAF6 primed MDS HSPC [8].	
	Ubiquitin C-terminal hydrolases (UCH)	
BAP1	*BAP1deubiquitinates the cell cycle regulator HCF-1 and its binding partner OGT1 to suppress proliferation and cell cycle progression, limiting myeloid transformation [9]. *Mutant ASXL1-MT/BAP1 complex promotes leukaemia transformation[8, 32].	
	Josephin/MDJ	
JOSD1	*JOSD1 interacts with and stabilises JAK2-V617F mutant to promote AML cells survival[29].	SB1-F-70; XL-9872-106C [29]
	JAMM/MPN+ metalloproteases	
BRCC36 (BRCC3)	-Deficiency of JAK2 K63-polyUb deubiquitination by BRCC36, as part of the BRISC complex, stabilises JAK2 signalling, promoting HSC expansion[28]. *BRCC36 is mutated in MDS and AML [8].	
MYSM1	-MYSM1 deubiquitinates H2AK119Ub, regulating transcriptional programs in HSPC [15, 19]. -Inactivating *MYSM1* mutations cause an inherited bone marrow failure syndrome (IBMFS) [15]. -MYSM1-deficient HSC exhibit increased oxidative stress [15, 20], γH2AX DNA damage mark, ribosomal stress and p53 activation [15, 37]. -By sustaining protein synthesis, MYSM1 protects hHSCs from ferroptosis [20]. -Deficiency of MYSM1 activity causes loss of HSC quiescence and depletion of hematopoietic lineages [15,38].	

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