PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2016, 43(9): 864~879
www.pibb.ac.cn

泛素链水解酶的选择性和产生机制 *

郑清芸[1,2)**] 王 涛[1)**] 潘 漫[2)] 李宜明[1,2)***] 田长麟[3)***]
([1)] 合肥工业大学生物与医学工程学院，合肥 230009；[2)] 清华大学化学系，北京 100084；[3)] 中国科学技术大学生命科学学院，合肥 230026)

摘要 底物蛋白的多聚泛素链修饰参与调节多种生命运动过程(包括蛋白质降解、自噬、DNA 损伤修复、细胞周期、信号转导、基因表达、转录调节、炎症免疫等). 去泛素化酶通过水解底物蛋白的单泛素和泛素链修饰，对泛素相关过程进行反向调节. 人类基因组中约含 90 余种去泛素化酶，它们通过对自身酶活性和底物识别特异性的调节，实现了对细胞内复杂泛素过程的精密且层次性的调控. 本文针对去泛素化酶对不同泛素链的识别选择性，综述目前已知泛素链水解酶的选择性和产生机制.

关键词 去泛素化酶，泛素链水解酶，多聚泛素化修饰，蛋白质泛素化
学科分类号 Q5，O6 **DOI**: 10.16476/j.pibb.2016.0112

泛素化(ubiquitylation)是一种真核细胞中广泛存在的蛋白质翻译后修饰. 该修饰通过泛素蛋白(ubiquitin，Ub)C 端羧基和底物蛋白赖氨酸侧链氨基共价连接形成异肽键. 目前泛素化修饰已被报道参与蛋白质降解、自噬、DNA 损伤修复、细胞周期、信号转导、基因表达、炎症免疫等重要生命过程[1-2]. 质谱组学研究表明，细胞内不仅存在底物蛋白质的单泛素化修饰，还存在以泛素自身 N端氨基和赖氨酸侧链氨基为异肽键连接位点的多聚泛素化修饰(Met1、Lys6、Lys11、Lys27、Lys29、Lys33、Lys48、Lys63). 这些多聚泛素化修饰依据链接类型的不同又可分为同源多聚泛素链和异源多聚泛素链，前者指单一泛素链上仅存在一种链连接类型，后者则包含多种不同连接类型的混合泛素链以及多位点的分叉泛素链修饰[3](图 1).

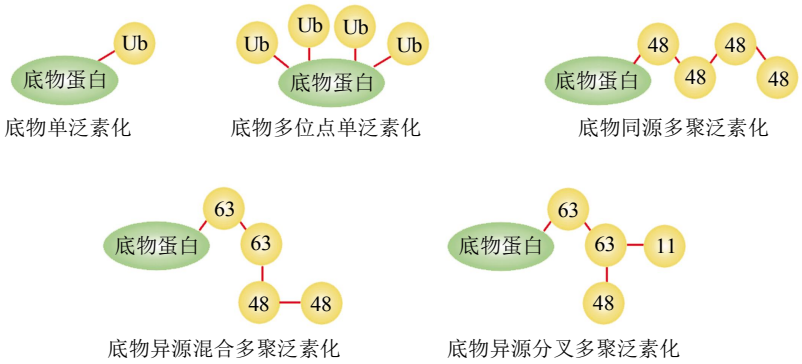

Fig. 1 Different linkages of ubiquitylation
图 1 泛素化修饰种类

* 国家自然科学基金资助项目(21372058, 21572043).
** 共同第一作者.
** 通讯联系人. Tel: 0551-65595608
李宜明. E-mail: ymli@hfut.edu.cn
田长麟. E-mail: cltian@ustc.edu.cn
收稿日期：2016-04-07，接受日期：2016-07-11

泛素化修饰的多样性使其能够参与大多数生命活动的调控．例如，Lys48 泛素链修饰的底物蛋白能被 26S 蛋白酶体识别并降解．Lys63 泛素链能在非蛋白降解过程如信号转导中发挥重要作用．不仅如此，Lys11、Lys63 和 Met1 泛素链调节 NF-κB 活性参与炎症免疫过程．Lys6 泛素链在线粒体自噬中发挥了关键作用．组蛋白上 Lys27 和 Lys63 泛素链修饰能应答 DNA 损伤修复．目前，除了 Lys63 泛素链，另外 7 种类型泛素链几乎都参与了蛋白质降解过程[4]．除此之外，分叉泛素和混合泛素修饰参与的生理过程还有待进一步研究[5]．

真核细胞的泛素化水平通过 E1-E2-E3 酶合成系统和去泛素化酶(deubiquitinating enzymes，DUBs)系统共同调控[6]．E1 激活酶在 ATP 供能下自身半胱氨酸和泛素 C 端甘氨酸反应形成高能硫酯中间体，活化 Ub；E2 结合酶通过同样的硫酯中间体将 Ub 转移到自身半胱氨酸上；E3 连接酶分别识别 E2 和底物蛋白，催化底物蛋白赖氨酸侧链氨基亲和进攻 E2 硫酯形成异肽键，从而实现底物蛋白的泛素修饰．目前人体中已发现有 2 种 E1 酶、40 种 E2 酶和大约 600 种 E3 酶．而与泛素化过程相反，DUBs 通过水解底物蛋白上的单泛素和泛素链修饰，对泛素相关过程进行反向调节．人类基因组中编码约 90 余种去泛素化酶[7]．根据结构相似性和作用机制，可将 DUBs 分为以下 5 种类型：泛素羧基末端水解酶家族(ubiquitin C-terminal hydrolases，UCHs)、卵巢肿瘤相关蛋白酶(ovarian tumour proteases，OTUs)、泛素特异性加工酶家族(ubiquitin-specific proteases, USPs)、machado-Josephin 蛋白酶家族(MJD)和 JAB1/MPN/Mov34 蛋白酶家族(JAMM)．其中，UCH、USP、OTU 和 MJD 家族属于半胱氨酸蛋白酶，JAMM 家族属于锌金属蛋白酶．

与种类丰富的 E3 酶不同，人源 DUBs 总量不足 E3 酶数量的 1/6．然而，它们仍能够精确地、层次性地调控细胞内不同的泛素相关过程：包括对泛素链的特异性识别、泛素链外切酶和内切酶活性选择[8-9]、蛋白质底物特异性识别、特异性去除底物单泛素化、水解泛素前体生成自由 Ub 单体等[10-12](图 2)．这就表明，去泛素化酶系统内存在一套独特的特异性识别底物和泛素链的密码，解析这套密码对于我们研究生命过程调控机理、寻找新的药物靶点、疾病诊断和治疗均具有重要意义．本文针对已发现的 90 余种去泛素化酶，总结了每种 DUB 能够选择性识别的泛素链，并对产生选择性的泛素链水解酶的作用机制进行系统综述．

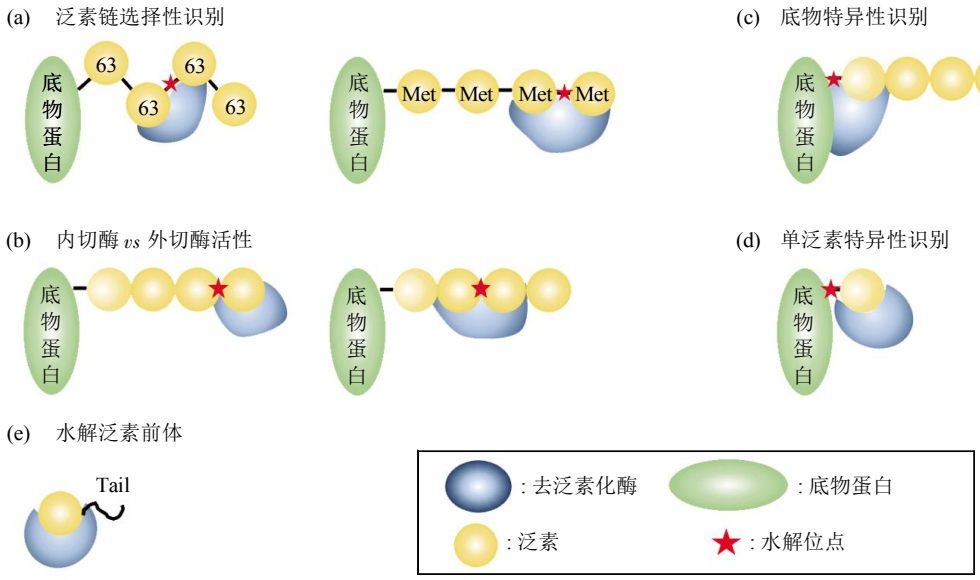

Fig. 2　Layers of DUB specificity

图 2　去泛素化酶识别多样性

1 泛素羧基末端水解酶 (UCHs) 家族

迄今为止，人类基因组已发现 4 种隶属 UCH 家族的去泛素化酶基因，包括 UCH-L1、UCH-L3、UCH-L5 和 BAP1(BRCA1-associate protein1)．这 4 个家族成员均存在一个保守的 UCH 催化结构域(约 230 个氨基酸)，其中，UCH-L1 和 UCH-L3 只包含这 1 个功能结构域，而 UCH-L5 则由 N 端 UCH 结构域(UCH-L5N)和 C端延伸结构域两部分组成．BAP1 定位在核内，其组成结构中还包括一段核定位信号[13].

UCH-L1 作为被发现的第一个 UCH 家族去泛素化酶，主要作用于泛素前体(Ub adducts)，该酶切开 Ub C 端与一些未折叠短肽形成的化学键，产生泛素单体[14]．UCH-L3 作用机制与之类似，通过水解泛素 C 端连接的离去基和短肽，生成自由单泛素[15]．尽管体外实验表明这 4 种 UCH 去泛素化酶全长蛋白均不具备水解泛素链的能力(表 1)，但当 UCH-L5 参与组成 19S 调节颗粒后(26S 蛋白酶体一个亚单位)，便能水解泛素链；BAP1 在和 BARD1 蛋白(BRCA1-associated RING domain)的 RING 结构域相互作用后也能够调节转录辅因子 HCF1(host cell factor 1)的多聚泛素化[16-17].

UCH-L1、UCH-L3 与 UCH-L5 的晶体结构[18-20]显示，UCH 家族中存在一个关键的交叉环(crossover loop)结构，该结构对于 UCH 家族去泛素化酶的底物识别特异性起到了关键作用：UCH-L1 在未连接泛素时，交叉环阻碍活性位点使蛋白处于失活状态；而当泛素前体结合到催化位点，UCH-L1 构象发生改变，环的直径增宽进而暴露活性位点，水解底物．UCH-L3 的交叉环则更加柔性，在不结合底物时完全不可见，和泛素底物相互作用时，环状结构则会包裹泛素 C 端的离去部分，帮助底物定位到酶催化中心[21]．尽管 UCH 家族蛋白均不具备独立水解泛素链的能力，但体外实验结果表明：随着交叉环链长的增加，UCH 结构域水解 Lys 48 diUb 的能力也逐渐增强．UCH-L5N 能够将 Lys 48 diUb 水解成单泛素，而 UCH-L1 和 UCH-L3 则不具备这样的活性．此外，降低 UCH-L5N 交叉环长度能降低水解 diUb 的活性；增加 UCH-L1 和 UCH-L3 的交叉环长度能增加其水解活性．然而，UCH-L5 全长蛋白因其 C 端结构域包含的 KEKE 基序自抑制 N 端 UCH 结构域活性，导致全长 UCH-L5 单独存在时不能水解泛素链．

除了长度因素，环链的柔韧程度也是影响 UCH 对异肽键的催化活性和底物特异性的重要因素之一[22]．一定长度的交叉环限制了能进入到活性催化区域 P′位点(protein conjugating binding site)的底物大小，由于这一原因，UCH-L1、UCH-L3 以及未和相应伴侣蛋白作用的 UCH-L5、BAP1 均只能水解泛素连接的小分子和短肽，并不参与水解泛素链和泛素化蛋白．因此，关注 UCH-L5 和 BAP1 在和各自伴侣蛋白相互作用时，交叉环结构的构象变化是阐明 UCH 家族对泛素链的水解选择性和产生机制的必要条件．

2 卵巢肿瘤相关蛋白酶家族

人类卵巢肿瘤相关蛋白酶(OTU)家族包含 16 个成员，根据序列相似性可将它们细化为 4 个亚家族：Otubain 亚家族(OTUB1 和 OTUB2)；OTUD 亚家族(OTUD1、OTUD2/YOD1、OTUD3、OTUD4、OTUD5/DUBA、OTUD6A、OTUD6B、ALG13)；类 A20 亚家族(A20、Cezanne、Cezanne2、TRABID、VCPIP)和 OTULIN 亚家族(OTULIN)．其中，大部分 DUB 除了包含核心 OTU 结构域之外，还拥有一个或多个泛素结合结构域(ubiquitin binding domain，UBD)．相比于 USP 家族，OTU 家族大部分成员都具有很强的泛素链识别选择性(表 1)．然而，这种选择性和特异性并非绝对存在，当增加酶浓度、延长孵育时间后 OTUs 最终会水解所有类型泛素链[23].

不同的异肽键连接方式导致泛素链具有多种多样的构型，这种多样性使得 DUBs 水解泛素链的机理也会不同．在水解二泛素过程中，2 个泛素亚基都会与 DUB 催化结构域作用，我们将远端泛素(提供羧基的泛素分子，Distal Ub)靶向的 DUB 催化位点称为 S1 位点，近端泛素(提供赖氨酸侧链氨基或 N 端氨基，proximal Ub)靶向的 DUB 催化位点称为 S1′位点．一般来说，远端泛素在二泛素形成中只提供 C 端羧基，在 8 种泛素链保持相似的构型，因此 S1 位点与泛素的相互作用并不能很好地解释去泛素酶的水解选择性，但与 S1′位点相互作用的近端泛素需要提供不同位置的氨基形成异肽键和肽键，因此，近端泛素的空间指向和定位是我们理解

去泛素化酶水解选择性机理的关键因素之一．总体而言，OTU 家族去泛素化酶倾向于通过以下几种机理实现对泛素链的水解选择性．

2.1 UBD 作为 S1′位点导致选择性水解泛素链

UBD 是一大类非共价结合泛素蛋白的模块化结构域，在 E1-E2-E3 酶系统、泛素底物蛋白与去泛素化酶中广泛存在．在 DUBs 识别泛素链过程中，近端泛素通过靶向 UBD 实现所需的空间指向和定位，促使 OTU 催化结构域能够快速识别泛素链并水解异肽键．例如，OTUD1能够特异性水解 Lys63 泛素链，但 UIM 结构域(一种 UBD 类型)的缺乏会导致 Lys63 特异性识别降低，OTUD2 选择性识别 Lys11、Lys27、Lys29 和 Lys33 泛素链，截断 C 端 ZnF 结构域或者对锌结合氨基酸进行突变，会导致其对 Lys27、Lys29 和 Lys33 泛素链识别降低[23]，TRABID 和 Lys33 二泛素的复合物晶体结构显示锚蛋白重复域(ankyrin-repeats domain)作为一种 UBD 形成 S1′位点，使得去泛素化酶选择性水解 Lys33 泛素链[24]．此外，TRABID 蛋白 N 端 UBD NZF1 能形成结合近端泛素和远端泛素的二齿配体，辅助 TRABID 水解 Lys29、Lys33 泛素链[25]．已知的 OTU 家族中至少一半成员蛋白质序列包含至少 1 个 UBD．因此，UBD 造成泛素链水解酶的水解选择性是一种被广泛认可的 DUBs 选择性产生机制．不仅如此，其他去泛素化酶家族中也存在一些 DUBs 采用相同的机制水解泛素链．

2.2 DUBs 通过特异性识别泛素序列水解泛素链

2013 年，Komander 等发现 OTUD2 通过识别 Lys11 泛素链序列水解 Lys11 二泛素[23]．突变 Lys11 附近的 Phe4、Val5、Thr7、Leu8、Thr12、Ile13和 Leu15 残基均使得 OTUD2 水解 Lys11 二泛素能力显著下降．OTUD2 和 Lys11 di-ub 的复合晶体结构显示，近端泛素 Lys11 上游的 4 个氨基酸和下游的 2 个氨基酸促进了异肽键作用到 OTU 结构域催化中心上．然而晶格的形成似乎影响了酶和泛素的结合，Phe4、Val5 和 Leu15 这几个在体外生化实验中潜在的、能对序列识别产生重要影响的几个氨基酸并未显示出明显有序的结构．

2.3 OTUD 亚家族核心结构域影响水解泛素链

除了 UBD 能够形成 S1′位点，OTU 催化结构域还可以通过催化中心限制异肽键构象，使得近端泛素和 OTU 结构域相互作用形成 S1′位点．这种构象的产生依赖于 3 个 loop 结构：Cys loop、His loop 和 V loop．在 OTUD1 和 OTUD3 中，3 个 loop 与催化结构域的 N 端螺旋共同靶向近端泛素形成 S1′位点，而 OTUD2 催化结构域的 C 端螺旋在结构上也发挥类似作用．序列分析 OTUD1、OTUD2、OTUD3 后发现，尽管组成 His 和 Cys loop 结构的氨基酸序列多变，但蛋白质结构具有很高的表面序列保守性，这种保守和变化共存的性质导致了酶的水解选择性．OTUB1 和 OTUB2 均不含有 UBD 结构域[23]，未连接泛素时均处于自抑制状态，和泛素相互作用后通过变构调节能产生水解活性．有趣的是，尽管这 2 个蛋白序列相似，但它们具有完全不同的水解泛素选择性(表 1)．Kessler 和 Ren 等[26]发现，OTUB2 和 OTUB1 之间选择性差异的产生源于蛋白 N 端区域，OTUB1 的 N 端螺旋结构能够辅助识别 Lys48 近端泛素，而 OTUB2 缺乏这一末端区域．OTULIN 是特异性水解线性泛素链的 DUB，它的水解特异性一方面来源于其 N 端螺旋参与形成 S1′位点，另一方面源于催化中心存在一个识别线性近端泛素 Glu16区域，能够作为双齿配体增强酶催化活性．

2.4 存在 S2 位点辅助结合泛素

在 OTUD2 识别泛素链的过程中，S2 位点由 OTUD2 C 端形成 α 螺旋的 2 个疏水氨基酸 Ile292 与 Val295 组成，将这 2 位点同时突变为 Gln 后发现突变体和野生型 OTUD2 水解 Lys11 二泛素链的活性变化不大，但野生型 OTUD2 水解 Lys11 三泛素和 Lys11 四泛素的速率明显快于 S2 位点突变的 OTUD2．这说明 S2 位点主要功能体现在辅助结合更长的 Lys11 泛素链[23]．此外，研究还发现 TRABID N 端存在的多个 NZF 结构域也同样起到稳定多聚泛素链的作用．

2.5 翻译后修饰影响水解泛素链活性

DUBs 蛋白的翻译后修饰也是影响其水解泛素链活性的重要因素之一．例如，真核细胞表达纯化的 OTUD5 蛋白能够水解多种泛素链，而在大肠杆菌中重组表达的 OTUD5 并未显示任何水解异肽键的能力．进一步实验结果表明，OTUD5 Ser177 位的磷酸化修饰是影响其水解泛素链活性的重要因素．晶体结构表明，这种选择性差异产生在于磷酸化修饰介导了去泛素化酶识别底物时构象的改变，当泛素底物与 OTUD5 结合后，磷酸基团能够通过

多个盐桥键和远端泛素以及 OTU 结构域中的 α6 螺旋相互作用，允许异肽键进入催化中心并最终释放反应产物[27].

此外，尽管人们早已发现 A20 在调节炎症免疫、肿瘤发生过程中扮演着十分重要的角色，但关于 A20 的机制研究仍然不清楚．体外实验表明 A20 作为去泛素化酶，高选择性水解 Lys48 泛素链而不是 Lys63 泛素链，与之相反的是体内信号通路研究则更多表明 A20 特异性水解 Lys63 泛素链．直到近期才有研究表明，可能是体内 A20 的磷酸化导致了这一实验结果的差异[28].

除了去泛素化作用，部分去泛素化酶还具有一些非典型的功能．例如 OTUD4 作为 USP7 和 USP9X 支架蛋白应答 DNA 损伤[29]．OTUB1 通过抑制 E2 酶信号调节 p53 活性，但调节作用与去泛素化活性无关[30].

3 泛素特异性加工酶家族(USPs)

USP 家族是最大的一类去泛素化酶，包含 56 个成员．它们的催化结构域采用类木瓜蛋白酶(papain-like)的折叠方式，这种同源保守的催化结构域使得 USPs 大多采用相似的机理水解异肽键．和其他去泛素化酶家族蛋白相比，USP 家族中大部分成员没有特异识别泛素链的能力，它们似乎对所有二泛素都具备水解活性，尤其是 Lys6、Lys11、Lys48 和 Lys63 二泛素．但是大部分 USP 在水解 Lys27 泛素链时普遍表现出较低的活性，还有部分 USP 在水解 Lys29 泛素链上也存在困难[31](表 1)．这种无差异性水解泛素链的能力导致部分 USPs (USP5、USP14)在维持体内单泛素和泛素链动态平衡中起着重要作用．不过，USP4、USP5、CYLD、USP14 等去泛素化酶通过在催化结构域插入其他结构序列的方式影响蛋白质的催化活性．例如 USP5 去泛素化酶的 USP domain 中就包含 2 个 UBA 结构域[32]，在识别 Lys48 和线性泛素过程中，N 端 ZnF-UBP 结构域靶向识别近端泛素(S1′位点)，而 2 个 UBA 结构域和 USP 结构域组成另外 3 个识别位点(S1、S2、S3)识别远端泛素．CYLD 的 B-box 结构域干扰了 USP 结构域折叠，影响其在亚细胞结构中的定位[33].

尽管 USPs 蛋白水解泛素链的选择性和产生机制并不明晰，但在功能调节中扮演了十分关键的作用：USP3、USP12、USP16、USP22、USP26、USP27、USP44、USP46 影响组蛋白泛素化，应答 DNA 损伤信号和细胞周期．USP2、USP5、USP10、USP13、USP29、USP42 调节 p53 信号通路，影响肿瘤发生．USP4、USP8、USP14、USP15、USP17、USP19、USP31、USP36、USP37 和免疫调节相关，影响细胞免疫应答(表 1)．因此，基于 USPs 作为新型药物靶点的药物设计研发是目前人们针对 USPs 的主要研究兴趣之一[34].

4 Machado-Josephin 蛋白酶家族(MJD)

Machado-Joseph 病是一种常染色体显性遗传性神经系统退化疾病．MJD 基因编码 ataxin-3，其正常功能尚不清楚，可能参与神经系统中蛋白质降解过程，其多聚谷氨酸链发生扩增导致发病．MJD 家族目前有 4 个成员：ataxin-3、the ataxin-3 like protein(ATXN3L)、Josephin-1 和 Josephin-2，它们的催化结构域 Josephin 结构域同源保守，例如 Ataxin-3 和 ATXN3L 的 Josephin 结构域序列相似性高达 85%，晶体结构也表明二者结构的相似性[35]. ATXN3L 螺旋 2、3 形成束发针(hairpin)结构，延伸在蛋白外，结合泛素后构象改变，产生水解酶活．酶活检测发现这 4 种蛋白均能有效水解 Lys48 和 Lys63 泛素链(表 1).

5 JAB1/MPN/Mov34 蛋白酶家族(JAMM)

不同于另外四类去泛素化酶亚家族属于半胱氨酸蛋白酶，JAMM 家族是唯一一类金属蛋白酶．Zn^{2+} 结合在活性位点中心，活化水分子作为亲和基团进攻异肽键．人类 AMSH 家族中包含 11 个成员，其中大部分具有水解 Lys63 泛素链特异性(表 1). 2008 年，Fukai 组报道了 AMSH-LP 和 Lys63 二泛素复合物晶体结构，结合 Zn^{2+} 的 Ins-2 loop 和与周围 loop 连接的 β6、α3 在蛋白表面形成一个凹面，靶向近端泛素确保只有 Lys63 构象的泛素链能够结合在该催化中心上．该结构也是首个报道的去泛素化酶和异肽键连接泛素链的复合物晶体结构(表 2).

Table 1 Summary of DUB linkage selectivity and DUBs mediated functions and diseases

表 1 去泛素化酶水解泛素链能力、相关生理过程和疾病总结

DUB	基因	水解泛素链能力								PDB ID	生理过程	疾病	备注
		K6-Ub_2	K11-Ub_2	K27-Ub_2	K29-Ub_2	K33-Ub_2	K48-Ub_2	K63-Ub_2	M1-Ub_2				
UCH	UCHL1	-	-	-	-	-	-	-	-	2etl	突触核蛋白降解	PD AD	水解 Ub 前体[14]二聚体具有 E3 活性
	UCHL3	-	-	-	-	-	-	-	-	1uch	NT[d]	宫颈癌乳腺癌	水解泛素前体识别类泛素 Nedd8[21]
	UCHL5	-	-	-	-	-	-	-	-	3ihr	蛋白酶体 TGF-β DSB 损伤[36]	食管鳞状细胞癌	结合伴侣蛋白后能水解泛素链[37]
	BAP1	-	-	-	-	-	-	-	-	NT	细胞周期	肺癌乳腺癌	结合伴侣蛋白后能水解泛素链[13]
OTU	OTUD1	-	-	-	-	-	-	+++	-	4bop	NT	甲状腺癌	[23, 38]
	OTUD2	-	++	+++	+++	+++	(+)	-	-	4boq	内质网降解	宫颈癌	[23]
	OTUD3	+++	+++	+++	-	-	+++	-	-	4bou	PI(3)K/Akt	乳腺癌	[39]
	OTUD4	-	(+)	-	-	-	+++	(+)	-	NT	DNA 烷基化损伤修复	NT	作为 USP7 和 USP9X 支架蛋白，不发挥 DUB 作用[29]
	OTUD5	-	-	-	-	-	-	-	-	3pfy	p53	NT	[27]
	(P)OTUD5	(+)	++	(+)	(+)	++	+++	+++	(+)	NT	NT	NT	[27]
	OTUD6A	-	++	+++	+++	+++	+++	-	-	NT	NT	NT	[23]
	OTUD6B	-	-	-	-	-	-	-	-	NT	B 淋巴系统	NT	[23, 40]
	ALG13	-	-	-	-	-	-	-	-	NT	NT	NT	[23]
	OTUB1	-	-	-	-	NT	+++	(+)	NT	2zfy	DNA 损伤 p53	肺癌、乳腺癌、前列腺癌	抑制 E2 酶信号但不发挥去泛素化酶活性[30, 41-42]
	OTUB2	++	++	(+)	(+)	(+)	++	+++	NT	4fjv 1tff	NF-κB	NT	[26, 43]
	OTILIN	-	-	-	-	-	-	-	+++	4ksj 3znv	NF-κB Wnt	肿瘤血管生成	[44]
	A20(OUT)	-	++	-	-	-	+++	-	-	3dkb	NF-κB[45-46]	NT	*in vivo* 水解 Lys63 泛素链，但 *in vitro* 水解 Lys48 泛素链[28]
	Cezanne	-	+++	-	-	-	-	(+)	-	NT	NF-κB	NT	[47]
	Cezanne2	-	+++	-	-	-	-	-	-	NT	NF-κB	肝细胞性肝癌	[48]
	VCPIP1/ VCIP135	-	+++	-	-	-	+++	-	-	NT	有丝分裂	NT	[23]
	TRABID	-	-	-	+++	+++	-	++	-	3zrh	Wnt	NT	[24−25, 49]
USP	USP1	NT	NT	NT	NT	NT	NT	NT	NT	NT	中心体复制基因损伤[50]	非小细胞性肺癌[51]	调节 FA 信号通路中 FANCD2 的单泛素化
	USP2	+++	+++	+	+	+++	+++	+++	-	3v6e 3v6c	Fas、p53 有丝分裂	三阴性乳腺癌, 前列腺癌	[52−54]
	USP3	NT	NT	NT	NT	NT	NT	NT	NT	NT	DSB 抗病毒免疫	冠心病	去 H2A, H2B 单泛素化或水解 H2A, H2AX K63 泛素链[55-56]
	USP4	NT	NT	NT	NT	NT	NT	NT	NT	2y6e	DNA-DSB Wnt, NF-κB TGF-β, Akt[57]	结直肠癌, 骨佩吉特氏病	自主去泛素化调节应答 DSB 修复[58-59]
	USP5	+++	+++	-	+++	+++	+++	+++	+++	3ihp	DNA-DSB p53[60-61]	炎症, 黑色素瘤, 成胶质细胞瘤	
	USP6	NT	NT	NT	NT	NT	NT	NT	NT	NT	囊泡运输 NF-κB 信号	骨囊肿	[62−63]
	USP7	+++	+++	+	+	++	+++	++	-	2f1z	p53, Akt 细胞周期 DNA 损伤	前列腺癌神经胶质瘤	去泛素化 Rad18[64] PCNA 应答 UV-DNA 损伤[65], 去泛素化 H2A, H2AX[66]

续表 1

DUB	基因	水解泛素链能力								PDB ID	生理过程	疾病	备注
		K6-Ub_2	K11-Ub_2	K27-Ub_2	K29-Ub_2	K33-Ub_2	K48-Ub_2	K63-Ub_2	M1-Ub_2				
USP	USP8	+++	+++	-	-	-	+++	+++	-	2gfo	免疫调节[67]、线粒体自噬	非小细胞型肺癌[68]、库欣病[69]	去泛素化 Parkin, 调节线粒体自噬[70-71]
	USP9X	NT	NT	NT	+++	NT	-[72]	-	NT	NT	DNA 损伤细胞周期自噬 TGF-β	PD, AD 淋巴瘤、亨廷顿舞蹈病[73]、	和 OTUD4 相互作用, 去泛素化 ALKBH2, ALKBH3[29]
	USP9Y	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	前列腺癌	[74−75]
	USP10	NT	NT	NT	NT	NT	NT	NT	NT	NT	转录调节 p53[76]	肾细胞癌	去泛素化 H2A, H2AZ 调节雄激素基因表达[77]
	USP11	+++	+++	+	+	+++	++	++	-	NT	HR-DSB p53[57]	胰腺癌[78]	去泛素化 γH2AX 和 BRCA2[79]
	USP12	NT	NT	NT	NT	NT	NT	NT	NT	NT	雄激素受体转录调控 Akt, Notch[80]	前列腺癌[81]	去泛素化 H2A, H2B[82]
	USP13	++	-	-	+++	-	-	++	-	NT	JAK-STAT p53, Akt, 自噬	黑色素瘤结直肠癌、乳腺癌[83]	稳定 PTEN 抑制肿瘤发生, 稳定 STAT1 应答抗病毒免疫[39, 84-85]
	USP14	NT	NT	NT	NT	NT	NT	NT	NT	2ayo 2ayn	Akt, NF-κB 突触传导[86]	上皮性卵巢癌[87-88] 肝细胞癌、	去泛素化 NLRC5 调控 NF-κB 活性[89]
	USP15	NT	NT	NT	NT	NT	+++	+++	++	4a3p	TGF-β RNA 剪接线粒体自噬 NF-κB[57, 70, 90-91]	恶性胶质瘤	SART3 募集 H2B-Ub, 去泛素化 H2B[92]
	USP16	+++	+++	+++	+++	+++	+++	+++	++	NT	转录调节细胞周期 DNA 损伤	慢性粒单核细胞白血病, 唐氏综合症[93]	H2A 去泛素化[94], 调节 Hox 基因表达
	USP17	NT	NT	NT	NT	NT	+++	+++	NT	NT	抗病毒免疫 DNA 损伤蛋白运输	乳腺癌[95]	[96]
	USP18	NT	NT	NT	NT	NT	-	+++	NT	NT	NF-κB, JAK-STAT, Ⅰ型 IFN	丙肝, 乙肝急性白血病[97-99]	去泛素化 NEMO, TAK1 应答免疫[100]
	USP19	NT	+++	NT	NT	NT	++	+++	NT	NT	自噬抗病毒免疫	肌肉萎缩[101]	通过调节 Beclin-1 泛素化实现自噬、抗病毒双功能调控[102]
	USP20	NT	NT	NT	NT	NT	+++	+++	NT	NT	β2 肾上腺受体, NF-κB ATR-DNA	NT	[103−104]
	USP21	+++	+++	NT	+++	NT	+++	+++	+++	NT	转录起始 NF-κB[106]	NT	H2A 去泛素化[105]
	USP22	NT	NT	NT	NT	NT	NT	NT	NT	NT	细胞周期	结直肠癌、肺腺癌[107-108]	H2A, H2B 去泛素化
	USP24	NT	NT	NT	NT	NT	NT	NT	NT	NT	紫外损伤	淋巴瘤 PD[109]	稳定 DDB2, 应答紫外损伤[110]
	USP25	+++	+++	++	++	++	++	++	-	NT	抗病毒免疫	NT	[111−112]
	USP26	NT	NT	NT	NT	NT	NT	NT	NT	NT	ERAD 降解蛋白酶降解 HR-DSB	男性不育	[113−115]
	USP27	NT	NT	NT	NT	NT	NT	NT	NT	NT	神经干细胞分化	NT	
	USP28	NT	NT	NT	NT	NT	NT	NT	NT	NT	DNA 损伤检验点 c-myc 降解	结肠癌, 乳腺癌, 非小细胞肺癌	[116−117]
	USP29	NT	NT	NT	NT	NT	NT	NT	NT	NT	p53, DNA 检验点[118]	NT	H2A 去泛素化

续表 1

DUB	基因	水解泛素链能力								PDB ID	生理过程	疾病	备注
		K6-Ub_2	K11-Ub_2	K27-Ub_2	K29-Ub_2	K33-Ub_2	K48-Ub_2	K63-Ub_2	M1-Ub_2				
USP	USP30	+++	++	+	+	+	++	+	-	NT	线粒体自噬	PD?	[70, 119−120]
	USP31	NT	NT	NT	NT	NT	NT	NT	NT	NT	NF-κB	NT	[121]
	USP32	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	[122]
	USP33	NT	NT	NT	NT	NT	+++	+++	NT	NT	β2 肾上腺受体, 先天性免疫, 中心体形成[123]	急性淋巴细胞白血病	调节 RALB 泛素化实现自噬和先天性免疫应答双调控[103, 124]
	USP35	NT	NT	NT	NT	NT	NT	NT	NT	NT	PARK2 相关线粒体自噬	NT	[125]
	USP36	NT	NT	NT	NT	NT	NT	NT	NT	NT	抗病毒免疫、RNA 合成选择性自噬	NT	[126]
	USP37	NT	+++	NT	NT	NT	+++	+	NT	NT	HR-DSB, c-myc[127]、细胞周期	肺癌	调节 cyclin A 泛素化调控细胞周期[128−129]
	USP38	NT	NT	NT	NT	NT	NT	NT	NT	4rxx	NT	哮喘	
	USP39	没有去泛素化酶活性								NT			[130]
	USP40	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	PD[109]	水解泛素前体
	USP41	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
	USP42	NT	NT	NT	NT	NT	NT	NT	NT	NT	p53[131]线粒体自噬转录调节	骨髓性白细胞[132]	去泛素化 H2B[133]
	USP43	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
	USP44	NT	NT	NT	NT	NT	NT	NT	NT	NT	DNA-DSB 纺锤体装配检查点[134]	NT	H2B 去泛素化[82]
	USP45	NT	NT	NT	NT	NT	NT	NT	NT	NT	DNA 修复	NT	
	USP46	NT	NT	NT	NT	NT	NT	+++	NT	5vcm	AMPA[135] γ- 氨基丁酸神经递质[136]	结肠癌[137]	去泛素化 H2A 和 H2B[82]
	USP47	+++	+++	-	-	-	+++	+++	- [138]	NT	碱基修复, Wnt, 氧化应激[139]	NT	去泛素化 DNA 聚合酶 β
	USP48	NT	NT	NT	NT	NT	+++	NT	NT	NT	NT	NT	[140−141]
	USP49	NT	NT	NT	NT	NT	NT	NT	NT	NT	mRNA 剪接	NT	去泛素化 H2B[142]
	USP50	没有去泛素化酶活性								NT	G2/M 检验点应答	骨髓性白细胞	[143]
	USP51	NT	NT	NT	NT	NT	NT	NT	NT	NT	神经干细胞分化	NT	[144]
	USP52	没有去泛素化酶活性								NT	NT	NT	[145]
	USP53	没有去泛素化酶活性								NT	NT	NT	
	USP54	没有去泛素化酶活性								NT	NT	NT	
	CYLD	-	-	-	-	-	+	+++	+++	2vhf	NF-κB、Wnt、JNK	家族性圆柱瘤, 成神经细胞瘤	[33, 146−147]
	USPL1	NT	NT	NT	NT	NT	NT	NT	NT	NT	snRNP, sno-RNP[148]	NT	去 SUMO 化[149]
JA MM	PSMD7/Mov34L	NT	NT	NT	NT	NT	NT	NT	NT	2o95	NT	NT	[150]
	AMSH	-	-	NT	-	NT	-	+++	-	3rzu 3rzv	EGFR, Myc 蛋白运输	小头畸形	[151−153]
	AMSH-LP	NT	NT	NT	NT	NT	-	+++	NT	2znr 2znv	蛋白运输	NT	[154]
	PSMD14	NT	NT	NT	NT	NT	NT	+++	NT	NT	NHEJ-DSB c-Jun	NT	[155−156]
	EIF3H	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	NT	
	BRCC36	NT	NT	NT	NT	NT	-	+++	NT	NT	HR 相关 DNA 损伤	三阴性乳腺癌、卵巢癌	去泛素化 H2A[156−158]

续表 1

DUB	基因	水解泛素链能力								PDBID	生理过程	疾病	备注
		K6-Ub_2	K11-Ub_2	K27-Ub_2	K29-Ub_2	K33-Ub_2	K48-Ub_2	K63-Ub_2	M1-Ub_2				
JA MM	CSN5/Jab1	NT	NT	NT	NT	NT	NT	+++	NT	4f7o	p53, 细胞周期, JNK	鼻咽癌亨廷顿氏舞蹈病[159]	CSN 复合体组成亚基，参与类泛素(去 Neddy 化)调节[160-161]
	CSN6/ HVIP	NT	NT	NT	NT	NT	NT	NT	NT	4qft	p53, c-Jun, DNA 损伤 HER2-Akt	宫颈癌、结直肠癌	[162−163]
	MPND	NT	NT	NT	NT	NT	NT	NT	NT	NT			
	PRPF8	NT	NT	NT	NT	NT	NT	NT	NT	NT			
	MYSM1	NT	NT	NT	NT	NT	NT	NT	NT	NT			
MJD	Ataxin-3	NT	NT	NT	NT	NT	+++	++	NT	1yzb 2klz	神经系统蛋白降解	MJD	
	Ataxin-3-like	NT	NT	NT	NT	NT	+++	+++	NT	3o65		MJD	
	JosD1	NT	NT	NT	NT	NT	++	++	NT	NT		MJD	
	JosD2	NT	NT	NT	NT	NT	++	+++	NT	NT		MJD	

NT：未有文献报道. +++：很强的水解泛素链活性. ++：较强的水解泛素链活性. +：弱的水解泛素链活性. (+)：背景水解活性. AD：阿尔茨海默病. PD：帕金森病. DSB：DNA 双链断裂. HR：同源性重组. TGF-β：转化生长因子. p53：人体抑癌基因. NF-κB：转录因子蛋白家族, 参与免疫早期和炎症反应. IFN：干扰素. EGFR：表皮生长因子受体. CtIP：转录因子, CtBP 作用蛋白. PCNA：增殖细胞核抗原. NEMO：NF-κB 必要控制子；FANCD2：范科尼贫血 D2 蛋白.

Table 2　Crystal structures of complexes of PolyUb chains with DUBs

表 2　去泛素化酶与泛素链复合物晶体结构

链种类	去泛素化酶	PDB ID	DUB 家族
Met1	OTULIN	4KSL/3ZNZ	OTU
Lys29	TRABID	4S22	OTU
Lys33	TRABID	5AF4	OTU
Lys63	AMSH-LP	2ZNV	JAMM
Lys63	CYLD	3WXG	USP
Met1	CYLD	3WXE/3WXF	USP
Met1 diUb aldehyde	USP21	2Y5B	USP
Lys11	OTUD2	4BOZ	OTU

6　展　　望

蛋白质翻译后修饰被视为中心法则调控规律的重要补充，实现了真核生物对复杂生命过程的调控．解密这套生命密码无疑会为人类理解生命过程起到极大的推动作用[164]．而在众多翻译后修饰过程中，泛素化凭借能对底物进行多层次修饰(单泛素、8 种泛素链、混合泛素链、分叉泛素链)，与去泛素化酶协同形成了独特的泛素网络，共同调控细胞生命过程．去泛素化酶的功能失调与癌症、心血管、神经退行性等多种重大疾病相关[34, 165-166]．因此，作为一类新兴的药物研发靶点，理解去泛素化酶选择性识别泛素链及其产生机制对于我们进行基于靶点的理性药物设计至关重要．

去泛素化酶早期研究遇到的主要困难是难以获取大量(毫克量级)、性质均一(单泛素链)、高纯度的泛素底物．基因重组表达技术无法获得单一修饰的泛素链，且链的长度不可控．利用酶法(体外加入特异性 E1-E2-E3 酶系统)合成泛素链是一类能大幅提高产率的方法，近年来研究人员通过改造酶、定点突变泛素、非天然氨基酸插入等方法已经能够成功获取除 Lys27 泛素链以外其他 7 种泛素链[167]．然而，目前酶法获取泛素链最明显的缺点是纯化困难，分离的步骤十分繁琐，此外，酶法仍未完全解决混合链和链长不可控等问题[168]．相比之下，多肽 / 蛋白质固相全合成和半合成法提供了一种能够大量获得单一、高纯泛素链的可行思路．最近一系列新方法的发展使得化学合成法获取泛素链的研究有了长足的进步[169-173]：2007 年，Muir 小组[174]利用溴乙酸在 N，N- 二异丙基碳二亚胺(DIC)存在条件下和赖氨酸侧链氨基发生溴乙酰化的反应制备得到溴乙酰赖氨酸，光依赖型辅基亲和取代溴原子后在光照条件下和能泛素硫脂反应，通过 S-N 酰基迁移反应形成特定位点异肽键连接的二泛素.该合成方法效率依赖于辅基，因此通过优化辅基分子能够

提高获取二泛素能力[175]. 2010 年，Brik 小组通过将 δ- 巯基半胱氨酸(δ-mercaptolysine)引入到特定位点(Ub Lys 6、Lys 11、Lys 27、Lys 29、Lys 33、Lys 48、Lys 63 位点)替代赖氨酸，然后利用异肽键连接法(isopeptide chemical ligation，ICL)与另一 Ub 分子的末端硫脂形成共价连接形成异肽键，再通过脱硫反应去除 δ- 巯基半胱氨酸上巯基[176]能够一次性得到所有连接类型的天然 di-Ub[177]. 此外，Komander 小组利用正交保护活化连接法(genetically encoded orthogonal protection and activated ligation，GOPAL)将改造过的 tRNA- 氨酰 tRNA 合成酶引入 *E. coli.*，通过基因密码子拓展在特定 Ub 赖氨酸位点上引入 Boc 保护的赖氨酸，其余氨基酸侧链用 benzyloxycarbamate (CBz)保护，脱 Boc 保护后游离侧链氨基通过 ICL 反应结合泛素硫脂成功地合成 Lys 6 和 Lys 29 di-ub，并解析得到了晶体结构[178]. 事实上，该方法也可直接用于固相合成，在供体泛素的异肽键连接位点引入 Boc 保护的赖氨酸，其余赖氨酸用 CBz 保护，脱 Boc 保护后侧链自由氨基和受体泛素末端硫脂 ICL 连接，也能得到特定位点连接的二泛素. 而随着对反应体系不断的优化，包括添加新的辅基分子和芳基硫脂，引入假二肽等，人们已经能够较高效地得到所有单一泛素链，以及各种混合、分叉修饰泛素链[179−182]. 泛素链和泛素探针的另一重要用途是发展去泛素化酶 assay，用于筛选小分子抑制剂，研究特异性靶向去泛素化酶的药物.

去泛素化酶在细胞增殖、分化过程中扮演着重要角色，其功能变化与许多重大疾病(癌症、神经退行性疾病、传染性疾病)的发生、发展直接相关(表 1)，因此，去泛素化酶是一大类极具吸引力的、潜在的药物靶点，筛选这些去泛素化酶的小分子抑制剂，开发针对去泛素化酶的药物是未来去泛素化酶研究的一大热点. 而且，目前对于去泛素化酶的功能研究还不够全面，有一大批去泛素化酶的生理功能仍然不明，阐明这些去泛素化酶的具体生理作用，验证其是否是好的药物靶点将是去泛素化酶研究的另一热点. 不仅如此，解析去泛素化酶和泛素链的复合物晶体，从结构和化学层面上来阐明去泛素化酶水解泛素链的选择性机制，能帮助我们更好地理解细胞中的泛素调控密码，从而辅助小分子的理性设计乃至发现新的尚未理解的生命调控机制.

参 考 文 献

[1] Swatek K N, Komander D. Ubiquitin modifications. Cell Res, 2016, **26**(4): 399−422

[2] Chen Z J, Sun L J. Nonproteolytic functions of ubiquitin in cell signaling. Mol Cell, 2009, **33**(3): 275−286

[3] Komander D, Rape M. The ubiquitin code. Ann Rev Biochem, 2012, **81**(7): 203−229

[4] Ikeda F, Dikic I. Atypical ubiquitin chains: new molecular signals. 'Protein Modifications: Beyond the Usual Suspects' review series. EMBO Rep, 2008, **9**(6): 536−542

[5] Emmerich C H, Bakshi S, Kelsall I R, *et al*. Lys63/Met1-hybrid ubiquitin chains are commonly formed during the activation of innate immune signalling. Biochem Biophys Res Commun, 2016, **474**(3): 452−461

[6] Husnjak K, Dikic I. Ubiquitin-binding proteins: decoders of ubiquitin-mediated cellular functions. Ann Rev Biochem, 2012, **81**(7): 291−322

[7] Coyne E S, Wing S S. The business of deubiquitination - location, location, location. F1000Research, 2016, **5**: 163

[8] Hu M, Li P W, Song L, *et al*. Structure and mechanisms of the proteasome-associated deubiquitinating enzyme USP14. EMBO J, 2005, **24**(21): 3747−3756

[9] Komander D, Lord C J, Scheel H, *et al*. The structure of the CYLD USP domain explains its specificity for Lys63-linked polyubiquitin and reveals a B box module. Mol Cell, 2008, **29**(4): 451−464

[10] Komander D. Mechanism, Specificity and Structures of the Deubiquitinases [M]//GROETTRUP M. Conjugation and Deconjugation of Ubiquitin Family Modifiers.Landes Bioscience and Springer Science Business Media, 2010: 69−87

[11] Reyes-Turcu F E, Ventii K H, Wilkinson K D. Regulation and cellular roles of ubiquitin-specific deubiquitinating enzymes. Ann Rev Biochem, 2009, **78**(1): 363−397

[12] Komander D, Clague M J, Urbe S. Breaking the chains: structure and function of the deubiquitinases. Nat Rev Mol Cell Biol, 2009, **10**(8): 550−563

[13] Fang Y, Fu D, Shen X Z. The potential role of ubiquitin C-terminal hydrolases in oncogenesis. Biochim Biophys Acta, 2010, **1806**(1): 1−6

[14] Setsuie R, Wada K. The functions of UCH-L1 and its relation to neurodegenerative diseases. Neurochem Int, 2007, **51**(2−4): 105−111

[15] Larsen C, Krantz B, Wilkinson K. Substrate specificity of deubiquitinating enzymes: ubiquitin C-terminal hydrolases. J Am Chem Soc, 1998, **37**(10): 3358−3368

[16] Misaghi S, Ottosen S, Izrael-Tomasevic A, *et al*. Association of C-terminal ubiquitin hydrolase BRCA1-associated protein 1 with cell cycle regulator host cell factor 1. Mol Cell Biol, 2009, **29**(8): 2181−2192

[17] Yao T, Song L, Xu W, *et al*. Proteasome recruitment and activation of the Uch37 deubiquitinating enzyme by Adrm1. Nat Cell Biol, 2006, **8**(9): 994−1002

[18] Das C, Hoang Q, Kreinbring C, *et al*. Structural basis for conformational plasticity of the Parkinson's disease-associated ubiquitin hydrolase UCH-L1. Proc Natl Acad Sci USA, 2006, **103**(12): 4675-4680

[19] Johnston S C, Larsen C N, Cook W J, *et al*. Crystal structure of a deubiquitinating enzyme (human UCH-L3) at 1.8Å resolution. EMBO J, 1997, **16**(13): 3787-3796

[20] Maiti T K, Permaul M, Boudreaux D A, *et al*. Crystal structure of the catalytic domain of UCHL5, a proteasome-associated human deubiquitinating enzyme, reveals an unproductive form of the enzyme. FEBS J, 2011, **278**(24): 4917-4926

[21] Popp M W, Artavanis-Tsakonas K, Ploegh H L. Substrate filtering by the active site crossover loop in UCHL3 revealed by sortagging and gain-of-function mutations. J Biol Chem, 2009, **284**(6): 3593-3602

[22] Zhou Z R, Zhang Y H, Liu S, *et al*. Length of the active-site crossover loop defines the substrate specificity of ubiquitin C-terminal hydrolases for ubiquitin chains. Biochem J, 2012, **441**(1): 143-149

[23] Mevissen T E, Hospenthal M K, Geurink P P, *et al*. OTU deubiquitinases reveal mechanisms of linkage specificity and enable ubiquitin chain restriction analysis. Cell, 2013, **154** (1): 169-184

[24] Licchesi J D, Mieszczanek J, Mevissen T E, *et al*. An ankyrin-repeat ubiquitin-binding domain determines TRABID's specificity for atypical ubiquitin chains. Nat Struct Mol Biol, 2012, **19**(1): 62-71

[25] Michel M A, Elliott P R, Swatek K N, *et al*. Assembly and specific recognition of k29- and k33-linked polyubiquitin. Mol Cell, 2015, **58**(1): 95-109

[26] Altun M, Walter T S, Kramer H B, *et al*. The human otubain2-ubiquitin structure provides insights into the cleavage specificity of poly-ubiquitin-linkages. PloS One, 2015, **10**(1): e0115344

[27] Huang O W, Ma X, Yin J, *et al*. Phosphorylation-dependent activity of the deubiquitinase DUBA. Nat Struct Mol Biol, 2012, **19** (2): 171-175

[28] Wertz I E, Newton K, Seshasayee D, *et al*. Phosphorylation and linear ubiquitin direct A20 inhibition of inflammation. Nature, 2015, **528**(7582): 370-375

[29] Zhao Y, Majid M C, Soll J M, *et al*. Noncanonical regulation of alkylation damage resistance by the OTUD4 deubiquitinase. EMBO J, 2015, **34**(12): 1687-1703

[30] Sun X X, Dai M S. Deubiquitinating enzyme regulation of the p53 pathway: A lesson from Otub1. World J Biol Chem, 2014, **5** (2): 75-84

[31] Faesen A C, Luna-Vargas M P, Geurink P P, *et al*. The differential modulation of USP activity by internal regulatory domains, interactors and eight ubiquitin chain types. Chem Biol, 2011, **18**(12): 1550-1561

[32] Reyes-Turcu F E, Shanks J R, Komander D, *et al*. Recognition of polyubiquitin isoforms by the multiple ubiquitin binding modules of isopeptidase T. J Biol Chem, 2008, **283**(28): 19581-19592

[33] Sato Y, Goto E, Shibata Y, *et al*. Structures of CYLD USP with Met1- or Lys63-linked diubiquitin reveal mechanisms for dual specificity. Nat Struct Mol Biol, 2015, **22**(3): 222-229

[34] Sipp W G, Collura V, Colland F. Ubiquitin-specific proteases as cancer drug targets. Future Oncol, 2011, **7**(5): 619-632

[35] Weeks S D, Grasty K C, Hernandez-Cuebas L, *et al*. Crystal structure of a Josephin-ubiquitin complex: evolutionary restraints on ataxin-3 deubiquitinating activity. J Biol Chem, 2011, **286** (6): 4555-4565

[36] Nishi R, Wijnhoven P, Le Sage C, *et al*. Systematic characterization of deubiquitylating enzymes for roles in maintaining genome integrity. Nat Cell Biol, 2014, **16**(10): 1016-1026, 1011-1018

[37] Chen Y J, Ma Y S, Fang Y, *et al*. Power and promise of ubiquitin carboxyl-terminal hydrolase 37 as a target of cancer therapy. Asian Pacific J Cancer Prevention, 2013, **14**(4): 2173-2179

[38] Carneiro A P, Reis C F, Morari E C, *et al*. A putative OTU domain-containing protein 1 deubiquitinating enzyme is differentially expressed in thyroid cancer and identifies less-aggressive tumours. Brit J Cancer, 2014, **111**(3): 551-558

[39] Yuan L, Lv Y, Li H, *et al*. Deubiquitylase OTUD3 regulates PTEN stability and suppresses tumorigenesis. Nat Cell Biol, 2015, **17**(9): 1169-1181

[40] Xu Z P, Zheng Y F, Zhu Y F, *et al*. Evidence for OTUD-6B participation in B lymphocytes cell cycle after cytokine stimulation. PloS One, 2011, **6**(1): e14514

[41] Edelmann M J, Iphofer A, Akutsu M, *et al*. Structural basis and specificity of human otubain 1-mediated deubiquitination. Biochem J, 2009, **418**(2): 379-390

[42] Wang T, Yin L, Cooper E M, *et al*. Evidence for bidentate substrate binding as the basis for the K48 linkage specificity of otubain 1. J Mol Biol, 2009, **386**(4): 1011-1023

[43] Nanao M H, Tcherniuk S O, Chroboczek J, *et al*. Crystal structure of human otubain 2. EMBO Rep, 2004, **5**(8): 783-788

[44] Keusekotten K, Elliott P R, Glockner L, *et al*. OTULIN antagonizes LUBAC signaling by specifically hydrolyzing Met1-linked polyubiquitin. Cell, 2013, **153**(6): 1312-1326

[45] Lin S C, Chung J Y, Lamothe B, *et al*. Molecular basis for the unique deubiquitinating activity of the NF-kappaB inhibitor A20. J Mol Biol, 2008, **376**(2): 526-540

[46] Hymowitz S G, Wertz I E. A20: from ubiquitin editing to tumour suppression. Nat Rev Cancer, 2010, **10**(5): 332-341

[47] Bremm A, Freund S M, Komander D. Lys11-linked ubiquitin chains adopt compact conformations and are preferentially hydrolyzed by the deubiquitinase Cezanne. Nat Struct Mol Biol, 2010, **17**(8): 939-947

[48] Xu Z, Pei L, Wang L, *et al*. Snail1-dependent transcriptional repression of Cezanne2 in hepatocellular carcinoma. Oncogene, 2014, **33**(22): 2836-2845

[49] Kristariyanto Y A, Abdul Rehman S A, Campbell D G, *et al*. K29-selective ubiquitin binding domain reveals structural basis of specificity and heterotypic nature of k29 polyubiquitin. Molcell, 2015, **58**(1): 83-94

[50] Jung J K, Jang S W, Kim J M. A novel role for the deubiquitinase USP1 in the control of centrosome duplication. Cell Cycle, 2016, **15**(4): 584-592

[51] Iraia G S, Godefridus J P, Jose AR, *et al*. USP1 deubiquitinase: cellular functions, regulatory mechanisms and emerging potential as target in cancer therapy. Mol Cancer, 2013, **12**(1): 91

[52] Castaneda C A, Dixon E K, Walker O, *et al*. Linkage *via* K27 bestows ubiquitin chains with unique properties among polyubiquitins. Struct, 2016, **24**(3): 423−436

[53] Qu Q, Mao Y, Xiao G, *et al*. USP2 promotes cell migration and invasion in triple negative breast cancer cell lines. Tumour Biol 2015, **36**(7): 5415−5423

[54] Nelson W G, De Marzo A M, Yegnasubramanian S. USP2a activation of MYC in prostate cancer. Cancer Discov, 2012, **2**(3): 206−207

[55] Sharma N, Zhu Q, Wani G, *et al*. USP3 counteracts RNF168 *via* deubiquitinating H2A and gammaH2AX at lysine 13 and 15. Cell Cycle, 2014, **13**(1): 106−114

[56] Nicassio F, Corrado N, Vissers J H, *et al*. Human USP3 is a chromatin modifier required for S phase progression and genome stability. CurrBiol, 2007, **17**(22): 1972−1977

[57] Vlasschaert C, Xia X, Coulombe J, *et al*. Evolution of the highly networked deubiquitinating enzymes USP4, USP15, and USP11. BMC Evol Biol, 2015, **15**(1): 230

[58] Liu H, Zhang H, Wang X, *et al*. The deubiquitylating enzyme USP4 cooperates with CtIP in DNA double-strand break end resection. Cell Rep, 2015, **13**(1): 93−107

[59] Wijnhoven P, Konietzny R, Blackford A N, *et al*. USP4 auto-deubiquitylation promotes homologous recombination. Mol Cell, 2015, **60**(3): 362−373

[60] Nakajima S, Lan L, Wei L, *et al*. Ubiquitin-specific protease 5 is required for the efficient repair of DNA double-strand breaks. PloS One, 2014, **9**(1): e84899

[61] Potu H, Peterson L F, Pal A, *et al*. Usp5 links suppression of p53 and FAS levels in melanoma to the BRAF pathway. Oncotarget, 2014, **5**(14): 5559−5569

[62] Funakoshi Y, Chou M M, Kanaho Y, *et al*. TRE17/USP6 regulates ubiquitylation and trafficking of cargo proteins that enter cells by clathrin-independent endocytosis. J Cell Sci, 2014, **127** (Pt 21): 4750−4761

[63] Ye Y, Pringle L M, Lau A W, *et al*. TRE17/USP6 oncogene translocated in aneurysmal bone cyst induces matrix metalloproteinase production *via* activation of NF-kappaB. Oncogene, 2010, **29**(25): 3619−3629

[64] Zlatanou A, Sabbioneda S, Miller E S, *et al*. USP7 is essential for maintaining Rad18 stability and DNA damage tolerance. Oncogene, 2016, **35**(8): 965−976

[65] Qian J, Pentz K, Zhu Q, *et al*. USP7 modulates UV-induced PCNA monoubiquitination by regulating DNA polymerase eta stability. Oncogene, 2015, **34**(36): 4791−4796

[66] Zhu Q, Sharma N, He J, *et al*. USP7 deubiquitinase promotes ubiquitin-dependent DNA damage signaling by stabilizing RNF168. Cell Cycle, 2015, **14**(9): 1413−1425

[67] Dufner A, Kisser A, Niendorf S, *et al*. The ubiquitin-specific protease USP8 is critical for the development and homeostasis of T cells. Nat Immun, 2015, **16**(9): 950−960

[68] Jeong C H. Inhibition of ubiquitin-specific peptidase 8 suppresses growth of gefitinib-resistant non-small cell lung cancer cells by inducing apoptosis. J Cancer Prevention, 2015, **20**(1): 57−63

[69] Huang C X, Shi Y Y, Zhao Y. USP8 mutation in Cushing's disease. Oncotarget, 2015, **6**(21): 18240−18241

[70] Durcan T M, Fon. E. A. The three 'P' s of mitophagy: PARKIN, PINK1, and post-translational modifications. Gene Devel, 2015, **29**(10): 989−999

[71] Durcan T M, Tang M Y, Perusse J R, *et al*. USP8 regulates mitophagy by removing K6-linked ubiquitin conjugates from parkin. EMBO J, 2014, **33**(21): 2473−2491

[72] Al-Hakim A K, Zagorska A, Chapman L, *et al*. Control of AMPK-related kinases by USP9X and atypical Lys (29)/Lys (33)-linked polyubiquitin chains. Biochem J, 2008, **411**(2): 249−260

[73] Murtaza M, Jolly L A, Gecz J, *et al*. La FAM fatale: USP9X in development and disease. Cell Mol Life Sci, 2015, **72** (11): 2075−2089

[74] Krausz C, Degl'innocenti S, Nuti F, *et al*. Natural transmission of USP9Y gene mutations: a new perspective on the role of AZFa genes in male fertility. Human Mol Genet, 2006, **15** (18): 2673−2681

[75] Zhu Y, Ren S, Jing T, *et al*. Clinical utility of a novel urine-based gene fusion TTTY15-USP9Y in predicting prostate biopsy outcome. Urologic Oncology, 2015, **33**(9): 384 e389−320

[76] Yuan J, Luo K, Zhang L, *et al*. USP10 regulates p53 localization and stability by deubiquitinating p53. Cell, 2010, **140**(3): 384−396

[77] Draker R, Sarcinella E, Cheung P. USP10 deubiquitylates the histone variant H2A.Z and both are required for androgen receptor-mediated gene activation. Nucl Acids Res, 2011, **39** (9): 3529−3542

[78] Burkhart R A, Peng Y, Norris Z A, *et al*. Mitoxantrone targets human ubiquitin-specific peptidase 11 (USP11) and is a potent inhibitor of pancreatic cancer cell survival. Mol Cancer Res, 2013, **11**(8): 901−911

[79] Yu M, Liu K, Mao Z, *et al*. USP11 is a negative regulator to gammaH2AX ubiquitylation by RNF8/RNF168. J Biol Chem, 2016, **291**(2): 959−967

[80] Urszula L, Mcclurg, Emma E, *et al*. Deubiquitinating enzyme Usp12 regulates the interaction between the androgen receptor and the Akt pathway. Oncotarget, 2014, **5**(16): 7081−7092

[81] Urszula L M, Walker S, Harle V J, *et al*. Ubiquitin-specific protease 12 interacting partners Uaf-1 and WDR20 are potential therapeutic targets in prostate cancer. Oncotarget, 2015, **6**(35): 37724−37736

[82] Joo H Y, Jones A, Yang C, *et al*. Regulation of histone H2A and H2B deubiquitination and *Xenopus* development by USP12 and USP46. J Biol Chem, 2011, **286**(9): 7190−7201

[83] Zhang Y H, Zhou C J, Zhou Z R, *et al*. Domain analysis reveals that a deubiquitinating enzyme USP13 performs non-activating catalysis for Lys63-linked polyubiquitin. PloS One, 2013, **6**(12): e29362

[84] Yeh H M, Yu C Y, Yang H C, *et al*. Ubiquitin-specific protease 13 regulates IFN signaling by stabilizing STAT1. J Immun, 2013, **191**(6): 3328−3336

[85] Zhang J, Zhang P, Wei Y, *et al*. Deubiquitylation and stabilization

of PTEN by USP13. Nat Cell Biol, 2013, **15**(12): 1486-1494

[86] Xu D C, Shan B, Liu M, *et al*. Phosphorylation and activation of ubiquitin-specific protease-14 by Akt regulates the ubiquitin-proteasome system. eLife, 2015, **4**(s1-2): 130-139

[87] Wang Y, Wang J, Zhong J, *et al*. Ubiquitin-specific protease 14 (USP14) regulates cellular proliferation and apoptosis in epithelial ovarian cancer. Med Oncology, 2015, **32**(1): 379-380

[88] Huang G, Li L M, Zhou W P. USP14 activation promotes tumor progression in hepatocellular carcinoma. Oncology Rep, 2015, **34**(6): 2917-2924

[89] Zhu. Y, Zhang. C, Gu. C, *et al*. Function of deubiquitinating enzyme USP14 as oncogene in different types of cancer. Cell Phys Biochem, 2016, **38**(3): 993-1002

[90] Eichhorn P J, Rodon L, Gonzalez-Junca A, *et al*. USP15 stabilizes TGF-beta receptor Ⅰ and promotes oncogenesis through the activation of TGF-beta signaling in glioblastoma. Nat Med, 2012, **18**(3): 429-435

[91] Pauli E K, Chan Y K, Davis M E, *et al*. The Ubiquitin-specific protease USP15 promotes RIG-I-mediated antiviral signaling by deubiquitylating TRIM25. SciSignaling, 2014, **7**(307): 162-171

[92] Long L, Thelen J P, Furgason M, *et al*. The U4/U6 recycling factor SART3 has histone chaperone activity and associates with USP15 to regulate H2B deubiquitination. J Biol Chem, 2014, **289** (13): 8916-8930

[93] Adorno M, Sikandar S, Mitra S S, *et al*. Usp16 contributes to somatic stem-cell defects in Down's syndrome. Nature, 2013, **501**(7467): 380-384

[94] Yang W, Lee Y H, Jones A E, *et al*. The histone H2A deubiquitinase Usp16 regulates embryonic stem cell gene expression and lineage commitment. Nature Commun, 2014, **5**(5): 3818

[95] Borbely G, Haldosen L A, Karin D W, *et al*. Induction of USP17 by combining BET and HDAC inhibitors in breast cancer cells. Oncotarget, 2015, **6**(32): 33623-33635

[96] Ni Y, Tao L, Chen C, *et al*. The deubiquitinase USP17 regulates the stability and nuclear function of IL-33. Int J Mol Sci, 2015, **16**(11): 27956-27966

[97] Guo Y, Dolinko A V, Chinyengetere F, *et al*. Blockade of the ubiquitin protease UBP43 destabilizes transcription factor PML/RARalpha and inhibits the growth of acute promyelocytic leukemia. Cancer Res, 2010, **70**(23): 9875-9885

[98] Kim J H, Luo J K, Zhang D E. The level of hepatitis B virus replication is not affected by protein ISG15 modification but is reduced by inhibition of UBP43 (USP18) expression. J Immun, 2008, **181**(9): 6467-6472

[99] Chen L, Li S, Mcgilvray I. The ISG15/USP18 ubiquitin-like pathway (ISGylation system) in hepatitis C virus infection and resistance to interferon therapy.Int J Biochem Cell Biol, 2011, **43**(10): 1427-1431

[100]Yang Z, Xian H, Hu J, *et al*. USP18 negatively regulates NF-kappaB signaling by targeting TAK1 and NEMO for deubiquitination through distinct mechanisms. Sci Rep, 2015, **5**: 12738

[101]Bedard N, Jammoul S, Moore T, *et al*. Inactivation of the ubiquitin-specific protease 19 deubiquitinating enzyme protects against muscle wasting. FASEB J, 2015, **29**(9): 3889-3898

[102]Jin S, Tian S, Chen Y, *et al*. USP19 modulates autophagy and antiviral immune responses by deubiquitinating Beclin-1. EMBO J, 2016, **35**(8): 866-880

[103]Berthouze M, Venkataramanan V, Li Y, *et al*. The deubiquitinases USP33 and USP20 coordinate beta2 adrenergic receptor recycling and resensitization. EMBO J, 2009, **28**(12): 1684-1696

[104]Yasunaga J, Lin F C, Lu X, *et al*. Ubiquitin-specific peptidase 20 targets TRAF6 and human T cell leukemia virus type 1 tax to negatively regulate NF-kappaB signaling. J Virology, 2011, **85**(13): 6212-6219

[105]Ye Y, Akutsu M, Reyes-Turcu F, *et al*. Polyubiquitin binding and cross-reactivity in the USP domain deubiquitinase USP21. EMBO Rep, 2011, **12**(4): 350-357

[106]Xu G, Tan X, Wang H, *et al*. Ubiquitin-specific peptidase 21 inhibits tumor necrosis factor alpha-induced nuclear factor kappaB activation *via* binding to and deubiquitinating receptor-interacting protein 1. J Biol Chem, 2010, **285**(2): 969-978

[107]Hu J, Yang D, Zhang H, *et al*. USP22 promotes tumor progression and induces epithelial-mesenchymal transition in lung adenocarcinoma. Lung Cancer, 2015, **88**(3): 239-245

[108]Ao N, Liu Y Y, Bian X C, *et al*. Ubiquitin-specific peptidase 22 inhibits colon cancer cell invasion by suppressing the signal transducer and activator of transcription 3/matrix metalloproteinase 9 pathway. MolMed Rep, 2015, **12**(2): 2107-2113

[109]Wu Y R, Chen C M, Chen Y C, *et al*. Ubiquitin specific proteases USP24 and USP40 and ubiquitin thiolesterase UCHL1 polymorphisms have synergic effect on the risk of Parkinson's disease among Taiwanese. Clin Chim Acta, 2010, **411** (13-14): 955-958

[110]Zhang L, Lubin A, Chen H, *et al*. The deubiquitinating protein USP24 interacts with DDB2 and regulates DDB2 stability. Cell Cycle, 2012, **11**(23): 4378-4384

[111]Blount J R, Burr A A, Denuc A, *et al*. Ubiquitin-specific protease 25 functions in Endoplasmic Reticulum-associated degradation. PloS One, 2012, **7**(5): e36542

[112]Zhong H, Wang D, Fang L, *et al*. Ubiquitin-specific proteases 25 negatively regulates virus-induced type Ⅰ interferon signaling. PloS One, 2013, **8**(11): e80976

[113]Typas D, Luijsterburg M S, Wiegant W W, *et al*. The de-ubiquitylating enzymes USP26 and USP37 regulate homologous recombination by counteracting RAP80. Nucl Acids Res, 2015, **43**(14): 6919-6933

[114]Asadpor U, Totonchi M, Sabbaghian M, *et al*. Ubiquitin-specific protease (USP26) gene alterations associated with male infertility and recurrent pregnancy loss (RPL) in Iranian infertile patients. J Assisted Reproduction Genetics, 2013, **30**(7): 923-931

[115]Dirac A M, Bernards R. The deubiquitinating enzyme USP26 is a regulator of androgen receptor signaling. Mol Cancer Res, 2010, **8**(6): 844-854

[116]Diefenbacher M E, Popov N, Blake S M, *et al*. The deubiquitinase

USP28 controls intestinal homeostasis and promotes colorectal cancer. J Clin Investigation, 2014, **124**(8): 3407−3418

[117]Zhang L, Xu B, Qiang Y, *et al*. Overexpression of deubiquitinating enzyme USP28 promoted non-small cell lung cancer growth. J Cell Mol Med, 2015, **19**(4): 799−805

[118]Martin Y, Cabrera E, Amoedo H, *et al*. USP29 controls the stability of checkpoint adaptor Claspin by deubiquitination. Oncogene, 2015, **34**(8): 1058−1063

[119]Cunningham C N, Baughman J M, Phu L, *et al*. USP30 and parkin homeostatically regulate atypical ubiquitin chains on mitochondria. Nat Cell Biol, 2015, **17**(2): 160−169

[120]Thobois S. USP30: a new promising target for Parkinson's disease? Movement Disorders, 2015, **30**(3): 340

[121]Tzimas C, Michailidou G, Arsenakis M, *et al*. Human ubiquitin specific protease 31 is a deubiquitinating enzyme implicated in activation of nuclear factor-kappaB. Cell Signalling, 2006, **18**(1): 83−92

[122]Akhavantabasi S, Akman H B, Sapmaz A, *et al*. USP32 is an active, membrane-bound ubiquitin protease overexpressed in breast cancers. Mammalian Genome, 2010, **21**(7−8): 388−397

[123]Li J, D'angiolella V, Seeley E S, *et al*. USP33 regulates centrosome biogenesis *via* deubiquitination of the centriolar protein CP110. Nature, 2013, **495**(7440): 255−259

[124]Shenoy S K, Modi A S, Shukla A K, *et al*. Beta-arrestin-dependent signaling and trafficking of 7-transmembrane receptors is reciprocally regulated by the deubiquitinase USP33 and the E3 ligase Mdm2. Pro Natl Acad Sci USA, 2009, **106**(16): 6650−6655

[125]Wang Y, Serricchio M, Jauregui M, *et al*. Deubiquitinating enzymes regulate PARK2-mediated mitophagy. Autophagy, 2015, **11**(4): 595−606

[126]Taillebourg E, Gregoire I, Viargues P, *et al*. The deubiquitinating enzyme USP36 controls selective autophagy activation by ubiquitinated proteins. Autophagy, 2012, **8**(5): 767−779

[127]Sun X X, Sears R C, Dai M S. Deubiquitinating c-Myc: USP36 steps up in the nucleolus. Cell Cycle, 2015, **14**(24): 3786−3793

[128]Huang X, Summers M K, Pham V, *et al*. Deubiquitinase USP37 is activated by CDK2 to antagonize APC (CDH1) and promote S phase entry. Mol cell, 2011, **42**(4): 511−523

[129]Yeh C, Coyaud E, Bashkurov M, *et al*. The deubiquitinase USP37 regulates chromosome cohesion and mitotic progression. Curr Biol, 2015, **25**(17): 2290−2299

[130]Van Leuken R J, Luna-Vargas M P, Sixma T K, *et al*. Usp39 is essential for mitotic spindle checkpoint integrity and controls mRNA-levels of aurora B. Cell Cycle, 2008, **7**(17): 2710−2719

[131]Hock A K, Vigneron A M, Carter S, *et al*. Regulation of p53 stability and function by the deubiquitinating enzyme USP42. EMBO J, 2011, **30**(24): 4921−4930

[132]Zagaria A, Anelli L, Coccaro N, *et al*. 5' RUNX1-3' USP42 chimeric gene in acute myeloid leukemia can occur through an insertion mechanism rather than translocation and may be mediated by genomic segmental duplications. Mol Cytogenetics, 2014, **7**(1): 66

[133]Hock A K, Vigneron A M, Vousden K H. Ubiquitin-specific peptidase 42 (USP42) functions to deubiquitylate histones and regulate transcriptional activity. J Biol Chem, 2014, **289** (50): 34862−34870

[134]Stegmeier F, Rape M, Draviam V M, *et al*. Anaphase initiation is regulated by antagonistic ubiquitination and deubiquitination activities. Nature, 2007, **446**(7138): 876−881

[135]Ribeiro F C, Santos L E, Ferreira S T. USP46: a new piece of the memory puzzle?. J Neurochem, 2015, **134**(6): 979−981

[136]Huo Y, Khatri N, Hou Q, *et al*. The deubiquitinating enzyme USP46 regulates AMPA receptor ubiquitination and trafficking. J Neurochem, 2015, **134**(6): 1067−1080

[137]Li X, Stevens P D, Yang H, *et al*. The deubiquitination enzyme USP46 functions as a tumor suppressor by controlling PHLPP-dependent attenuation of Akt signaling in colon cancer. Oncogene, 2013, **32**(4): 471−478

[138]Piao J, Tashiro A, Nishikawa M, *et al*. Expression, purification and enzymatic characterization of a recombinant human ubiquitin-specific protease 47. J Biochem, 2015, **158**(6): 477−484

[139]Peschiaroli A, Skaar J R, Pagano M, *et al*. The ubiquitin-specific protease USP47 is a novel beta-TRCP interactor regulating cell survival. Oncogene, 2010, **29**(9): 1384−1393

[140]Armando I, Villar V A, Jones J E, *et al*. Dopamine D3 receptor inhibits the ubiquitin-specific peptidase 48 to promote NHE3 degradation. FASEB J, 2014, **28**(3): 1422−1434

[141]Schweitzer K, Naumann M. CSN-associated USP48 confers stability to nuclear NF-kappaB/RelA by trimming K48-linked Ub-chains. Biochim Biophys Acta, 2015, **1853**(2): 453−469

[142]Zhang Z, Jones A, Joo H Y, *et al*. USP49 deubiquitinates histone H2B and regulates cotranscriptional pre-mRNA splicing. Genes Develop, 2013, **27**(14): 1581−1595

[143]Aressy B, Jullien D, Cazales M, *et al*. A screen for deubiquitinating enzymes involved in the G(2)/M checkpoint identifies USP50 as a regulator of HSP90-dependent Wee1 stability. Cell Cycle, 2010, **9**(18): 3815−3822

[144]Kobayashi T, Iwamoto Y, Takashima K, *et al*. Deubiquitinating enzymes regulate Hes1 stability and neuronal differentiation. FEBS J, 2015, **282**(13): 2411−2423

[145]Bett J S, Ibrahim A F, Garg A K, *et al*. The P-body component USP52/PAN2 is a novel regulator of HIF1A mRNA stability. Biochem J, 2013, **451**(2): 185−194

[146]Masoumi K C, Masoumi R. CYLD and SUMO in neuroblastoma therapy. Oncoscience, 2016, **3**(1): 3−4

[147]Hrdinka M, Fiil B K, Zucca M, *et al*. CYLD limits Lys63- and Met1-linked ubiquitin at receptor complexes to regulate innate immune signaling. Cell Rep, 2016, **14**(12): 2846−2858

[148]Hutten S, Chachami G, Winter U, *et al*. A role for the Cajal-body-associated SUMO isopeptidase USPL1 in snRNA transcription mediated by RNA polymerase Ⅱ. J Cell Sci, 2014, **127**(5): 1065−1078

[149]Schulz S, Chachami G, Kozaczkiewicz L, *et al*. Ubiquitin-specific protease-like 1 (USPL1) is a SUMO isopeptidase with essential, non-catalytic functions. EMBO Rep, 2012, **13**(10): 930−938

[150]Sanches M, Alves B S, Zanchin N I, *et al*. The crystal structure of

the human Mov34 MPN domain reveals a metal-free dimer. J Mol Biol, 2007, **370**(5): 846-855

[151]Mccullough J, Clague M J, Urbe S. AMSH is an endosome-associated ubiquitin isopeptidase. J Cell Biol, 2004, **166** (4): 487-492

[152]Davies C W, Paul L N, Kim M I, *et al*. Structural and thermodynamic comparison of the catalytic domain of AMSH and AMSH-LP: nearly identical fold but different stability. J Mol Biol, 2011, **413**(2): 416-429

[153]Mcdonell L M, Mirzaa G M, Alcantara D, *et al*. Mutations in STAMBP, encoding a deubiquitinating enzyme, cause microcephaly-capillary malformation syndrome. Nat Genet, 2013, **45**(5): 556-562

[154]Sato Y, Yoshikawa A, Yamagata A, *et al*. Structural basis for specific cleavage of Lys 63-linked polyubiquitin chains. Nature, 2008, **455**(7211): 358-362

[155]Morris J R. Attenuation of the ubiquitin conjugate DNA damage signal by the proteasomal DUB POH1. Cell Cycle, 2012, **11**(22): 4103-4104

[156]Cooper E M, Cutcliffe C, Kristiansen T Z, *et al*. K63-specific deubiquitination by two JAMM/ MPN complexes: BRISC-associated Brcc36 and proteasomal Poh1. EMBO J, 2009, **28**(6): 621-631

[157]Patterson-Fortin J, Shao G, Bretscher H, *et al*. Differential regulation of JAMM domain deubiquitinating enzyme activity within the RAP80 complex. J Biol Chem, 2010, **285**(40): 30971-30981

[158]Cooper E M, Boeke J D, Cohen R E. Specificity of the BRISC deubiquitinating enzyme is not due to selective binding to Lys63-linked polyubiquitin. J Biol Chem, 2010, **285**(14): 10344-10352

[159]Cong S Y, Pepers B A, Zhou T T, *et al*. Huntingtin with an expanded polyglutamine repeat affects the Jab1-p27 (Kip1) pathway. Neurobiol Disease, 2012, **46**(3): 673-681

[160]Liu Y, Shah S V, Xiang X, *et al*. COP9-associated CSN5 regulates exosomal protein deubiquitination and sorting. Am J Pathol, 2009, **174**(4): 1415-1425

[161]Pan Y, Zhang Q, Tian L, *et al*. Jab1/CSN5 negatively regulates p27 and plays a role in the pathogenesis of nasopharyngeal carcinoma. Cancer Res, 2012, **72**(7): 1890-1900

[162]Gao S J, Fang L K, Phan L M, *et al*. COP9 signalosome subunit 6 (CSN6) regulates E6AP/UBE3A in cervical cancer. Oncotarget, 2015, **6**(29): 28026-28041

[163]Fang L, Lu W, Choi H H, *et al*. ERK2-dependent phosphorylation of CSN6 is critical in colorectal cancer development. Cancer Cell, 2015, **28**(2): 183-197

[164]Lothrop A P, Torres M P, Fuchs S M. Deciphering post-translational modification codes. FEBS Lett, 2013, **587** (8): 1247-1257

[165]Mcclurg U L, Robson C N. Deubiquitinating enzymes as oncotargets. Oncotarget, 2015, **6**(12): 9657-9668

[166]Kee Y, Huang T T. Role of deubiquitinating enzymes in DNA repair. Mol Cell Biol, 2016, **36**(4): 524-544

[167]Faggiano S, Alfano C, Pastore A. The missing links to link ubiquitin: Methods for the enzymatic production of polyubiquitin chains. Anal Biochem, 2016, **492**: 82-90

[168]Eger S, Scheffner M, Marx A, *et al*. Synthesis of defined ubiquitin dimers. J Am Chem Soc, 2010, **132**(46): 16337-16339

[169]Dawson P E, Muir T W, Clark-Lewis I, *et al*. Synthesis of proteins by native chemical ligation. Science, 1994, **266**(5186): 776-779

[170]Kent S B H. Total chemical synthesis of proteins. Chem Soc Rev, 2009, **38**(2): 338-351

[171]Fang G M, Wang J X, Liu L, Convergent chemical synthesis of proteins by ligation of peptide hydrazides. Angew Chem Int Ed 2012, **51**(41): 10347-10350

[172]Huang Y C, Fang G M, Liu L, Chemical synthesis of proteins using hydrazide intermediates. Natl Sci Rev, 2016, **3**(1): 107-116

[173]Huang Y, Liu L. Chemical synthesis of crystalline proteins.Sci China Chem 2015, **58**(12): 1779-1781

[174]Chatterjee C, McGinty R K, Pellois J P, *et al*. Auxiliary-mediated site-specific peptide ubiquitylation. Angew Chem Int Ed, 2007, **119**(16): 2814-2818

[175]Weller C E, Huang W, Chatterjee C. Facile synthesis of native and protease-resistant ubiquitylated peptides. ChemBioChem 2014, **15**(9): 1263-1267

[176]Yan L Z, Dawson P E. Synthesis of peptides and proteins without cysteine residues by native chemical ligation combined with desulfurization. J Am Chem Soc, 2001, **123**(4): 526-533

[177]Kumar K S, Spasser L, Erlich L A, *et al*. Total chemical synthesis of di-ubiquitin chains. Angew Chem Int Ed, 2010, **49**(48): 9126-9131

[178]Virdee S, Ye Y, Nguyen D P, *et al*. Engineered diubiquitin synthesis reveals Lys-29 isopeptide specificity of an OUT deubiquitinase. Nat Chem Biol, 2010, **6**(10): 750-757

[179]Castaneda C, Liu J, Chaturvedi A, *et al*. Nonenzymatic assembly of natural polyubiquitin chains of any linkage composition and isotopic labeling scheme. J Am Chem Soc, 2011, **133**(44): 17855-17868

[180]Hemantha H P, Brik A. Non-enzymatic synthesis of ubiquitin chains: where chemistry makes a difference. Bioorg Med Chem, 2013, **21**(12): 3411-3420

[181]Jaime L M, Ivan D. Deciphering functions of branched ubiquitin chains, Cell, 2014, **157**(4): 767-769

[182]Tobias W, Swatek K N, Wagstaff J L, *et al*. Ubiquitin Ser65 phosphorylation affects ubiquitin structure, chain assembly and hydrolysis. EMBO J, 2014, **34**(3): 307-325

Selectivity and Mechanism of Polyubiquitin Chain Hydrolases*

ZHENG Qing-Yun[1,2)**], WANG Tao[1)**], PAN Man[1,2)], LI Yi-Ming[1,2)***], TIAN Chang-Lin[3)***]

([1)] *School of Medical Engineering, Hefei University of Technology, Hefei* 230009, China;

[2)] *Department of Chemistry, Tsinghua University, Beijing* 100084, China;

[3)] *School of Life Science, University of Science and Technology of China, Hefei* 230026, China)

Abstract Ubiquitylation is involved in most part of cellular processes, including protein degradation, autophagy, DNA damage repair, cell cycle, signaling transduction, gene expression, transcription regulation, inflammation and immune response. Instead of the formation of ubiquitylation, deubiquitinating enzymes (DUBs) hydrolyze monoubiquitylation and polyubiquitylation of substrates in response to ubiquitin-mediated pathways. There exits approximately 90 DUBs in human genome which regulate the enzymatic activity and recognition of substrates to control with precision the multi-layer complex cellular ubiquitin network. DUBs play diverse roles in cellular process, their dysfunctions direct to many serious diseases (like cancers, neurodegenerative disorders and infection diseases). Therefore, DUBs represent novel candidates for target-directed drug development. However, many physiologic functions of DUBs are still unknown. Whether they recognize different polyubiquitin chains and how to response signaling transduction accurately are unlear. In this review, we systematically surveyed the selectivity of the DUBs that we have known in hydrolyzing different ubiquitin chains and their mechanisms.

Key words deubiquitinating enzyme, polyubiquitin chain hydrolases, polyubiquitylation, protein ubiquitylation

DOI: 10.16476/j.pibb.2016.0112

* This work was supported by a grant from The National Natural Science Foundation of China(21372058, 21572043).

**These authors contributed equally to this work.

***Corresponding author. Tel: 86-551-65595608

LI Yi-Ming. E-mail: ymli@hfut.edu.cn

TIAN Chang-Lin. E-mail: cltian@ustc.edu.cn

Received: April 7, 2016 Accepted: July 11, 2016