去泛素化酶与细胞周期调控

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摘要 泛素化是蛋白质翻译后的一种重要修饰方式。通过泛素化, 蛋白质被标记上多聚泛素化链, 而被蛋白酶体所识别并导致其降解。细胞周期是细胞增殖的基础, 泛素化介导的蛋白质降解在驱动真核生物细胞周期的有序运行中起着极其重要的作用。近年来的研究表明, 泛素化是一个可逆的过程, 去泛素化酶能够逆转泛素化过程而稳定蛋白质, 并与细胞周期各时相的调控密切相关。因此, 该文就去泛素化酶在细胞周期调控中的最新研究进展进行了综述。

关键词 细胞周期; 去泛素化酶; 泛素化

Deubiquitinase and Cell Cycle Regulation

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Abstract Ubiquitination is an important modification of protein translation. Through ubiquitination, proteins are labeled with polyubiquitinated chains that are recognized by the proteasome and cause degradation. The cell cycle is the basis of cell proliferation, and ubiquitin-mediated protein degradation plays an extremely important role in driving the orderly operation of cell cycle in eukaryotes. In recent years, studies have shown that ubiquitination is a reversible process. Deubiquitinase can reverse the ubiquitination process and stabilize proteins, and are closely related to the regulation of cell cycle phases. Therefore, the recent advances of deubiquitinases in cell cycle regulation are reviewed.

Keywords cell cycle; deubiquitinase; ubiquitination

1 泛素–蛋白酶体途径(ubiquitin-proteasome pathway, UPP)

泛素(ubiquitin)是广泛存在于真核生物中的高度保守的小分子多肽, 共由76个氨基酸组成, 包括7个可直接参与泛素化过程的赖氨酸残基(Lys6、Lys11、Lys27、Lys29、Lys33、Lys48和Lys63)[1]。蛋白质的泛素化过程是在ATP参与下, 由泛素活化酶E1(ubiquitin-activating enzyme E1)通过形成酰基–腺苷酸中介物使泛素分子C-端活化, 活化的泛素分子进而与泛素结合酶E2(ubiquitin-conjugating enzyme E2)的半胱氨酸残基结合, 最后在泛素连接酶E3(ubiquitin-ligase E3)的参与下, 将E2结合的泛素羧基与靶蛋白赖氨酸残基侧链的氨基脱水缩合形成异肽键。通过细胞内的蛋白酶体能够识别并结合多聚泛素化的蛋白, 进而将靶蛋白进行水解并释放出泛素分子[2-4]。泛素–蛋白酶体通路是细胞内蛋白质降解的最主要途径, 介导细胞内多种生理过程的调控, 如细胞周期、细胞凋亡、损伤应激、基因转录调节、免疫应答等。

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2 去泛素化

去泛素化是指泛素化的蛋白质在去泛素化酶的作用下被移除泛素的过程。人类基因组可编码约100种去泛素化酶(deubiquitinase, DUB)[1]。根据序列和结构的相似性, 可将去泛素化酶分为七大家族: 泛素特异性蛋白酶家族(ubiquitin-specific proteases, USPs)、泛素C-末端水解酶家族(ubiquitin C-terminal hydrolases, UCHs)、卵巢癌蛋白酶家族(ovarian tumor proteases, OTUs)、Machado-Joseph疾病蛋白酶家族(Machado-Joseph disease proteases, MJDs)、MINDY蛋白酶家族(MIU-containing novel DUB family)、JAMM家族(Jad1/Pad/MPN domain-containing metallo enzymes)以及最新发现的ZUFSP/Mug105家族(zinc finger with UFM1-specific peptidase domain protein/C6orf113/ZUP1)[5]。在细胞内去泛素化酶的功能可大致分为以下几种[1](图1): (1)加工泛素前体, 产生自由的泛素分子; (2)移除蛋白质上的泛素链, 避免蛋白质被蛋白酶体降解, 从而稳定蛋白质; (3)移除蛋白质上连接的非降解泛素化信号; (4)通过阻止泛素分子与底物蛋白质一起被降解, 确保细胞内泛素分子的稳态; (5)参与细胞内游离泛素链的解体; (6)通过剪切泛素链, 编辑泛素链的类型。

3 细胞周期

细胞增殖是细胞生命活动的重要特征之一。通常将从一次细胞分裂结束开始, 经过物质准备, 直到下一次细胞分裂结束为止, 称为一个细胞周期(cell cycle)。根据细胞周期运行中的特点, 可将这一连续的过程分为4个时相: G_1期、S期、G_2期和M期。参与细胞周期运行和调控的蛋白质主要有周期蛋白(cyclin)、周期蛋白依赖性激酶(cyclin-dependent kinases, CDKs)和周期蛋白依赖性激酶抑制剂(CDK inhibitors, CKIs)。作为细胞周期调控的核心, CDKs与相应的Cyclin成复合物, 通过Cyclin的周期性表达和降解, 进而促进细胞周期的有序运行[6]。作为细胞周期负性调控因子, CKIs主要在细胞周期检验点处与CDK-Cyclin复合物相结合并抑制其活性, 阻滞细胞周期的运行。研究表明, 细胞周期相关蛋白的泛素化降解在细胞周期的有序运行中起着极其重要的作用, 它们不仅组成驱动真核细胞周期运行的核

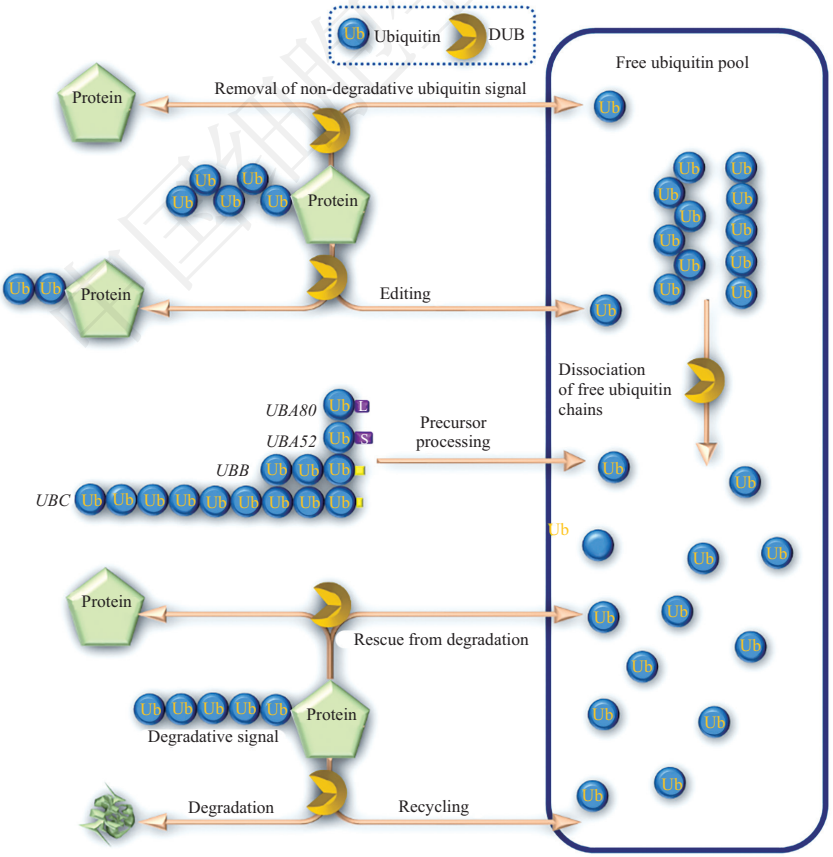

图1 去泛素化酶的功能

Fig.1 General roles of deubiquitinases

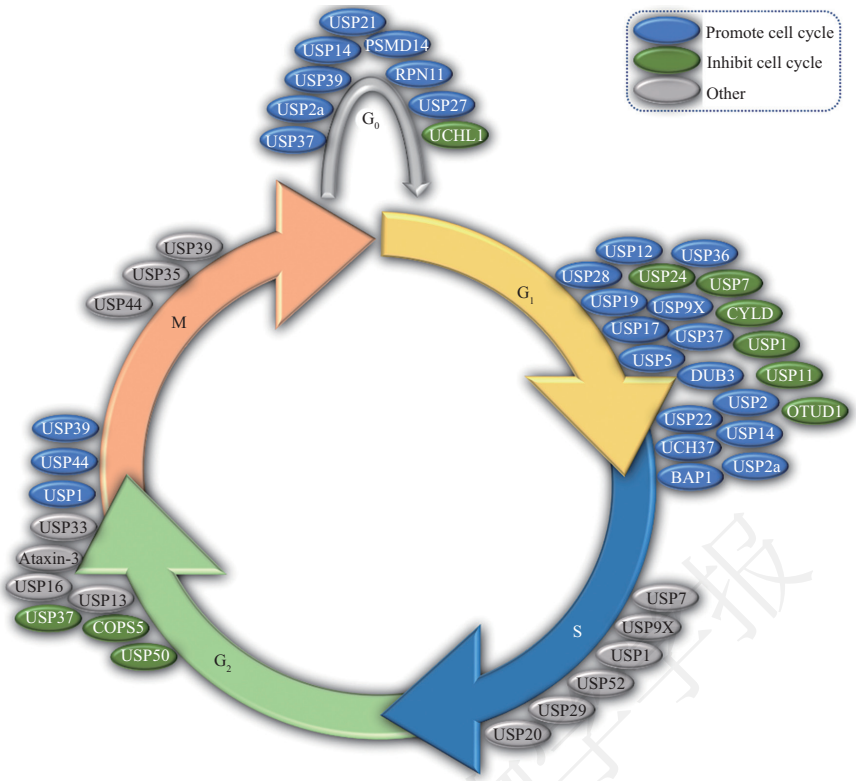

图2 细胞周期调节中的去泛素化酶

Fig.2 Deubiquitinases in cell cycle regulation

心振荡器, 而且也是确保基因组完整性的关键。近年来的研究显示去泛素化酶能够逆转蛋白泛素化过程, 介导细胞内蛋白水平的动态平衡, 并在细胞周期各时相中起着同样重要的作用[7](图2)。

4 去泛素化酶与细胞周期

4.1 G_0/G_1

G_0期是细胞周期外的一个时期, 处于这个时期的细胞会暂时脱离细胞周期, 停止分裂, 但依然能够进行代谢活动, 执行特定的生物学功能。处于G_0期的细胞一旦得到特定信号, 就会快速返回细胞周期, 继续进行分裂增殖。参与G_0/G_1期调控的周期蛋白主要有Cyclin E、Cyclin D1, 这些周期蛋白与相应的CDK形成复合物促进该时期的转化; $p21^{cip}$、$p27^{kip}$是参与该时期转化的负性调控因子。去泛素化酶可通过直接或间接影响这些蛋白质进而调控G_0/G_1期转化。从目前研究来看, 有较多的去泛素化酶可调控G_0/G_1期转化, USP27可通过去泛素化稳定Cyclin E促进G_0/G_1期转化[8]; MEK2作为MAPK信号通路中的关键成员在细胞增殖和细胞周期运转中发挥着重要的作用, USP21能够去泛素化稳定MEK2, 进而促进G_0/G_1期转化[9]。敲低USP39可上调$p21^{cip1}$、$p27^{kip1}$和Bax并抑制胞外信号调节激酶1/2(ERK1/2)的磷酸化从而抑制G_0/G_1期转化[10]; 与敲低USP39类似, 敲低PSMD14也可上调$p21^{cip1}$、$p27^{kip1}$, 同时下调Cyclin B1-CDK1-CDC25C和Cyclin D1进而促进细胞衰老并抑制G_0/G_1期转化[11]; 抑制USP2a的酶活性也可导致Cyclin D1下调并抑制G_0/G_1期转化[12]。此外, 一些去泛素化酶参与了G_0/G_1的调节过程, 如过表达去泛素化酶UCHL1可将细胞周期阻滞在G_0/G_1期[13], 同样敲低去泛素化酶UCH37[14]、RPN11[15]和USP14[16]也能将细胞周期阻滞在G_0/G_1期。

4.2 G_1/S期

G_1期是进行蛋白质与RNA合成的时期, 该时期主要为DNA的复制提供物质准备。在细胞发生G_1/S期转化时, CDK4/6与Cyclin D形成复合物促进Rb的磷酸化, 进而激活转录因子E2F1, 促进G_1/S转化的相关基因表达。G_1/S检验点是细胞增殖的关键节点, 许多去泛素化酶通过调控CKI、转录因子、周期蛋白以及一些凋亡相关因子等来参与这一过程。

去泛素化酶对G_1/S期的调控一般可通过这几种途径: (1)直接或间接调控CKI, 如USP11对$p21^{Cip1}$的

去泛素化稳定作用[17]以及USP19可以通过稳定$p27^{kip1}$的E3泛素连接酶KPC1, 从而引起$p27^{kip1}$的降解[18]; (2)通过去泛素化作用调控一些转录因子, 如OTUD1等去泛素化酶对p53的稳定作用[19]、USP28[20]和USP37[21]对c-Myc的稳定作用以及UCH37对E2F1的激活作用[22], 此外USP24也能够下调转录因子E2F4促进G_1/S期转换, 但具体作用机制目前还不清楚[23]; (3)去泛素化稳定周期相关蛋白, 如USP37对Cyclin A的调控[24]、USP2和USP2a对Cyclin D1的调控[12,25]以及DUB3对CDC25A的调控[26]; (4)对其他蛋白质的调控, 如USP22和USP9X对β-catenin的调控[27-28]、USP5对FoxM1的调控[29]和BAP1对HCF-1的调控[30]均可促进细胞周期的运行; 而USP17对Ras的调控[31]、USP7对Rb和Chfr的调控[32-33]以及泛素C-末端水解酶CYLD对Bcl-3入核的抑制作用[34]则能抑制细胞周期的运行。USP36对DHX33的去泛素化稳定作用能够维持细胞周期的正常运转, 而细胞中缺失USP36可通过p53依赖性方式将细胞周期阻滞在G_0/G_1期[35]。同时, 也有研究指出, 缺失USP12[36]、USP14[16]均能使细胞周期阻滞在G_1期, 而过表达DUB-1[37]可将细胞周期阻滞在G_1期, 但它们的具体作用机制目前还不清楚。

4.3 S期

S期是分裂细胞进行遗传物质复制的时期。在此期间为保证DNA的精准复制, 细胞会启动多种复杂的DNA修复机制。进入S期后, Cyclin E降解, CDK2从Cyclin E-CDK2复合物中脱离下来并与Cyclin A结合, 进而使细胞周期向G_2期运行。在S期后期, S期检验点检查遗传物质的复制是否完全, 为进入下一个细胞周期时相——G_2期做准备。有多种去泛素化酶参与调控DNA的复制和损伤修复过程。

Claspin作为S期检验点关键的蛋白质, 主要参与DNA复制以及DNA损伤修复调节过程[38-40]。研究发现, 有较多的去泛素化酶, 如USP9X[41]、USP7[42]、USP29[43]和USP20[44-46], 能够去泛素化稳定该蛋白; 此外, 去泛素化酶也可通过其他途径参与DNA损伤修复以及S期的调控, 如USP1可通过FA(fanconi anemia)途径和TLS(translesion synthesis)途径参与DNA损伤修复[47-48]以及USP52能在S期稳定组蛋白伴侣ASF1A, 而缺失USP52阻碍染色体组装和DNA复制, 进而可将细胞阻滞在S期[49]。

4.4 G_2/M期

细胞周期由G_2期向M期过渡时, 存在G_2/M期DNA损伤检验点。该检验点的作用是阻止带有DNA损伤的细胞进入分裂期并阻止染色体的异常分离。在DNA损伤时, 细胞内会发生一系列的变化, 启动损伤修复机制并阻止细胞进入M期。共济失调毛细血管扩张症突变基因(ataxia telangiectasa mutated, *ATM*)和ATM及Rad3同源基因(ATM and Rad3 related, *ATR*)是参与DNA损伤的主要信号因子[50], 这两者所引发的信号通路相互作用形成复杂的信号网络, 对G_2/M期转换至关重要[51]。有多种周期蛋白和蛋白激酶参与了这一过程的调控, 如检验点蛋白激酶1(checkpoint kinase1, Chk1)、细胞周期蛋白B1(cyclin B1)等。

去泛素化酶对G_2/M期DNA损伤检验点的调控有很多经典的例子。如泛素化酶COPS5对PEA15的稳定作用可阻滞细胞的G_2/M期转换[52], PEA15的缺失可导致c-JUN转录因子激活CDK6的表达促使带有DNA损伤的细胞顺利通过G_2/M检验点[53]。USP50对Weel蛋白的稳定作用使得细胞在DNA损伤检验点激活之前就被滞留在G_2期[54]。ATR-Chk1信号通路是DNA损伤修复的经典通路之一, DNA损伤时, Chk1会激活G_2/M检验点, 将细胞周期阻滞在G_2/M期并促进损伤的DNA修复[55]。去泛素化酶Ataxin-3对Chk1稳定作用可参与调控这一过程[56]。USP13也可参与到G_2/M期的调控。USP13通过去泛素化稳定RAP80, 参与到BRCA1对DNA损伤修复过程的调节, 可增强卵巢癌细胞对放化疗的耐受性[57-58]。去泛素化酶对G_2/M的调控作用也可通过CKI实现。如敲低USP39能够通过$p21^{Cip1}$依赖性的途径阻滞G_2/M的转换, 抑制细胞增殖[59]。USP37可以与REST结合从而逆转$p27^{kip1}$的泛素化降解过程, 从而达到稳定$p27^{kip1}$的作用[60-61]。

癌基因诱导的细胞衰老(oncogene-induced senescence, OIS)能有效地阻止癌前细胞发生转化。目前已知OIS与慢性DNA损伤信号通路以及组蛋白翻译后修饰有关, 因此一些去泛素化酶也可通过调控OIS相关蛋白来调控G_2/M期转换。如OIS诱导去泛素化酶USP1水平的下调, 进而引发了一系列的细胞内响应, 如DNA损伤等, 最终导致多倍体细胞增多以及G_2/M转换迟滞[62]。组蛋白的泛素化修饰对于染色体结构和功能具有重要的调节作用。H2A的泛素化对细胞周期运行非常关键, USP16对其去泛素化从而参与G_2/M的转换[63-64]。

研究表明, USP44通过阻滞非整倍体的形成来参与纺锤体检验点的构成[65], USP44还可以稳定肿瘤促进蛋白securin来促进胶质瘤细胞的增殖并提高其恶性程度[66]。在胶质瘤细胞中敲低USP44可抑制细胞的增殖以及癌细胞的迁移和侵袭, 进而将细胞周期阻滞在G_2/M期[67]。CP110表达水平对细胞维持中心体正常复制非常重要。USP33特异性的结合并去泛素化稳定CP110, 从而对细胞周期的G_2/M期产生影响[68]。

4.5 分裂期

分裂期是细胞周期进程中时间较短的一个阶段, 很多重要的细胞事件都在这相对短暂的时间内发生[69]。分裂期过程中细胞的表现为核膜的解离、染色单体的缩合、微管的附着以及胞质分裂等。为保证这些细胞事件准确无误地持续进行, 细胞具有分裂中期检验点又称为纺锤体检验点, 其主要作用是检查微管中所有配对姐妹染色单体在其着丝粒上的连接是否正确[70]。

极光激酶B(aurora B)可参与各种细胞分裂事件, 如纺锤体组装、动粒附着等。敲低 USP39能够引起aurora B的mRNA和蛋白水平的下降, 但USP39对细胞分裂的影响并不依赖其去泛素化酶活性[71]。而USP35能够去泛素化稳定aurora B, 确保染色体的正常分离[72]。另外, USP44能够通过去泛素化作用稳定CDC20, 进而增加有丝分裂后期促进复合物APC/C的活性, 促进细胞周期由中期到后期的转化和胞质分裂的过程[65]。

5 结语与展望

细胞周期进程涉及多层次多方面的调控, 去泛素化酶作为参与蛋白质翻译后修饰的一类重要的水解酶, 在细胞周期调控过程中发挥了重要的作用(表1)。一方面去泛素化酶通过调控细胞周期正向调控蛋

表1 去泛素化酶与细胞周期调的关系

Table 1 The relationship between deubiquitinases and cell cycle regulation

细胞周期 Cell cycle	去泛素化酶 DUB	分子机制 Molecular mechanism	生物学效应 Biological effect	参考文献 Reference
G_1/S	USP17	USP17-depleted cells have elevated levels of $p21^{cip1}$ and $p27^{kip1}$	USP17 silencing impairs G_1/S transition	[31]
	USP19	Stabilizing KPC1, a Ubiquitin Ligase for $p27^{Kip1}$	Supporting cell proliferation	[18]
	USP5	Loss of USP5 leads to accumulation of $p27^{kip1}$ and down regulation of Cyclin D1	USP5 silencing leads to cell cycle block in G_1/S phase	[29]
	USP11	Deubiquitination and stabilization of $p21^{cip1}$	Loss of USP11 induces the G_1/S transition	[17]
	USP28	Deubiquitination and stabilization of c-Myc	USP28 loss leads to cell cycle arrest in G_0/G_1	[20]
	OTUD1	Deubiquitination and stabilization of p53	Inhibits cell proliferation	[19]
	USP37	Deubiquitination and stabilization of Cyclin A	Accelerating S phase entry	[21]
	USP2	Deubiquitination and stabilization of Cyclin D	Accelerating G_1/S transition	[25]
	USP2a	Deubiquitination and stabilization of Cyclin D	Accelerating G_1/S transition	[12]
	CYLD	Remove of a lysine 63-linked ubiquitin chain from Bcl-3 and inhibits activity of Bcl-3	Delay in the G_1/S transition	[34]
	DUB-3	Deubiquitination and stabilization of CDC25A	Promotes oncogenic transformation BAP1	[26]
	BAP1	Deubiquitination and tabilization of HCF-1, a cell-cycle regulator	depletion inhibits cell proliferation	[30]
	UCH37	Knockdown Uch37 can stabilized $p27^{Kip1}$; UCH37 remove of a lysine 63-linked ubiquitin chain from E2F1; Activation of E2F1	Uch37 loss leads to cell cycle arrest in G_0/G_1; Accelerating G_1/S transition	[14]
	USP24	Decreasing in the USP24 level educes the expression of E2F4 and its partner TFDP1	Increases the G_1/S transition	[23]
	USP7	Deubiquitination and tabilization of Rb and Chfr, which is further upregulated of $p21^{Cip1}$	Cell cycle arrest at G_1 phase	[32]
	USP9X	Deubiquitination and stabilization of β-catenin	USP9X depletion inhibits G_1/S transition	[28]
	USP36	Deubiquitination and stabilization of DHX33	Depletion of USP36 cause cell cycle arrest at G_1 phase	[35]

(续表 1)

细胞周期 Cell cycle	去泛素化酶 DUB	分子机制 Molecular mechanism	生物学效应 Biological effect	参考文献 Reference
	USP14	Over-expression of USP14 in lung adenocarcinoma increase the accumulation of β-catenin, a key member in the Wnt pathway that promotes proliferation in different types of tumors	Promotes proliferation	[16]
	USP12	USP12 enhance the transcript level of BMI-1, c-Myc and Cyclin D2	Overexpression of USP12 promoted HeLa cell growth	[36]
	USP22	Upregulation FoxM1 expression via promoting β-catenin nuclear localization	Promotes the G_1/S phase transition	[27]
	DUB-1	Not yet been identified	Growth suppression	[37]
	USP9X	USP9X regulated the expression and stability of Claspin in an S-phase–specific manner	USP9X depletion results in the accumulation of DNA damage	[41]
	USP29	Deubiquitination and stabilization of Claspin, which is a key protein in the DDR and its levels are controlled after DNA damage and during the cell cycle	USP29 depleted cells show a major defect in the S-phase progression	[43]
S	USP20	Silencing the expression of USP20 in GC cells could reduce Claspin protein	Inhibition of proliferation, G_1/S transition delay	[44]
	USP1	Deubiquitinates ub-FANCD2、ub-FANCI、FANCD2 and FANCI	Reverting the critical event in the activation of the FA pathway	[48]
	USP52	Deubiquitination and stabilization of ASF1A, a protein can regulates chromatin structure organization and stabilization of ASF1A	USP52 deficiency impaired chromatin assembly and DNA replication as well as S-phase progression	[49]
	USP7	Deubiquitination and stabilization of Claspin	Maintenance of genomic integrity	[42]
	USP37	USP37 links REST to the control of $p27^{Kip1}$ stability and cell proliferation in medulloblastoma cells	Blocked cell proliferation	[60]
	COPS5	Deubiquitination and stabilization of PEA15, the loss of PEA15 accelerates cell cycle progression by activating CDK6 expression via the c-JUN transcription factor	Negatively regulates cell cycle progression	[52]
	USP50	USP50 depletion causes a loss in accumulation of the HSp90 client Wee1, which is an essential component of the G_2/M cell cycle arrest	As a negative regulator of the G_2/M checkpoint pathway	[54]
	Ataxin-3	Deubiquitination and stabilization of Chk1, Chk1 activates G_2/M checkpoint to delay cell cycle progression and to facilitate DNA repair	ATX3 deficiency impairs Chk1-mediated G_2/M DNA damage checkpoint	[56]
	USP13	Deubiquitination and stabilization of RAP80	Knockout USP13 compromised the DNA damage-induced G_2/M checkpoint	[57]
	USP16	Deubiquitination and activation of H2A and Plk1	Knockdown of USP16 results in a decrease in the proportion of cells at G_2/M	[63]
G_2/M	USP39	The protein expression of Cyclin A2 and $p21^{cip1}$ significantly increased in the USP39 silencing U2OS cells	Knockdown of USP39 expression decreased cell proliferation	[59]
	USP1	Limiting the production of dysfunctional aggregation of chromatin-bound mono-ubiquitinated FD2 protein, polyploidy, replication stress, and chronic DNA damage signaling	USP1 depletion causes G_2/M cell-cycle block and an increase in ploidy	[62]
	USP33	Deubiquitination and activation of CP110, a centriolar protein that positively regulates centriole duplication while restricting centriole elongation and ciliogenesis	Inhibits centrosome amplification and mitotic defects	[68]
	USP44	Deubiquitination and activation of tumor-promoter securin securing	Knockdown of USP44 inhibited proliferation	[67]
	USP39	Depletion of USP39 reduce the mRNA level of aurora B	Maintain the spindle checkpoint and support successful cytokinesis	[71]
M	USP35	Deubiquitination and activation of aurora B	Allowing proper chromosome segregation and ensuring faithful mitotic progression	[72]
	USP44	Deubiquitination and activation of CDC20	Prevents premature activation of the APC	[65]

白, 如Cyclin A、Cyclin E、c-Myc和E2F1等, 促进细胞周期的运行; 另一方面去泛素化酶通过调控细胞周期负向调控因子, 如$p21^{cip1}$、$p27^{kip1}$等, 抑制细胞周期的运行。不仅如此, 去泛素化酶对细胞周期的调控也是多层次多方面的。同一去泛素化酶可调控多个细胞周期过程, 如USP14、UCH37可在G_0/G_1期以及G_1/S期细胞周期调控。去泛素化酶在不同的周期调控中发挥不同的生物学功能, 如USP44在G_2/M期以及USP39在G_0/G_1和G_2/M期均能够促进细胞周期运行, 而在M期则通过调控染色体的分离过程影响细胞周期进程; USP9X和USP1在S期主要参与DNA损伤修复过程影响细胞周期在S期的运行, 而USP9X在G_1/S期以及USP1在G_2/M期则是通过其他途径促进细胞周期运行。

细胞中的去泛素化酶通过多种途径调控细胞周期的运行, 一旦细胞中的去泛素化酶失调将会导致细胞周期运行紊乱并产生多种疾病, 如肿瘤等。随着研究的日益深入, 去泛素化酶对细胞周期的调控可作为癌症等相关疾病的治疗靶点。目前已经研究报道了多种抑制剂能够影响去泛素化酶活性, 如b-AP15能够通过抑制蛋白酶体19S RP进而能够影响UCHL5和USP14的去泛素化酶活性从而发挥其抗癌作用[73]。我们可以通过去泛素化酶与细胞周期调控间的关系, 结合肿瘤的发生机制, 以期挖掘出更多针对肿瘤治疗的药物靶点。

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