泛素连接酶复合物骨架蛋白CUL4B功能研究进展

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摘要 CUL4B属于Cullin家族, 该家族成员作为骨架蛋白参与构成真核生物中最大的一类泛素连接酶复合物——Cullin-RING E3泛素连接酶复合物(Cullin-RING E3 ligases, CRLs). 由CUL4B作为骨架蛋白构成的CRLs称为Cullin 4B-RING泛素连接酶复合物, 简称CRL4B复合物, 通过催化底物蛋白多泛素化或单泛素化而在多种组织、病理生理过程中发挥重要作用. 本实验室和英国研究组几乎同时发现CUL4B突变导致人类智力低下综合征, 从此开启了研究CUL4B功能的新方向. 利用细胞模型研究发现, CRL4B复合物在调控细胞增殖和DNA损伤修复等生物学过程中发挥重要作用. 同时发现, CUL4B在多种实体瘤组织中高表达, 通过表观转录调控多种抑癌基因表达来调控肿瘤进程. 利用小鼠(*Mus musculus*)模型研究发现CUL4B在胚胎发育、神经系统发育、生殖细胞发育、髓系细胞分化、脂肪细胞分化、胰岛delta细胞发育等多种组织发育和细胞分化过程中发挥重要作用. 本文主要介绍了以上CUL4B功能的研究进展.

关键词 CUL4B, 智力低下, 肿瘤, 细胞增殖, 胚胎发育

CUL4B属于Cullin家族, 该家族成员作为骨架蛋白参与构成真核生物中最大的一类泛素连接酶复合物Cullin-RING E3 ligases(CRLs). 人类Cullin家族有8个成员, 分别为CUL1, CUL2, CUL3, CUL4A, CUL4B, CUL5, CUL7以及CUL9^[1], 其中CUL4A和CUL4B同源性最高, 蛋白质同源性高达83%, 在低等生物中只有一个CUL4. 因此, 在发现CUL4B突变导致人类疾病之前, 研究者通常将CUL4A和CUL4B作为一体进行研究. 由CUL4A和CUL4B作为骨架蛋白构成的CRLs分别称为Cullin 4A-RING泛素连接酶复合物(CRL4A)和Cullin 4B-RING泛素连接酶复合物(CRL4B), 两者以类似的方式

构成CRL复合物, 即其C末端与RING蛋白Rbx/ROC结合, N末端则与连接蛋白DDB1(DNA damage-binding protein 1)结合, 连接蛋白DDB1进一步与底物受体蛋白DCAF(DDB1-CUL4 associated factors)结合, 通过DCAF特异性地结合底物蛋白^[2](图1). 2007年, 我们和英国研究小组几乎同时发现CUL4B突变导致人类智力低下综合征, 随后的研究证实CUL4B突变是X连锁智力低下综合征的最常见的三个病因之一, 从此开启了研究CUL4B功能的新方向. 研究发现由CUL4B参与构成的CRL4B复合物通过催化底物蛋白多泛素化或单泛素化而在多种组织和病理生理过程中发挥重

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图1 CUL4B泛素连接酶复合物组织方式示意图

要作用, 本文对CUL4B的功能研究进展进行了综述。

1 CUL4B基因丧失功能突变导致智力低下综合征

CUL4B最初引起研究者的关注是由于发现CUL4B基因突变导致X连锁智力低下综合征。智力低下是一组以智力、情感和社会适应能力障碍为主要特征的精神障碍性疾病, 在人群中的发病率高达1%~3%^[3]。X连锁基因缺陷在智力低下发生中发挥重要作用, 这类智力低下被称为X连锁智力低下(X-linked mental retardation, XLMR), 占有智力低下病例的10%^[4,5]。X连锁智力低下又根据临床表现分为综合征型X连锁智力低下(syndromic X-linked mental retardation, MRXS)和非综合征型X连锁智力低下(non-syndromic or non-specific X-linked mental retardation, MRX), 前者除智力低下外还伴有其他临床症状和生化指标的异常, 而后者智力低下是其唯一共有表型^[6]。

2007年英国一研究组和本实验室同时发现CUL4B基因突变导致X连锁智力低下综合征。英国研究组在对250个X连锁智力低下综合征家系患者进行X连锁基因突变筛查时发现, 8个家系中存在CUL4B基因

的突变, 其中包括3个无义突变、2个剪接位点突变和3个错义突变。8个家系患者的共同特征为智力低下、语言发育迟缓、运动发育迟缓, 此外多数患者有行为异常、身材矮小、肥胖等症状^[7]。

本实验室在山东省邹城市发现了一个X连锁智力低下综合征大家系^[8], 患者除智力低下外, 还伴有身材矮小、巨舌、失语、短指、双足内翻以及外周血中单核细胞增多等症状。利用连锁分析方法将该家系致病基因定位于Xq25上10.18 Mb的区域^[9,10], 随后采取候选基因分析策略对位于该区域内的候选基因进行突变分析, 发现CUL4B基因存在c.1162C→T的突变, 该突变致使第388位精氨酸密码子变为终止密码子, 并导致无义突变介导的mRNA降解途径, 使含有突变的CUL4B mRNA降解, 因此该突变为丧失功能突变。此外还发现女性携带者外周血白细胞表现为X染色体失活完全偏倚^[11]。

随后CUL4B基因突变在更多的X连锁智力低下综合征家系患者中得到证实。2010年在对波兰的同一家系的3位患有X连锁智力低下综合征的兄弟进行基因测序时发现, 3人均在CUL4B基因第18位外显子上发生c.2107A→T的突变, 该突变导致第703位赖氨酸密码子变成终止密码子, 患者表现为智力低下、语言障碍、多动、意向性震颤、腹股沟疝、小脚、颅面部异常等异常^[12]。

2010年在对一名确诊为综合征型智力低下的男孩进行研究时发现, 患者除了智力低下外还有身材矮小、较少的颅面部发育异常、青春期延迟、性腺功能减退、相对大头畸形、步态共济失调、高足弓等临床特征, 经证实该患者是由CUL4B基因中60 kb的片段缺失引起^[13]。

2012年在对患有智力低下综合征的一对同卵双胞胎兄弟进行研究时发现, 患者的CUL4B基因中有28 kb的缺失, 患者均表现为智力低下、身材矮小、语言发育迟缓、运动发育迟缓、步态共济失调、震颤、癫痫、肥胖症、高足弓、下唇凸出、相对大头畸形等临床症状^[14]。

2014年在对一个综合征型智力低下的大家系分析时发现8位患者患有严重的智力低下、身材矮小、失语、小头畸形、颅面部发育异常、骨骼发育异常等临床症状。患者存在CUL4B基因第7内含子中剪接位点突变c.974+1G>T, 该突变会导致第7外显子的移除^[15]。

2015年发现了11个*CUL4B*基因突变的家系, 共25名患者, 患者主要表现为智力低下、语言发育迟缓、身材矮小、行为异常、震颤、癫痫、步态异常、颅面部发育异常、性腺功能减退、男性乳房发育、短指、高足弓等临床症状。此外, 影像学检查发现部分患者存在脑白质缩小、皮质畸形、巨脑室等异常表现^[16]。

*CUL4B*基因成为继*FMRI*, *ATRX*基因之后最常见的X连锁智力低下综合征致病基因之一^[17]。对智力低下患者进行针对包括*CUL4B*在内的智力低下致病基因的靶向二代测序成为分子诊断的有效策略^[18]。在对50个智力低下家系和100名散发智力低下患者的靶向二代测序分析(针对107个X连锁智力低下基因)时发现, 有2个家系和一名散发患者是由*CUL4B*基因突变导致的^[19]。在对986名智力低下患者进行的靶向二代测序分析(针对565个已知或候选的智力低下相关基因)时发现, 有5名患者存在*CUL4B*基因突变^[20]。

对已报道的35个*CUL4B*基因突变导致的X连锁智力低下综合征家系的分析发现, 突变类型有33种, 包括缺失、无义突变、错义突变和剪接位点突变等(表1)。*CUL4B*基因突变所产生的一致临床表现为智力低下, 此外大部分患者还表现出发育迟缓、身材矮小、癫痫等症状(表2), 提示*CUL4B*基因在多种组织的发育中都发挥着重要的作用。但在最近的一篇报道中, 携带*CUL4B*基因突变(p.Leu329Gln)的患者具有较轻的表型, 并且没有智力低下的症状^[21]。

2 利用细胞模型研究CUL4B功能

细胞模型是人们认识基因功能的必经途径, 利用细胞模型研究发现*CUL4B*在调控细胞增殖、DNA损伤修复和细胞分化过程中发挥重要作用。

2.1 CRL4B复合物在调控细胞增殖中的作用

Cyclin E具有调节细胞周期的功能^[23]。在人H1299细胞中, *CUL4B*缺失导致Cyclin E累积, *CUL4B*复合物可以多泛素化Cyclin E^[24]。干扰*CUL4B*表达抑制人宫颈癌细胞(HeLa细胞)的增殖、细胞S期延长, 且Cyclin E发生累积^[25]。由于*CUL4B*缺失导致的S期延长的细胞中, γ -H2AX阳性微核的细胞比例显著升高^[26]。此外, *CUL4B*对细胞增殖的调控依赖于该分子的核定位, 而*CUL4B*的入核是通过其N末端的核定位信号

(³⁷KKRK⁴⁰)介导的^[25]。

*CRL4B*复合物可以转录抑制miR-372和miR-373, 并通过抑制miR-372和miR-373的表达而正向调控CDK2。CDK2可以磷酸化CDC6, 使其免受APC-CDH1介导的蛋白酶体降解。CDC6促进MCM2与染色质的结合, 并最终调控DNA的复制起始^[27]。

在小鼠(*Mus musculus*)成纤维细胞中敲除*CUL4B*会导致CDK抑制因子p21和p57表达上调, 进而导致细胞增殖明显减缓和G1期细胞周期停滞^[28]。此外, 在Wnt信号诱导下, *CUL4A*和*CUL4B*导致p27^{KIP1}降解和S期延长^[29]。

通过全基因组CRISPR-Cas9扫描的方法发现并证实, Cdc25A是*CRL4B*^{DCAF8}泛素连接酶复合物的底物。在HEK293T细胞中, Cdc25A可以与DCAF8, DDB1或*CUL4B*发生相互作用。过表达DCAF8, DDB1或*CUL4B*都可以增加Cdc25A的泛素化水平, 而敲低DCAF8, DDB1或*CUL4B*都可以使Cdc25A的半衰期延长^[30]。

2.2 CRL4B复合物在调控DNA损伤修复中的作用

*CUL4B*在紫外线损伤的DNA位点与DDB2共定位。在功能性DDB2存在时, *CUL4B*作为*CRL4B*^{DDB2} E3连接酶的一部分结合到紫外线损伤的染色质上。*CRL4B*^{DDB2}可以在体外单泛素化组蛋白H2A, 并且比DDB1-*CUL4A*^{DDB2}更有效。表明*CRL4B*^{DDB2} E3连接酶可能在改变紫外线损伤部位的染色质结构方面具有独特的功能, 从而促进有效的核苷酸切除修复^[31]。紫外线损伤后, *CRL4B*^{DDB2} E3连接酶特异性结合到含有环丁烷嘧啶二聚体或6-4光产物光合作用的核小体中, 并对组蛋白H2A Lys-119/Lys-120进行单泛素化, 并使核小体去稳定化, 以便进行核苷酸切除修复^[32]。

携带有*CUL4B*基因突变的X连锁智力低下综合征患者的淋巴母细胞对喜树碱(CPT)的敏感性增加, 喜树碱诱导的拓扑异构酶 I (Topo I) 降解延缓、泛素化水平降低, 从而证明TOPO I 是*CRL4B*复合物的底物。*CUL4B*突变的淋巴母细胞中喜树碱诱导的DNA断裂水平升高, 此外还发现这些细胞中Cdt1, p21和p27表达升高^[33]。

在DNA损伤和进入S期时, *CRL4*^{Cdt2}复合物可以降解DNA聚合酶 δ 的亚基p12, 使DNA聚合酶 δ 由异源四聚体变为三聚体, 并改变酶特性参与到DNA损伤

表 1 *CUL4B* 基因突变位点汇总

文献	家系数量	患者人数	突变位点	产物改变	突变类型
Zou ^[11]	1	7	c.1162C>T	p.R388X	无义突变
		7	c.1007_1011Del 5		缺失
		3	c.1162C>T	p.R388X	无义突变
		8	c.2566C>T	p.R856X	无义突变
Tarpey ^[7]	8	3	c.901-2A>G	SS exon 7	剪接突变
		3	c.2493G>A		产生新剪接位点
		6	c.1714C>T	p.R572C	错义突变
		4	c.2234T>C	p.V745A	错义突变
		5	c.638C>T	p.T213I	错义突变
Badura-Stronka ^[12]	1	3	c.2107A>T	p.K703X	无义突变
Isidor ^[13]	1	1	60 kb deletion	Deletion of 3' portion of <i>CUL4B</i> gene	缺失
Ravn ^[14]	1	2	chrX:119,657,376-119,685,013 c.727-?_2742+?del	del exon 4-22	缺失
Londin ^[15]	1	8	c.974+1G>T	SS exon 7	剪接突变
		3	c.2590_2593 del	p.Leu864Ala fs*13	缺失
		3	c.2353_2355 del	p.Leu785 del	缺失
		2	c.1860_1862 dup	p.Ala621 dup	重复
		2	c.1007_1011 del	p.Ile336Lys fs*2	缺失
		3	c.2493+3 A>G	SS exon 20	剪接突变
Silfhout ^[16]	11	3	c.2361dup	p.Gly788Trp fs*4	重复
		2	c.1906+1 G>T	canonical SS exon15	剪接突变
		2	c.481_482 del	p.Ser161Tyr fs*24	缺失
		2	del 119,578,701-119,584,448	Deletion upstream of exon 2	缺失
		2	c.149C>T	p.Pro50Leu	错义突变
		1	c.2626C>T	p.Gln876*	无义突变
Redin ^[18]	1	1	c.811_812del	p.Gln271Aspfs*11	缺失
		2	c.2469C>G	p.Ile823Met	错义突变
Tzschach ^[19]	3	2	c.2060G>A	p.Trp687*	无义突变
		1	c.429_431dup	p.Ser146dup	重复
		1	c.803dup	p.Leu268Phefs*5	重复
		1	c.2161-2A>G		剪接突变
Grozeva ^[20]	5	1	c.1396C>T	p.Arg466*	无义突变
		1	c.434T>A	p.Leu145*	无义突变
		1	c.1428G>A	p.Trp476*	无义突变
Weissbach ^[21]	1	1	c.986T>A	p.Leu329Gln	错义突变
Okamoto ^[22]	1	1	c.2698G>T	p.Glu900*	无义突变

修复中^[34]。

p53通常在健康细胞内维持低水平, 肿瘤发生时表达异常^[35]。在HEK293细胞系中CRL4B复合物可以与p53相互作用, 并促进p53的多泛素化^[36]。在压力诱导的正常人类成纤维细胞(normal human fibroblasts, NHFB)中, CRL4B复合物可以多泛素化p53并将其进行蛋白降解, 并负向调节p53-ROS正反馈环, 从而抑制

压力诱导的细胞衰老^[37]。

HUWE1在调节DNA损伤修复和细胞凋亡过程中发挥非常重要的作用。HeLa细胞在受到DNA损伤后CRL4B复合物以NEDD8依赖的方式被激活, 同时HUWE1表达降低, 并证实CRL4B复合物与HUWE1相互作用, 促进HUWE1多泛素化和蛋白降解。在干扰*CUL4B*的HeLa, MCF-7和U2OS细胞系中均发现

表 2 智力低下患者临床症状汇总^{a)}

文献	Zou ^[11]	Tarpey ^[7]	Ravn ^[14]	Isidor ^[13]	Badura-Stronka ^[12]	Silfhout ^[16]	Londin ^[15]	Tzschach ^[19]	Okamoto ^[22]	Weissbach ^[21]
家系数量	1	8	1	1	1	11	1	3	1	1
临床表现										
智力低下	6/6	22/22	2/2	+	3/3	24/24	8/8	5/5	1/1	0/1
语言发育迟缓	6/6	18/18	2/2	+	3/3	23/23	8/8	NR	1/1	0/1
运动发育迟缓	6/6	5/5	2/2	+	NR	23/23	NR	NR	1/1	0/1
颅脑影像学异常	0/6	0/22	NR	—	NR	10/15	NR	NR	1/1	1/1
步态异常	6/6	6/12	2/2	+	NR	10/21	NR	NR	0/1	1/1
行为异常	5/6	12/15	1/2	+	3/3	13/22	NR	NR	0/1	1/1
身材矮小	6/6	7/11	2/2	+	2/3	17/22	8/8	3/5	1/1	0/1
下唇凸出	0/6	6/17	2/2	+	3/3	18/23	8/8	1/5	1/1	NR
大头畸形	0/6	8/11	2/2	—	1/3	7/22	NR	4/5	1/1	NR
脚趾异常	5/5	11/13	NR	+	3/3	14/19	8/8	NR	NR	NR
高足弓	NR	7/8	2/2	+	NR	2/11	NR	NR	NR	NR
震颤	1/5	11/13	2/2	+	2/3	9/20	NR	NR	1/1	0/1
癫痫	4/5	8/11	2/2	—	NR	7/22	NR	NR	0/1	NR
肥胖	NR	15/19	2/2	—	2/3	11/21	NR	3/5	NR	0/1
失语	5/6	11/15	2/2	+	2/3	NR	8/8	NR	1/1	0/1

a) NR表示未报道。

HUWE1的稳定性增加, 在293T和HeLa细胞中过表达CUL4B导致HUWE1蛋白水平降低. U2OS细胞中敲低CUL4B通过上调HUWE1增加DNA损伤诱导的凋亡^[38].

3 CUL4B在多种实体瘤组织中高表达, 通过表观转录调控多种抑癌基因表达而调控肿瘤进程

3.1 CUL4B在多种实体瘤组织中高表达

CUL4B在多种实体瘤组织中高表达, 包括食管癌^[39](包括食管鳞状细胞癌^[40])、肺癌^[39,41](如非小细胞肺癌^[42])、胃癌^[39,43,44]、结肠癌^[39,45]、胰腺癌^[39,46,47]、宫颈癌^[39,48]、肾癌^[39]、膀胱癌^[39,49]、前列腺癌^[50]和神经胶质瘤^[51]等. CUL4B的表达与食管癌^[39]、结肠癌^[45]、肺癌^[41]等的肿瘤分级成正相关. CUL4B可以作为胃癌^[43]、结肠癌^[45]、肺癌^[41]、胆管癌^[52]等的预后标志物.

3.2 CRL4B复合物通过表观调控机制抑制抑癌基因表达

CRL4B复合物可以催化H2AK119单泛素化, 并且与转录抑制复合物PRC2协同作用促进H3K27三甲基

化(H3K27me3), 进而抑制一系列抑癌基因的转录(如*p16*和*PTEN*等), 并促进食管癌细胞的增殖、侵袭和肿瘤发生^[39].

CRL4B复合物可以与SUV39H1/HP1/DNMT3A复合物协同作用, 通过催化H2AK119单泛素化, 促进H3K9三甲基化(H3K9me3)和DNA甲基化, 沉默一系列抑癌基因的表达(如*IGFBP3*等), 进而促进宫颈癌细胞增殖和侵袭^[48].

3.3 CRL4B复合物调控Wnt/ β -catenin通路

CUL4B和 β -catenin在肝细胞癌中均高表达, 并且二者的表达成正相关. CRL4B复合物与PRC2复合物协同作用促进H2AK119单泛素化和H3K27三甲基化(H3K27me3), 抑制Wnt通路、抑制分子DKK1和PPP2R2B的表达, 进而保护 β -catenin免受GSK3介导的降解, 激活Wnt/ β -catenin通路, 促进肝细胞癌细胞的增殖和侵袭^[53]. 以上结果提示CUL4B在Wnt/ β -catenin通路依赖的肝细胞癌发生中作为一个表观驱动因子发挥作用^[54].

CUL4B通过激活Wnt/ β -catenin信号通路促进胰腺癌细胞的上皮间质转化, 进而促进胰腺癌细胞的转移和增殖^[46]. miR-300通过下调CUL4B表达, 抑制Wnt/ β -

catenin信号通路, 进而抑制胰腺癌细胞的增殖、迁移、侵袭和上皮间质转化, 促进其凋亡^[47]. 在食管鳞状细胞癌细胞中, miR-133b通过下调CUL4B表达, 抑制AKT/GSK3 β /catenin信号通路, 进而抑制食管鳞状细胞癌细胞的增殖, 促进其凋亡^[40].

此外, CUL4B还可以通过激活Wnt/ β -catenin信号通路促进胃癌^[44]、结直肠癌^[55]、膀胱癌^[49]和非小细胞肺癌^[51]细胞的增殖、迁移、侵袭和上皮间质转化过程.

3.4 CUL4B复合物调控microRNA表达

CUL4B可以促进非小细胞肺癌细胞的增殖、迁移和移植瘤的形成. miR-194可以直接结合CUL4B的3'-UTR区并下调其表达. 同时, CUL4B复合物可以催化H2AK119单泛素化、与PRC2复合物协同作用促进H3K27三甲基化, 进而表观抑制miR-194的转录. 因此miR-194与CUL4B形成一个负反馈环, 其调节异常会导致非小细胞肺癌的发生^[42].

体内外实验表明CUL4B可促进胃癌细胞的迁移及转移, CUL4B表达沉默可抑制肿瘤细胞的上皮间质转化(EMT). 染色质免疫共沉淀实验证实CUL4B可通过直接靶向miR125a启动子区来抑制miR125a的表达, 进而正调控其靶基因HER2的表达. 通过Herceptin或si-Her2敲低HER2表达可部分逆转CUL4B过表达所致的EMT和体内肿瘤转移^[43].

3.5 CUL4B调控肿瘤进程的其他途径

CUL4B可以促进人骨肉瘤细胞的增殖, 抑制其凋亡^[56]. 在人骨肉瘤细胞中, CUL4B复合物可以与DCAF11相互作用, 泛素化降解p21^{Cip1}, 进而调节细胞周期进程^[57]. TNF α /NF- κ B通路的激活可以导致CUL4B^{DCAF11}活性增强, 进而导致p21^{Cip1}蛋白降解, 因此调控人骨肉瘤细胞的细胞周期进程^[58]. 在人骨肉瘤细胞中, CUL4B^{DCAF13}E3泛素连接酶复合物特异性地识别并降解肿瘤抑制因子PTEN, 从而促进人骨肉瘤细胞的生长^[59].

此外, CUL4B还可以促进胆管癌细胞的增殖、迁移、侵袭和上皮间质转化过程^[52], 以及促进神经胶质瘤细胞的增殖^[60].

通过CUL4B转基因小鼠模型研究发现, CUL4B转基因小鼠自发肝癌, 并且促进DEN诱导的肝细胞癌发

生. CUL4B转基因小鼠的肝脏组织中细胞增殖加快, 细胞周期调控蛋白CDK1, CDK4和Cyclin D1表达升高, 抑制蛋白p16表达降低. DEN诱导损伤后, CUL4B转基因小鼠的肝脏组织代偿性增殖加快, 并伴随AKT, ERK, p38和NK- κ B信号通路被激活. 同时还发现, CUL4B转基因小鼠的肝脏组织中, CUL4B复合物的蛋白降解底物Prdx3水平降低, DEN诱导的活性氧(reactive oxygen species, ROS)水平升高^[61]. 生物信息学分析发现, CUL4B参与肝细胞癌的TGF- β 信号的激活^[62].

4 利用小鼠模型研究CUL4B的功能

为阐明CUL4B基因的生理功能及其在X连锁智力低下综合征发生过程中的作用机制, 目前至少有3个实验室利用Cre/loxP系统建立了Cul4b敲除小鼠模型, 并利用小鼠模型系统阐明了Cul4b在小鼠多个组织系统发生和功能调控中的作用.

4.1 在胚胎发育中的作用

本实验室构建了Cul4b基因打靶小鼠(即Cul4b floxed小鼠), 然后将Cul4b floxed基因打靶小鼠与Ella-Cre转基因小鼠交配, 由于Ella-Cre转基因小鼠的Cre重组酶在小鼠组织中广泛表达, 包括在生殖细胞中表达, 因此可以获得Cul4b基因全身性敲除小鼠. 研究结果发现, Cul4b基因全身性敲除小鼠在胚胎早期表现出严重的发育停滞, 并且在胚胎发育9.5天之前死亡. 敲除小鼠胚胎细胞的增殖活性降低, 细胞凋亡增加, 并且造成CUL4B复合物底物Cyclin E的累积. 同时Cul4b杂合子雌鼠表现出胚胎期发育迟缓、胎盘发育异常等缺陷^[63]. 表达分析发现, CUL4B参与到胎盘疾病特别是宫内生长受限的发病过程^[64].

Liu等人^[65]构建了Cul4b基因打靶小鼠(即Cul4b floxed小鼠), 并将Cul4b floxed基因打靶小鼠与CAG-Cre转基因小鼠交配(Cre酶在受精卵时就开始表达), 以获得Cul4b基因全身性敲除小鼠. 研究发现Cul4b基因全身性敲除小鼠在胚胎发育9.5天之前死亡. 随后将Cul4b floxed基因打靶小鼠与Sox2-Cre转基因小鼠交配获得Cul4b^{Sox2-Cre}小鼠, 该小鼠的胚体中Cul4b被敲除, 而胚外组织(胎盘)中Cul4b表达正常, 该小鼠可以存活, 提示CUL4B在小鼠胚外组织发育中发挥重要的作用, Cul4b基因全身性敲除小鼠在胚胎期死亡是由于胚外

组织发育异常导致的。该小鼠成为研究人类X连锁智力低下综合征的一个很好的小鼠模型^[66]。

4.2 在神经系统发育中的作用

鉴于*Cul4b*基因全身性敲除小鼠在胚胎期死亡, Chen等人^[67]将构建的*Cul4b floxed*基因打靶小鼠与*Sox2-Cre*转基因小鼠交配, 获得能够存活的*Cul4b^{Sox2-Cre}*小鼠用于表型分析。*Cul4b^{Sox2-Cre}*小鼠表现出学习和记忆能力缺陷, 更容易被戊四唑(PTZ)诱导出癫痫症状, 海马中PV-阳性的GABA能中间神经元数量减少, 海马神经元树突分支减少、直径减小, 树突棘密度增加、形态异常。

本实验室^[68]构建了神经系统特异性*Cul4b*基因敲除小鼠(*Cul4b^{Nestin-Cre}*小鼠), 发现*Cul4b^{Nestin-Cre}*小鼠表现出学习和记忆能力缺陷。体外结果发现, 与野生型神经前体细胞相比, *Cul4b*缺失的神经前体细胞向GFAP阳性细胞分化增多。与体外实验结果一致, *Cul4b^{Nestin-Cre}*小鼠的多个脑区中GFAP阳性细胞都明显多于同窝对照小鼠。同时发现敲除*Cul4b*基因后前列腺素D2合成酶PTGDS的表达明显升高, 并证实CUL4B调控GFAP阳性细胞分化是通过PTGDS介导的: 用PTGDS的活性抑制剂AT56处理神经前体细胞可以有效拯救GFAP阳性细胞分化增多的表型; 在*Cul4b*缺失的神经前体细胞中通过感染含有shRNA的病毒来敲低PTGDS的表达也可拯救GFAP阳性细胞分化增多的表型; 加入外源性PTGDS蛋白可显著增加野生型神经前体细胞向GFAP阳性细胞分化的比例。ChIP实验结果显示CUL4B可直接结合至*Ptgds*启动子上, CUL4B/PRC2复合物可通过促进H2AK119单泛素化和H3K27三甲基化来抑制*Ptgds*转录。

4.3 在生殖细胞发育中的作用

CUL4B在精子发生过程中起到非常重要的作用。雄性*Cul4b*敲除小鼠(*Cul4b^{Sox2-Cre}*)不育, 成年*Cul4b*敲除的雄性小鼠附睾中成熟精子数量减少, 并存在明显的形态异常。雄性*Cul4b*敲除小鼠的精细胞顶体和核形态存在异常。在精子形成过程中, 特别是在顶体期和头帽期, 精细胞表现出明显高水平的凋亡。比较蛋白质组学分析发现, *Cul4b*敲除小鼠的睾丸在减数分裂后的精子发育早期存在大量的蛋白表达变化, 如ARF3表达上调。*Cul4b*敲除小鼠的精细胞中经典的和非经典的组蛋

白水平均发生改变。该研究揭示在单倍体雄性生殖细胞分化过程中, CUL4B在顶体形成、核固缩、组蛋白动态调节过程中发挥重要作用, 并导致雄性不育^[69]。

与此同时发现, 生殖细胞特异性*Cul4b*敲除雄性小鼠(*Cul4b^{Vasa-Cre}*)不育, 尽管其睾丸形态正常、精子数量与野生型小鼠相当。由于线粒体活性和糖酵解水平降低, *Cul4b^{Vasa-Cre}*小鼠的精子运动能力降低。此外, *Cul4b^{Vasa}*小鼠的精子超微结构存在异常, 其鞭毛轴丝的微管及鞭毛纤维排列存在异常。质谱分析发现, 在雄性生殖细胞中INSL6是CUL4B复合物的底物, CUL4B可以泛素化并降解INSL6。该研究显示CUL4B在精子发生过程中是必不可少的, 它在雄性生殖细胞中的细胞自主性功能确保精子的运动能力, 在体细胞中的非细胞自主性功能维持精原细胞干性^[70]。

4.4 在髓系细胞分化中的作用

由于CUL4B突变患者具有单核细胞增多表型, 我们建立了血液系统特异性*Cul4b*基因敲除小鼠(*Cul4b^{Tek-Cre}*), 表型分析发现血液系统敲除*Cul4b*导致小鼠CD11b+Gr1+细胞增多, 其免疫抑制活性增强。利用移植瘤模型研究发现, 敲除小鼠增加的CD11b+Gr1+细胞促进移植瘤的生长和转移, 提示CUL4B在肿瘤微环境调控中发挥重要作用。重要的是, 与其在肿瘤组织中发挥癌基因作用相反, 在肿瘤微环境中CUL4B发挥抑癌基因作用。机制研究发现, CUL4B复合物通过转录抑制磷酸酶PP2A和PHLPP1/2转录而正调控AKT/ β -catenin信号通路, 因此, CUL4B的缺失导致髓源抑制细胞的累积并且活性增强, 进而促进肿瘤的发生发展。因此, CUL4B可以通过阻止肿瘤支持微环境的建立而发挥抗肿瘤的作用^[71]。此外, 有研究显示髓系特异性敲除*Cul4b*会加重脂多糖(lipopolysaccharides, LPS)诱导的腹膜炎^[72]。

4.5 在脂肪细胞分化中的作用

脂肪细胞特异性*Cul4b*敲除小鼠(*Cul4b^{Fabp4-Cre}*)在高脂饲料喂养情况下更易肥胖, 有更多的皮下脂肪(腹腔沟脂肪)积累, 脂肪细胞直径明显减小, 提示*Cul4b^{Fabp4-Cre}*小鼠倾向于肥胖是由于脂肪细胞分化加快而不是脂肪细胞体积增大引起的。在喂养高脂饲料条件下, *Cul4b^{Fabp4-Cre}*小鼠比野生型小鼠有更低的血糖

和胰岛素水平,表明*Cul4b*敲除小鼠改善高脂诱导的胰岛素抵抗,其原因可能是由于敲除小鼠脂肪组织炎症减轻.机制研究发现*Cul4b^{Fabp4-Cre}*小鼠中PPAR γ 蛋白水平升高,CUL4B作为泛素连接酶复合物的成员促进PPAR γ 蛋白的降解.以上结果提示CUL4B在肥胖和胰岛素抵抗中发挥重要作用^[73].

4.6 在胰岛delta细胞发育中的作用

为进一步明确*Cul4b*在代谢中的调控作用,本实验室^[74]建立了胰岛beta和delta细胞特异性敲除*Cul4b*基因的小鼠,发现特异性敲除胰岛delta细胞*Cul4b* (*Cul4b^{Sst-Cre}*)导致胰岛生长抑素分泌增加,进而通过旁分泌作用使胰岛素分泌下降,最终引起葡萄糖不耐受.机制研究显示,CRL4B复合物通过转录抑制L型钙通道Cav1.2和腺苷酸环化酶AC6转录进而调控生长抑素分泌.在人的胰岛delta细胞中,也验证了CUL4B表达下调或活力降低促进生长抑素的分泌.以上结果提示CUL4B对胰岛稳态与血糖稳态的维持过程具有重要的生理和病理生理意义.

5 CUL4B的同源蛋白CUL4A研究进展

如前所述,在Cullin家族中,CUL4A与CUL4B同源性最高.与CUL4A相比,CUL4B具有一个含有149个氨基酸的特异性N末端^[25].CUL4B在进化上高度保守(图2),但是仅在哺乳动物和斑马鱼(*Danio rerio*)中存在CUL4A和CUL4B两个基因,在植物和果蝇(*Drosophila melanogaster*)、线虫(*Caenorhabditis elegans*)、裂殖酵母(*Schizosaccharomyces pombe*)等低等真核生物中,仅有一个CUL4基因.因此早期大多数研究都将CUL4A和CUL4B作为一体(CUL4)进行研究^[75].CUL4B与CUL4A的功能和分子机制有重叠交叉,也有差异差别,因此对二者进行比较分析能够更好地加深对CUL4B的了解.

CUL4B与CUL4A都在体内广泛表达,不同的是CUL4B的表达存在明显的组织差异,在脑、肾、脾、肝、小肠等组织中该蛋白表达水平较高,而在心脏和肌肉等组织中表达水平较低.CUL4A同样在体内广泛表达,但与CUL4B不同,该蛋白在组织间的表达差异较小.此外,CUL4B与CUL4A的亚细胞定位不同,CUL4A主要分布在细胞质,而CUL4B则主要分布在细胞核^[25].

由于CUL4A和CUL4B同源性很高,并且利用同一个连接蛋白DDB1来装配CRL4复合物,因此二者可能降解某些共同的底物,并且在某些细胞功能上相互代偿^[2].目前已发现的CRL4A复合物的底物包括DDB2^[76,77],CDT1^[78,79],p21^[80],p27^[29],p53^[81],c-Jun^[82],HOXA9^[83],STAT1^[84],H2A^[85],H3,H4^[86],Myc^[87]和CHK1^[88-90]等重要蛋白,这些底物在DNA修复、细胞周期调控、染色质重塑、细胞增殖和分化等重要生命活动中发挥重要作用^[89,91].虽然CRL4A复合物和CRL4B复合物有一些共同的底物,但同时也各自有一些特异性底物,提示二者各自的独特作用.

与*Cul4b*基因敲除小鼠在胚胎期致死不同,敲除*Cul4a*基因并不影响小鼠的生存.目前有3个独立的课题组分别建立了*Cul4a*基因敲除小鼠模型.敲除*Cul4a*基因第1外显子导致小鼠早期胚胎死亡^[92],然而后期的研究显示该敲除方法影响了邻近的*Pcid2*基因的功能从而导致了胚胎致死的表型^[93].敲除*Cul4a*基因第17~19外显子的基因敲除小鼠没有明显的异常表型,但可以增强DNA损伤修复能力,对紫外线诱导的皮肤癌的抵抗性增强^[93],此外敲除*Cul4a*基因会影响雄性小鼠的减数分裂和精子发生,导致雄性小鼠不育^[94].敲除*Cul4a*基因第4~8外显子的基因敲除小鼠出生后同样没有明显的异常表型,但是同样会影响雄性小鼠精子发生,导致雄性小鼠不育^[95].

与CUL4B基因突变导致人类的X连锁智力低下综合征不同,目前并未发现CUL4A基因的突变导致人类疾病的发生.但是与CUL4B类似,CUL4A在癌症的发生发展中发挥重要作用.CUL4A在多种肿瘤组织中高度表达,包括乳腺癌、肝细胞癌、鳞状细胞癌、肾上腺皮质癌和小儿髓母细胞瘤等^[96],并且CUL4A的高表达与病人的预后密切相关^[97].在多种癌症中发现CUL4A基因所在染色体区域(ch13q34)的拷贝数扩增^[98-101].皮肤组织特异性的*Cul4a*基因敲除小鼠对紫外线诱导的皮肤癌的抵抗性增强^[93].肺组织特异性的CUL4A转基因小鼠表现出肺部增生,并可自发肺部肿瘤^[102,103].CRL4A复合物的底物包括p27^{Kip1}和p53等,因而推测CUL4A的过表达可以导致p27^{Kip1}和p53的加速降解,从而促进肿瘤细胞的生长^[24,81,104-106].

因此,尽管过去认为CUL4A和CUL4B以几乎相同的方式参与同一生命活动的调节,但近年来的研究却显示二者功能及具体作用机制上存在较大差异.这些

图2 CUL4B蛋白序列进化保守性分析图

研究表明, 尽管CUL4A和CUL4B同源性较高, 但各自都具有其独特的作用.

6 展望

CUL4B作为泛素连接酶复合物的主要支架组分, 可以通过组织形成CUL4B泛素连接酶复合物对底物进行泛素化修饰及蛋白酶体降解, 这是其发挥功能的主要机制. CUL4B复合物通过催化底物蛋白的多泛素化降解或单泛素化修饰而在多种组织、多种病理生理过程中发挥重要作用. 目前已报道的CUL4B复合物的底物蛋白如表3所示. 此外, 用蛋白质组学方法分析CUL4B的相互作用蛋白对了解蛋白质平衡和致病机制具有重要作用^[124]. 研究CUL4B复合物的底物蛋白有

助于了解CUL4B的功能, 从而明确CUL4B突变导致X连锁智力低下综合征的作用机制^[125].

CUL4B复合物还可以通过表观遗传学方式调控基因转录. CUL4B复合物可以多泛素化降解组蛋白H3K4甲基转移酶复合物的核心亚基WDR5, 导致H3K4三甲基化(H3K4me3)水平降低, 进而抑制一系列基因的表达^[108]. CUL4B复合物可以催化H2AK119单泛素化, 协同多种转录抑制复合物发挥转录抑制作用. 例如, 协同转录抑制复合物PRC2促进H3K27三甲基化(H3K27me3)^[39]; 协同SUV39H1/HP1/DNMT3A复合物促进H3K9三甲基化(H3K9me3)和DNA甲基化^[48]; 协同SIN3A-HDAC复合物促进H3和H4去乙酰化^[28].

尽管目前已经发现了CUL4B基因的多种突变类型, 发现了CUL4B复合物的多种底物, 通过细胞学实

表3 CUL4B复合物的底物蛋白^{a)}

底物	E3连接酶	DCAFs	功能	参考文献
ERα	CUL4B	AhR	雌激素受体	[107]
Cyclin E	CUL4B	N.A.	细胞周期调控	[24,25]
TOPO I	CUL4B	N.A.	DNA拓扑异构酶	[33]
WDR5	CUL4B	N.A.	H3K4甲基转移酶复合物亚单位	[108]
PRXIII	CUL4B	N.A.	抗氧化蛋白	[109]
Jab1/CSN5	CUL4B	N.A.	正调控BMP信号通路	[110]
TSC2	CUL4A/4B	FBW5, Raptor, mLST8	mTOR通路抑制因子	[111~113]
p53	CUL4A/4B	Cdt2, PCNA, MDM2	转录因子、DNA损伤修复	[36,37,81]
RGS2	CUL4B	FBXO44	调节血管收缩	[114]
HUWE1	CUL4B	N.A.	DNA损伤修复	[38]
PPARγ	CUL4B	N.A.	脂肪分化	[73]
Cdc25A	CUL4B	DCAF8	细胞周期调控	[30]
γ-tubulin	CUL4A/4B	N.A.	着丝粒稳定性	[115]
CLC-1	CUL4A/4B	CRBN	电压门控氯离子通道	[116]
TR4	CUL4B	DCAF1	脂肪代谢	[117]
p12	CUL4A/4B	Cdt2	DNA损伤修复	[34]
APP	CUL4A/4B	CRBN	神经退行性病变	[118]
MRFAP1	CUL4B	N.A.	组蛋白乙酰转移酶复合物亚单位	[119]
TAT	CUL4B	N.A.	HIV-1潜伏	[120]
INSL6	CUL4B	N.A.	精子发生	[70]
p21 ^{Cip1}	CUL4B	DCAF11	细胞周期调控	[57,58]
PTEN	CUL4B	DCAF13	肿瘤抑制因子	[59]
LATS1	CUL4A/4B	N.A.	细胞凋亡	[121]
p73	CUL4A/4B	N.A.	细胞凋亡	[121]
Mcm10	CUL4A/4B	VprBP	DNA复制	[122]
p150 ^{Sal2}	CUL4A/4B	RBBP7	静止因子	[123]

a) N.A.表示未知.

验和小鼠模型对于CUL4B的功能研究已经取得了一些进展,但是仍有许多未解之谜等待研究者去揭示。

(i) *CUL4B*基因突变导致X连锁智力低下综合征的致病机制目前尚不清楚。利用神经系统不同细胞类型特异性的敲除小鼠模型,或者不同脑区特异性的敲除小鼠模型将有助于推动智力低下综合征的致病机制的研究,为人类相关疾病的治疗提供了重要线索。

(ii) CRL4B复合物与DNA结合的机制:尽管目前已有多个证据表明,CRL4B复合物在组蛋白甲基化、组蛋白乙酰化、DNA甲基化等表观遗传学修饰中发挥重要的作用,但是CRL4B复合物的成员均没有DNA结合结构域,那么CRL4B复合物是如何结合到染色质上去的?目前推测长链非编码RNA可能介导了这个过程,但是其具体机制目前并不明确。

(iii) 调控CUL4B表达的分子机制:目前CRL4B复合物调控底物蛋白和靶基因的分子机制已经逐渐明确,但是调控CUL4B表达的分子机制目前仍知之甚少,特别是在肿瘤中CUL4B为何高表达。目前已发现NF- κ B可以结合到*CUL4B*基因启动子区并直接调控*CUL4B*的表达^[58]。多种micro RNA可以在翻译水平调控CUL4B水平,如miR-194^[42],miR-421^[50],miR-133b^[40],miR-300^[47,59]和miR-101-3p^[126]可以结合到*CUL4B*的3'-UTR区域,并抑制其表达。但是调控CUL4B表达的分子机制仍需进一步明确。

CUL4B功能研究不仅对于明确X连锁智力低下综合征的致病机制和进行预防及治疗具有重要的临床价值,而且对于理解智力发育和认知功能的分子生物学基础也具有重要的理论和实际意义。

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Pathogenic roles of the Cullin E3 ligase scaffolding protein CUL4B

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CUL4B belongs to the Cullin family, the members of which are scaffolding proteins of Cullin-RING E3 ligases (CRLs), the largest ubiquitin ligase complex in eukaryotes. CUL4B is a scaffolding protein of Cullin 4B-RING ubiquitin ligase complex (CRL4B), which catalyzes the polyubiquitination or monoubiquitination of substrate proteins and plays important roles in various physiological and pathophysiological processes. The fact that mutations in *CUL4B* are causally associated with human mental retardation syndrome opened a new direction for the study on the function of CUL4B. Using cell models, it was found that the CRL4B complex plays important roles in regulating cell proliferation and DNA damage repair. At the same time, it was found that CUL4B is highly expressed in various solid tumor tissues, and regulates tumor progression by epigenetically regulating the transcription of multiple tumor suppressor genes. Research using mouse models found that CUL4B played important roles in embryonic development, nervous system development, germ cell development, myeloid cell differentiation, adipocyte differentiation, and pancreatic delta cell function. This review will summarize our current knowledge of CUL4B in the pathogenesis in human diseases and mouse models.

***CUL4B*, mental retardation, tumor, cell proliferation, embryonic development**

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