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评 述

# 肠道隐窝-绒毛轴上皮细胞更新及调控机制研究进展

邹立军[1,2,3], 熊霞[2*], 王小城[2], 杨焕胜[1], 李建中[1], 黄鹏飞[1], 印遇龙[1,2*]

1. 湖南师范大学生命科学学院, 动物营养与人体健康实验室, 长沙 410081;
2. 中国科学院亚热带农业生态研究所, 亚热带农业生态过程重点实验室, 长沙 410125;
3. 湖南第一师范学院, 基础生物学实验室, 长沙 410205

* 联系人, E-mail: xx@isa.ac.cn; yinyulong@isa.ac.cn

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**摘要** 肠道不仅是营养物质消化吸收的主要部位, 也是重要的免疫器官和内分泌器官. 小肠上皮细胞的分化对于肠道应激后的损伤修复、免疫屏障以及肠道功能的正常行使具有非常重要的意义. 近年来, 肠道上皮隐窝-绒毛轴干细胞自我更新、分化和调控的研究得到了快速发展. 本文结合本研究组的研究成果综述了哺乳动物肠道隐窝-绒毛轴上皮细胞分化过程中差异基因和蛋白表达; 信号通路、转录因子和表观遗传修饰对肠上皮细胞分化的影响以及营养因子对肠道细胞分化和损伤修复调控的最新研究进展, 以期在营养学和药理学方面, 为干预和治疗肠道损伤及相关疾病提供理论指导依据.

**关键词** 小肠, 隐窝-绒毛轴, 细胞更新, 损伤修复, 营养调控

肠道不仅是营养物质消化吸收的主要部位, 也是重要的免疫器官和内分泌器官[1]. 肠道稳态维持是通过肠道干细胞(intestinal stem cell)的增殖分化实现的, 肠上皮细胞沿隐窝端向绒毛端迁移, 逐渐分化成熟并最终在绒毛顶端脱落, 分化成熟后的肠上皮细胞有着显著的顶角结构和上千个微绒毛[2]. 小肠上皮作为哺乳动物更新速度最快(4~5天)的器官, 其分化对于肠道应激后的损伤修复、肠道屏障以及肠道功能的正常行使具有非常重要的意义[3]. 小肠隐窝干细胞具有非常精细的细胞增殖与分化的调控机制, Wnt/β-catenin、Notch、表皮生长因子(epidermal growth factor, EGF)和骨形成蛋白(bone morphogenetic protein, BMP)等信号在小肠干细胞命运调控中起着非常重要的作用[4]. 结合本研究组的成果, 本文综述了哺乳动物肠道隐窝-绒毛轴(crypt-villus axis)细胞的组成; 肠上皮细胞增殖分化和损伤修复过程中相关基因、蛋白的表达差异; 信号通路、转录因子和表观遗传修饰对肠上皮细胞分化的影响; 并从营养生理学的角度总结了各类营养调控因子促进肠道细胞增殖分化和肠道损伤修复等方面的研究进展.

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## 1 小肠隐窝-绒毛轴上皮细胞组成

肠道上皮由单层柱状上皮细胞层覆盖并可分为隐窝和绒毛两部分; 隐窝内陷进入了下层间充质, 绒毛朝向肠腔[5]. 肠上皮细胞快速和持续的更新动力依赖于隐窝处的Lgr5阳性(Lgr5$^+$)特异标记的底部柱状细胞(CBC细胞(crypt base columnar cell)), CBC细胞(干细胞)分裂增殖后产生的子代细胞继续分化为"短暂扩增细胞"(TA细胞(transit amplifying cell))并继续向上迁移分化[6]. 因此, 隐窝和绒毛分别包括干细胞增殖和分化细胞区域. 分化成熟的肠上皮细胞主要包括4种类型的细胞: 吸收型上皮细胞(absorptive enterocytes)和3种分泌型细胞(secretory lineages). 吸收型肠上皮细胞主要负责营养物质的吸收, 其数量约占绒毛细胞的95%以上[7]. 分泌型肠上皮细胞与肠道免疫屏障有密切关系, 其中, 杯状细胞(goblet cells)在整个上皮分布并分泌黏液; 潘氏细胞(paneth cells)在隐窝底部聚集, 产生抗菌肽并调节肠道微生物, 同时也产生生长因子维持附近干细胞的生长; 肠内分泌细胞(enteroendocrine cells)数量较少, 但负责调节肠道上皮的多种功能, 如分泌激素和消化酶调控饱腹感等功能[8,9]. 此外, 肠道上皮还存在M细胞(membranous cell)和塔夫细胞(tuft cells). M细胞存在于肠道相关的淋巴滤泡, 可能与肠腔内物质与免疫细胞的交流有关[10]. 塔夫细胞在肠道上皮中的数量较少, 目前它们的功能尚不清楚[11].

分化形成的各种细胞(除潘氏细胞外)在沿隐窝-绒毛轴向上移动的过程中逐渐成熟, 最后达到绒毛顶端区域并最终凋亡[2]. Canale-Zambiano等人[12]发现, DNA合成在隐窝-绒毛过渡期间迅速下降, 表明分化过程中伴随着细胞周期阻滞. 肠道某些特定基因的转录以及绒毛顶端细胞存在DNA碎片, 暗示着有细胞凋亡的发生, 这表明肠上皮细胞的衰老和凋亡是一个有序过程[13]. 但关于绒毛顶端mRNA表达的下降和细胞衰老之间的关系还需要进一步研究. 另外, 小肠绒毛细胞的脂质过氧化程度显著高于隐窝, 在绒毛区域产生大量自由基, 这可能是导致肠上皮细胞从绒毛顶端脱落发生凋亡的原因之一, 这也促进了肠上皮细胞的分化迁移[14]. 研究表明, 细胞平面极性信号通路(the planar cell polarity, PCP)对上皮细胞极性的建立和细胞迁移有重要作用; 其作用机制主要是通过激活小G蛋白RhoA和Rac1后再分别诱导下游Rho-相关激酶(Rho-associated coiled-coil protein kinase, ROCK)和c-Jun氨基末端激酶(c-Jun N-terminal kinas, JNK)的表达进而分别诱导细胞骨架重塑或引起转录反应[15]. 因此, 小肠上皮细胞沿绒毛分化迁移和在绒毛上脱落是个复杂严密的过程.

## 2 肠道隐窝-绒毛轴细胞基因及蛋白表达变化

### 2.1 消化吸收相关基因和蛋白的表达差异

肠道刷状缘是营养物质消化吸收的最终部位, 肠上皮细胞分化程度越高, 它的消化吸收能力越强. 肠碱性磷酸酶(alkaline phosphatase, ALP)是营养物质吸收的标志酶, 与蛋白质和脂质的代谢有密切关系, 其表达量和活性在肠上皮细胞顶端处最高, 隐窝区域最低[16]. 三磷酸腺苷酶($Na^+/K^+$-ATPase)是细胞膜上的糖蛋白, 主要作用是通过参与维持肠道上皮细胞膜内外的离子浓度差来吸收营养物质, 其表达沿隐窝-绒毛轴从上到下逐渐降低[17]. 一系列研究表明, 二糖酶蔗糖酶-异麦芽糖酶(sucrase isomaltase, SI)、乳糖酶-根皮苷水解酶(lactase-phlorizin hydrolase, LPH)、氨肽酶N(aminopeptidasen, APN)、蔗糖酶(invertase)和乳糖分解酶(lactase, Lct)等酶类在绒毛上的表达和活性均高于隐窝[7,18].

负责营养物质吸收转运的转运载体在隐窝-绒毛轴的分布也存在差异, 如溶质载体家族基因在隐窝-绒毛轴上的表达不同[19]. 易化性单糖转运体5(facilitated glucose transporter 5, GLUT-5)/溶质载体家族2成员5(solute carrier family 2 member 5, SLC2a5)和$Na^+$依赖性单糖转运体1($Na^+$-dependent glucose transporter, SGLT1)是哺乳动物体内的两种单糖转运体, 对碳水化合物的吸收起着重要作用. 研究表明, 大鼠(*Rattus norvegicus*) *GLUT-5*在绒毛3/4以上部位表达[20]. *SGLT1*在绒毛部位的表达量高于隐窝区域, 而其蛋白质的表达却没有差异, 表明隐窝-绒毛轴上的基因表达可能存在转录后或翻译后水平的调控[21].

通过DNA微矩阵检测大鼠空肠隐窝-绒毛轴细胞分化的实验表明, 从隐窝干细胞分化形成绒毛细胞的过程中与消化吸收相关的基因表达显著上调, 如单羧酸转运蛋白(*SLC16a3*)、钠偶联核苷转运蛋白(*SLC28a1*)

和铜转运蛋白(*SLC31a1*)等; 与淀粉和蔗糖的消化吸收有关的基因, 如*SLC2a5*, *SLC5a1*和*SI*等在绒毛中端表达最高; 而与脂肪吸收相关的基因, 如脂肪酸结合蛋白(fatty acid-binding protein 1, *Fabp1*)、载脂蛋白C3(apolipoprotein C3, *Apoc3*)和载脂蛋白A4(apolipoprotein A4, *Apoa4*)等的表达在绒毛顶部表达最高[22]. 肠脂肪酸结合蛋白(intestines fatty acids binding proteins, I-FABP)对肠道细胞内脂肪酸的利用过程起重要作用, 蛋白质组学和免疫荧光实验均证明I-FABP在整个隐窝-绒毛轴上均有表达, 且在绒毛顶端的表达量最高[3,23]. 因此, 营养物质和离子的消化吸收基因在隐窝-绒毛轴上表达模式的差异表明肠道不同部位的肠黏膜可能对这些过程有不同的贡献. 在哺乳动物中, 肠道沿隐窝-绒毛轴上基因表达模式的变化与上皮细胞更新之间的关系需要进一步研究, 以提高对营养物质和离子消化吸收的认识.

### 2.2 肠道细胞损伤修复过程中的基因和蛋白质表达变化

哺乳动物(大鼠、猪(*Sus scrofa domestica*)等)的早期断奶和细胞感染均能引起肠道功能障碍和损伤. 首先, 严重影响肠道的形态结构, 引起小肠黏膜的损伤, 包括绒毛萎缩以及隐窝增生, 导致ALP的表达和活性下降, 增加肠道疾病的易感性[24]. 其次, 能够影响肠道功能基因的表达, 全基因组芯片分析早期断奶仔猪肠道差异表达基因, 发现与凋亡相关基因, 如细胞色素C(cytochrome C, *CYCS*), Bcl-xl/Bcl-2相关死亡启动子(bcl-xl/bcl-2 associated death promoter, *BAD*)和共济失调毛细血管扩张症突变基因(ataxia telangiectasia-mutated gene, *ATM*)、促炎因子白细胞介素-8(interleukin-8, *IL-8*)和肿瘤坏死因子(tumor necrosis factor, *TNF*)表达上调, 而细胞周期调控相关基因E2F转录因子5(E2F transcription factor 5, *E2F5*)和SMAD家族成员4(SMAD family member 4, *Smad4*)表达下调; 表明断奶引起免疫抑制, 使细胞周期停滞, 加速凋亡[25]. 在脂多糖(lipopolysaccharides, LPS)诱导的仔猪肠道疾病模型实验中发现, 肠道免疫反应、黏膜生长、能量代谢、吸收、黏膜屏障功能、抗病毒功能等相关基因的表达均受到影响[26]. 另外, 断奶还使仔猪抗氧化能力显著下降; 肠道细胞内与氧化应激相关基因表达增加, 自由基代谢异常, 血清中NO和$H_2O_2$含量显著增加, 而体内自由基水平与肠道结构与功能有密切关联[27].

本研究组以哺乳动物猪为实验材料, 建立了仔猪肠道沿隐窝-绒毛轴细胞分级方法, 利用碱性磷酸酶和增殖细胞核抗原验证进行了哺乳阶段仔猪空肠沿隐窝-绒毛轴的蛋白质组学相关研究, 结果显示, 在肠道细胞分化过程中, 结构和酶调节的蛋白质表达显著下调; 而对营养物质的消化和吸收相关蛋白质的表达上调[3], 这是由于在猪肠上皮细胞快速更新的过程中对能量有显著需求所致[7]; 而饲粮中的氨基酸(如谷氨酸、天冬氨酸和谷氨酰胺)、葡萄糖和脂肪酸是猪肠黏膜的主要能量来源. 通过断奶和哺乳仔猪相比, 发现抗氧化能力随着肠上皮细胞分化而逐渐降低; 隐窝-绒毛轴的差异蛋白主要表现在参与碳代谢、氨基酸和蛋白质代谢、磷代谢和氧化磷酸化等(数据未发表); 参与β-氧化和糖酵解相关蛋白在绒毛细胞内的表达下降, 而糖酵解相关蛋白在隐窝区域表达上调, 表明能量代谢在小肠细胞的增殖和分化过程中发挥了重要作用[16]. 因此, 对肠道损伤机制及其营养调控修复的研究日益受到关注.

## 3 小肠隐窝-绒毛轴细胞分化的分子调控

### 3.1 调控小肠干细胞增殖分化的信号通路

小肠上皮细胞更新是由多种信号通路进行严格调控, 例如, Wnt, Notch, BMP和EGF等信号通路在维持小肠干细胞的自我更新、增殖和分化方面有重要作用. 上述信号通路调控小肠干细胞沿隐窝-绒毛轴分化的调控机制在Qi和Chen[4]的报道中已有详细阐述, 在此主要从Wnt和Notch信号通路协同调控的角度进行综述. 研究表明, Wnt信号作为维持小肠上皮细胞持续更新的主要动力, 其活性在小肠绒毛-隐窝轴上呈梯度递减性[28]; 而Notch信号的活性是决定干细胞分化为吸收型细胞和分泌型细胞的关键因素[29]. 最新研究证明, Wnt和Notch信号通路能协同调控肠道干细胞的自我更新和分化. Yin等人[30]通过筛选小鼠(*Mus musculus*) $Lgr5^+$干细胞进行体外3D培养, 并利用小分子调控Wnt和Notch信号通路, 从而控制肠道干细胞自我更新和分化的方向; 结果表明, Wnt和Notch信号一起激活能够维持干细胞自我增殖; 抑制Wnt信号, 激

活Notch信号可定向诱导肠上皮细胞分化; 激活Wnt信号, 抑制Notch信号可定向诱导潘氏细胞分化; 同时抑制Wnt和Notch信号则定向诱导杯状细胞分化; 而内分泌细胞分化需要抑制Notch信号通路, 但Wnt通路对其影响不大[31]. 因此, 信号通路的协同调控对维持小肠干细胞的正常功能以及肠上皮细胞之间的分化平衡起重要作用.

### 3.2 细胞分化相关转录因子调控

为了进一步了解隐窝-绒毛轴细胞更新过程中的基因差异表达的机制, 就必须探索基因与信号转导和转录因子之间的联系. Mariadason等人[32]通过DNA芯片技术检测小鼠肠道隐窝-绒毛轴肠上皮细胞差异基因的表达, 结果显示, 随着细胞的分化迁移, 与细胞周期进程、RNA转录和翻译相关的基因在成熟过程下调, 与细胞骨架组装和脂质摄入相关的基因上调, 其中Wnt信号基因表达差异最大, 其靶基因C-myc癌基因(myc proto-oncogene protein, *MYC*)和细胞周期蛋白D1(*cyclin D1*)等在绒毛顶端表达量最低. 随后发现, 在小鼠肠道细胞中转录因子胰腺和十二指肠同源基因(pancreas/duodenum homeobox protein 1, *Pdx1*)和甲状腺激素受体(thyroid hormone receptor α, *Thrα*)等沿隐窝-绒毛轴表达下调, 在绒毛顶端处表达量最低[33], 而蛋白磷酸酶(protein phosphatase 5C, *Ppp5c*)隐窝处表达最高[21], 而*Ppp5c*作为一种重要的核转录因子能通过诱导*Pdx1*和*Thra*的表达[34], 随后负调控靶基因*SI*和*Lct*在隐窝-绒毛轴上表达显著升高[35]. 结果表明, 肠道转录因子相关基因的差异表达在小肠上皮细胞分化过程中能够调控与细胞生长和消化吸收相关基因的表达.

尾型同源框转录因子2(homeobox protein CDX-2, *cdx2*)作为肠上皮细胞特异性核转录因子, 大量表达在绒毛末端处, 主要调控肠道上皮的增殖分化和迁移[36]; Shimakura等人[37]发现, 人肠道上皮细胞中氢/肽偶联转运载体Pept1(peptide transporter PEPT1, *SLC15A1*)基因表达受*cdx2*调控, 由于Pept1启动子上没有*cdx2*经典的TA序列结合区域从而通过结合转录因子*sp1*上的启动子区域间接地调控Pept1表达. 小鼠上皮细胞中*cdx2*可结合到短链脂肪酸转运载体(solute carrier family 5A member 8, *SLC5A8*)启动子区域, 上调其mRNA表达[38]. *cdx2*除了参与调控上述与肠道营养物质转运相关基因表达外, 还参与一些其他关于肠道上皮细胞增殖分化和肠道上皮结构基因的表达调控. Yamamoto等人[39]证明, *cdx2*能够通过结合肠道黏蛋白基因(Mucin-2, *MUC2*)的顺式作用原件来激活其表达, 而*MUC2*在杯状细胞中特异表达, 因此*cdx2*在杯状细胞分化中起重要作用. 作为钙依赖型凝集素超家族的一员再生基因4(regenerating islet-derived protein 4, *RegIV*), 是表皮生长因子受体(epidermal growth factor receptor, EGFR)/Akt/actibator protein-1信号通路潜在的激活者, 在结直肠肿瘤中异位高表达; *cdx2*可以直接结合到*Reg IV*的启动子区域并且调控其表达[40]. Coskun等人[41]分析了与*cdx2*结合相关的全基因组启动子, 发现细胞吞噬运动蛋白3(engulfment and cell motility protein 3, *ELMO3*)是*cdx2*的潜在靶基因, 并验证了*cdx2*和*sp1*能够协同调控*ELMO3*基因的表达, 从而调控细胞的迁移. 最新研究表明, *cdx2*能够直接结合到氨基酸转运蛋白基因*SLC7A7*启动子区域并激活其表达从而诱导猪小肠上皮细胞增殖[42].

转录因子GATA家族属于锌指蛋白家族, *GATA-4/-5/-6*在成熟的小肠上皮细胞内表达, *GATA-4*在小肠的近端到远端呈由高至低的表达梯度, 而*GATA-5*和*6*在空肠和回肠高表达, 它们能抑制或激活肠道特异性基因*Lct*, *Invertase*, *I-FABP*和顶端钠离子/胆汁酸转运体(apical sodium-dependent bile acid transporter, *ASBT*)的表达[43~45]. *GATA-4*和*GATA-6*在肠上皮细胞结构和细胞分化中起着重要的作用; 成年小鼠小肠上皮中条件性敲除*GATA-4*和*GATA-6*引起十二指肠和空肠内干细胞增殖, 肠内分泌细胞和潘氏细胞显著减少, 而杯状细胞大量增加[46]. Walker等人[47]证明, GATA转录因子通过对Notch信号配体*Dll1*的精细调控来抑制分泌型细胞的分化. 另外, *cdx2*还与*GATA-4*和肝细胞核因子-1α(hepatocyte nuclear factor-1α, *HNF-1α*)协同作用调控肠道细胞分化的开始并参与肠道上皮结构发育的调整[48,49].

### 3.3 表观遗传修饰对肠道细胞分化的调控

表观遗传修饰不仅可以影响细胞基因的表达, 而且这种影响还可随细胞分裂而遗传. 近年来, 表观遗传调控干细胞的分化机制已成为研究热点和前沿领域. 最近的研究表明, 基因表达的骤变经常发生在细胞分化过程中, 并且是伴随着主要的染色质结构变化所引发的修饰, 如组蛋白尾部的乙酰化和基因甲基化. 研究发现,

组蛋白去乙酰化酶1(histone deacetylase 1, *HDAC1*)、组蛋白乙酰转移酶2(histone acetyltransferase 2, *Myst2*)、CREB结合蛋白(CREB binding protein, *CBP*)和E1A结合蛋白p300(E1A binding protein p300, *EP300*)在沿小鼠肠道隐窝-绒毛轴的表达量迅速下降; 这些基因在肠道细胞分化过程中与*SI*和*SGLT1*的启动子/增强子区域结合从而诱导其高表达. DNA甲基化主要发生在CpG双核苷酸序列的胞嘧啶上; 肠道细胞更新的研究主要集中在DNA甲基化的变化伴随着隐窝干细胞分化[50]以及DNA甲基化在早期是如何介入并控制细胞的分化[51]. Ziller等人[52]证明, DNA甲基化具有组织差异性的特点. 随后一系列的研究揭示了DNA甲基化在小肠细胞分化过程中的作用. 首先, 通过基因组水平的DNA甲基化图谱揭示了DNA CpG岛的甲基化是在小肠干细胞的分化过程中的一种动态表观遗传学标记, 虽然只有50个甲基化区域存在显著差异, 也说明DNA甲基化动态变化在小肠干细胞分化中具有一定的作用; 而转录因子Tcf4(TCF4 transcription factor 4)的结合与低甲基化现象相关联, 则证明了Tcf4的结合是形成甲基化区域差异性的一个重要原因[53]. 其次, 发现了增强子上DNA低甲基化在小肠干细胞自我增殖过程中是必要的[54]; 条件性敲除小鼠肠道甲基转移酶基因(methyltransferase 1, *Dnmt1*), 发现小肠隐窝增生[55], 在沿着小肠干细胞分化方向存在DNA甲基化改变, 其中主要是形成超甲基化现象, 并且在基因组中超甲基化的富集与基因调控紧密联系[56].

## 4 小肠上皮细胞更新及损伤修复的营养调控

肠上皮细胞功能的变化必然引起营养素代谢的变化, 而产生的代谢物也在一定程度上调控肠上皮细胞的增殖分化和肠道发育. 现有的研究表明, EGF、转化生长因子(transforming growth factor, TGF)、氨基酸及其衍生物和核苷酸等营养因子以及代谢产物分别通过不同的作用机制调控哺乳动物肠道上皮细胞增殖分化和损伤后的重新修复.

### 4.1 EGF

EGF是由53个氨基酸残基组成的单链小分子多肽, 在胃肠道等组织分布较丰富. 外源性EGF能促进小鼠肠黏膜辐射损伤后小肠隐窝细胞的快速增生, 证明了足够量的外源性EGF能促进小肠干细胞的增殖和分化, 以此来修复受损的肠上皮细胞[57]. 使用表达EGF的乳酸菌发酵上清液饲喂早期断奶仔猪, 发现能够促进新生仔猪的肠道发育, 其中杯状细胞的数量, 绒毛的高度和抗炎症因子IL-13的表达都显著增加[58]. 另外, EGF在小肠内能增加肠谷氨酰胺酶活性, 为小肠提供能量及酰胺氮以促进细胞增殖[59]. 而肠道隐窝-绒毛轴上的内源EGF信号也能促进小肠干细胞和短暂扩增细胞(TA细胞)的增殖[60]; 其作用机制是特异性结合细胞膜上的糖蛋白受体(EGFR), 而后受体磷酸化, 激活酪氨酸激酶, 开启一系列下游信号通路(如PI3K/Akt和Ras/Raf/MEK/ERK等通路)作用于细胞核内转录因子, 通过调节下游基因的转录来调控细胞的增殖和分化[61]. 在隐窝干细胞中, EGF的下游通路Ras/Raf/MEK/ERK是处于激活状态的[62].

### 4.2 TGF-β

TGF-β是一种产生于整个胃肠道的多肽, 在肠道黏膜上皮细胞的更新、损伤修复的调控以及维持肠道细胞完整性方面发挥了重要作用. 外源性TGF-β1可通过活化丝裂原活化蛋白激酶(mitogen-activated protein kinases, MAPK)、Smad信号通路增加细胞紧密连接蛋白的表达量, 从而加强肠黏膜免疫屏障功能, 缓解肠黏膜炎症及通透性增加[63]. 内源性TGF-β在肠道上皮细胞的增殖分化、促进细胞修复方面也具有重要调节作用; 作为TGF-β超家族的重要成员——BMP, 参与了调控细胞的增殖、分化和凋亡等诸多过程, 在胚胎发育和成体干细胞维持中发挥着重要的作用[64]. 由BMP信号控制小肠上皮细胞发育分化过程中的稳态的机制已经得到阐明; 早期胚胎肠发育过程中, 内胚层诱导信号BMP-4在内壁中胚层表达, 从而调节中胚层细胞增殖[65]; 随后, BMPs在绒毛生长之前介导了绒毛和隐窝周边间充质浓缩[66,67]; 在成熟的肠道中, BMPs主要控制肠上皮细胞增生[68]. 进一步的研究证明, 激活的BMP信号是通过细胞内的PTEN/PI-3K/AKT和Smad等途径直接抑制β-catenin的代谢和活性[69], 从而拮抗Wnt信号通路, 维持上皮细胞增殖与分化的动态平衡. 而隐窝基底层和周边细胞会分泌Noggin和Chordin等BMP的拮抗分子来抑制隐窝附近的BMP信号, 使干细胞周围的BMP保持较低的浓度, 促进肠隐窝干细胞的自我更新和不断增殖[70,71].

### 4.3 氨基酸极其衍生物

在肠道干细胞的增殖和分化过程中, 氨基酸通过激活多条信号通路来共同完成调节. *L*-谷氨酰胺(*L*-glutamine, Gln)作为非必需氨基酸, 是肠道的主要功能物质. Gln缺乏时可诱导鼠肠上皮细胞发生凋亡, 从而促进肠道干细胞的分化来形成新的上皮细胞[72]. 补充Gln能通过上调紧密连接蛋白的表达来促进细胞生长和维持细胞膜的完整性, 提高氧化应激反应和黏膜屏障功能[73]. 在Gln补给肠切除的动物模型实验中发现Gln能特异增加肠黏膜生长因子(EGF和IGF-II)的表达; 而EGF, IGF-II能通过激活一系列信号通路促进肠道干细胞的增殖和分化, 重建受损的肠道上皮[74]. Marc Rhoads和Wu[75]发现, Gln能激活MAPK; 在Gln补给对大鼠肠道损伤恢复的实验发现, Gln能显著促进P-ERK的表达[76], 上述研究说明, Gln在激活ERK信号通路的同时, 也通过增加生长因子的表达来促进肠道干细胞的增殖和分化, 从而修复受损的肠上皮. 通过饲粮添加Gln和丙氨酰谷氨酰胺二肽(Ala-Gln)对LPS诱导的仔猪应激实验结果显示, 在肠腔和血浆中Gln浓度增加, 肠道细胞中半胱氨酸蛋白酶-3(caspase-3)活性, 核转录因子κB(nuclear facter of κB, *NF-κB*)和Toll样受体4(Toll-like receptor 4, *TLR4*)的表达显著降低, 从而预防和降低肠道氧化损伤和炎症, 增强细胞生长性能[77].

*L*-精氨酸(*L*-arginine, Arg)作为一种碱性必需氨基酸, 在促进动物肠道生长和结构改善, 加速受损黏膜修复, 维护肠道屏障功能方面有重要作用. 研究证明, Arg与感应器(CASTOR1)结合后激活雷帕霉素靶蛋白(mTOR)/P70(S6)激酶通路, 增强黏膜细胞迁移和损伤恢复[75,78]. 本研究组通过在饲粮中添加Arg可提高断奶仔猪肠道热休克蛋白70(heat shock protein 70, HSP70) mRNA和蛋白的表达, 增加肠道黏膜杯状细胞的数量, 提高营养物质利用率[79]; 并发现补充Arg可增加断奶仔猪肠道绒毛高度和黏膜血管内皮生长因子水平, 降低皮质醇、氨(NH3)和尿素浓度, 提高血浆胰岛素和Arg浓度, 促进肠道发育[80].

N-乙酰半胱氨酸(N-acetyl-*L*-cysteine, NAC)是一种强有效的抗氧化剂, 在断奶仔猪饲粮中添加NAC后发现, 肠道黏膜抗凋亡基因*Bcl-2*, 细胞周期调控基因*E2F5*, *Smad4*表达显著上调, 而凋亡相关基因死亡受体(factor associated suicide, *Fas*)和创伤愈合指示基因整合素*αvβ6*(alpha v beta 6 integrin)表达显著下调, 表明NAC在一定程度上可以缓减仔猪断奶应激, 提高仔猪抗氧化能力, 保护肠细胞结构的完整性[81]. 饲粮中添加NAC也可缓解LPS刺激导致的仔猪肠黏膜中的免疫因子(IL-2和IL-6)和细胞紧密连接蛋白(occludin和claudin-1)表达升高, 并降低小肠黏膜细胞中caspase-3的表达, 有效缓解肠道黏膜损伤[82]. 另外, 赖氨酸[83]、蛋氨酸[84]、α-酮戊二酸[85]等在对维持肠黏膜形态和功能的完整性以及肠上皮细胞的增殖有一定的促进作用.

### 4.4 外源性核苷酸

外源性核苷酸对小肠细胞分化也有重要影响, 通过核苷混合物(胞苷、尿苷、鸟苷和肌苷)对大鼠小肠隐窝上皮细胞(IEC-6)进行培养; 结果发现尿苷、鸟苷和肌苷在培养基中消失, 而胞苷浓度增加; 且分化的IEC-6细胞中核苷酸的浓度显著升高. 这些变化同时伴随着碱性磷酸酶活性的增加, 绒毛形态的延长和*RND3*基因表达下调. 表明外源核苷酸选择性的被IEC-6细胞吸收, 增加了细胞内的核苷酸量、GTP和能荷, 有利于分化过程中细胞的形态和功能变化[86]. 在体内实验中, 核苷酸显著提高早期断奶大鼠肠黏膜Invertase活性; 结果表明, 核苷酸的补充对提高小肠上皮细胞体内和体外的增殖和成熟有促进作用, 因此, 外源核苷酸可能在肠道细胞更新中起着重要的作用[87].

### 4.5 其他

脂肪酸, 糖类除了作为肠黏膜细胞主要能量来源外, 还能减少炎症发生, 促进肠细胞代谢、增殖和分化. 饲粮中添加丁酸钠可增加仔猪肠黏膜厚度和肠黏膜上绒毛高度和隐窝深度的比值, 并促进结肠内杯状细胞的分化, 增强肠道消化吸收功能[88]. 本研究组通过在饲粮中添加壳多糖可增强肠黏膜紧密连接蛋白(occludin和ZO-1)的表达, 提高肠黏膜紧密连接性, 阻缓肠黏膜通透性升高, 并对大肠杆菌攻毒的早期断奶仔猪小肠黏膜屏障功能的损伤有保护作用[89]; 补充黄芪多糖(astragalus polysacharin, APS)能够促进断奶仔猪的肠道细胞更新, 改善肠道消化功能、调节氨基酸代谢, 从而达到促生长的目的[90]. 另外, 在饲粮中添加适宜的营养性微量元素锌(Zn)能通过促进肠黏膜分泌型免疫球蛋白A(SIgA)和IL-2的表达来维持肠黏膜免

疫屏障的稳定性, 进而促进肠道细胞的分化[91].

## 5 总结和展望

众所周知, 小肠上皮的更新需要隐窝基底部干细胞不断增殖和分化的维持. 到目前为止, 随着对小肠隐窝干细胞增殖分化机制的研究, 逐步发现了包括多种营养因子和Wnt信号通路等一系列信号在小肠细胞的增殖和更新中发挥着重要的调控作用, 而这些信号的异常与肠道疾病发生有着密切的关系. 虽然人们对小肠细胞自我更新的调控有一定的了解, 但是对于调控小肠干细胞增殖和分化信号通路的具体机制, 肠道营养物质的吸收量对肠上皮细胞分化与细胞结构变化的影响, 以及干细胞分化与肠上皮细胞功能之间的关系等许多问题还没有解决. 总之, 对小肠上皮细胞自我更新调控机制进行深入研究, 不仅可以使人们更加了解其内在的分子调控机制, 更能在营养学和药理学方面, 为干预和治疗肠道损伤及相关疾病提供新的线索和依据.

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# Current advances in renewal mechanisms of intestinal epithelial cells along the crypt-villus axis

ZOU LiJun[1,2,3], XIONG Xia[2], WANG XiaoCheng[2], YANG HuanSheng[1], LI JianZhong[1], HUANG PengFei[1] & YIN YuLong[1,2]

*1 Laboratory of Animal Nutrition and Human Health, College of Life Sciences, Hunan Normal University, Changsha 410081, China;*
*2 Key Laboratory for Agro-Ecological Processes in Subtropical Regions, Institute of Subtropical Agriculture, the Chinese Academy of Sciences, Changsha 410125, China;*
*3 Laboratory of Basic Biology, Hunan First Normal College Changsha, 410205, China*

The small intestine is not only an organ for food digestion and nutrient absorption but also an integral part of the immune and endocrine systems. Differentiation of enterocytes plays an important role in the damage repair mechanism after immune response elicitation by the intestine, for restoring the intestinal barrier function and homeostasis. Recently, research related to self-renewal, differentiation, and regulation of the intestinal epithelial crypt stem cells has been developing rapidly. In this review, we attempt to sum up recent studies related to the expression pattern of genes and proteins along the crypt-villus axis, and the effect of signaling pathways, transcription factors, and epigenetic modifications on the differentiation of crypt stem cells. We also examine the mechanisms by which nutritional factors influence the differentiation and damage repair processes of the intestinal epithelial cells. This review could provide valuable theoretical framework in nutritional and pharmacological aspects and guidance for intervention and treatment of intestinal damage and related diseases.

**small intestine, crypt-villus axis, cell renewal, damage repair, nutrition regulation**

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