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评 述

微生物和肠道干细胞互作调控幼龄动物肠道发育的研究进展

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摘要 肠道干细胞通过增殖和分化沿隐窝-绒毛轴不断地向上迁移, 构成全部肠上皮细胞, 是生命早期肠道发育的结构基础. 肠道微生物可通过其表面分子或者代谢物调控肠道干细胞的命运与功能, 继而影响肠道发育轨迹. 本文对肠道干细胞稳恒及上皮更新作用驱动幼龄动物肠道发育、修复肠道稳态的研究进行综述. 重点解析肠道微生物表面鞭毛蛋白、肽聚糖、脂多糖以及微生物三大代谢产物短链脂肪酸、色氨酸代谢物、次级胆汁酸对肠道干细胞增殖与分化的调控机制. 探讨益生菌、益生元等营养物质及菌群移植对肠道干细胞发育的促进作用, 为研究生命早期微生物菌群与肠道发育互作提供参考.

关键词 肠道发育, 干细胞, 微生物, 代谢产物, 营养调控

生命早期动物的生长速度与肠道发育和成熟密切相关, 肠道微生物通过定植和代谢与宿主肠上皮细胞互作, 共同维持肠道内环境稳态[1~3]. 幼龄动物肠道发育不健全, 易受多种因素影响破坏肠道稳态, 从而减缓生长发育速度甚至诱发疾病. 肠道干细胞(intestinal stem cells, ISCs)是肠道发育成熟的基础, 一方面ISCs受激素和酶的调控. 例如, 甲状腺激素、胰高血糖素样肽2等激素能够促进ISCs发育; 生长抑素和褪黑素则抑制ISCs增殖[4]. ISCs中组蛋白甲基转移酶SETDB1缺失引起的基因组不稳定会导致Z-DNA结合蛋白1介导的坏死性凋亡, 进一步触发肠道炎症[5]. 另一方面, ISCs受微生物自身或代谢活性的调控. 肠道微生物缺失会抑制ISCs的增殖分化, 与常规饲养小鼠相比, 无菌小鼠肠道ISCs向潘氏细胞的分化受阻, 且隐窝-绒毛轴的上皮细胞更新周期更长[6]. 此外, 肠内营养物质可驱动微生物区系变化, 同时调节ISCs的增殖、分化与肠上皮细胞的重塑. 如外源添加岩藻糖可改变幼龄小鼠肠道菌群的组成和功能, 增加*Akkermansia*的丰度和丙酸盐浓度, 从而显著提高ISCs的增殖速率和分化细胞的数量[7]. 本文综述ISCs维持肠道稳态的关键功能、ISCs经典调控通路及肠道微生物与ISCs互作的研究进展, 重点阐述微生物自身直接调控和代谢产物间接调

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控肠道发育与成熟的机制，进一步探讨益生菌、益生元、菌群移植对ISCs命运与功能的干预作用，为采用营养手段调控ISCs功能从而维持幼龄动物肠道稳态提供理论依据，促进机体健康生长、预防和治疗肠道疾病.

1 肠道干细胞与肠道发育

在发育过程中，隐窝中ISCs增殖和分化驱动肠上皮快速更新以维持肠黏膜屏障的完整性[8]. ISCs可以通过对称分裂重塑肠道组成以响应上皮缺陷，形成两个子代干细胞或两个子代非干祖细胞向上迁移，每3~5天完成一次肠道上皮细胞的更新[9]. ISCs通过增殖和分化动态调控肠道上皮细胞吸收和分泌谱系的分布, 在维持肠道稳态中发挥重要作用, 保证肠道上皮对营养物质的消化吸收. 此外, 在肠道屏障受损时, ISCs既是攻击肠道内稳态和屏障完整性的目标，也是损伤后肠道修复再生的关键调控靶点[8].

1.1 ISCs的稳恒与上皮更新作用

根据功能和空间位置以及分子标记的不同，将隐窝中的ISCs分为两类. 一类是以LGR5，SOX9作为分子标记的隐窝基底柱状细胞，定义为活性干细胞，通过不断地自我更新保证黏膜完整[10,11]. 小鼠肠道谱系追踪实验显示，LGR5$^+$隐窝基底柱状细胞在60天内产生所有的上皮细胞谱系[10]. LGR5$^+$ ISCs首先分化为分泌和吸收系祖细胞，进而分化为分泌细胞，即杯状细胞、潘氏细胞、肠内分泌细胞、簇状细胞，以及吸收细胞即肠道上皮细胞[10,12,13].

每种肠上皮细胞可以细分为不同亚型. 肠道上皮的杯状细胞是由两种亚型构成的动态系统，其中富集Fcgbp和Clca1的典型杯状细胞被认为是分泌黏液屏障的主要贡献者，而富集Dmbt1和Gsdmc4的非典型杯状细胞可通过表达消化酶及其他代谢相关基因参与肠腔内的食物消化[14]. 肠内分泌细胞感应不同的食物摄入和化学刺激，释放血清素、饥饿素和胃泌素等，根据其分泌不同激素的潜力定义细胞亚型[15]. 簇状细胞可分为免疫相关和神经元发育相关的两个亚型，前者表达TSLP和CD45，这是首次在非造血干细胞谱系中发现的CD45阳性细胞[15].

另外一类ISCs是储备干细胞，表达Bmi1，Lrig1，Hopx，Tert[16]. 活性干细胞对DNA损伤敏感, 储备干细胞则具有损伤抗性[17]. 肠道处于稳态时，储备干细胞是静止的；肠道受到损伤时，储备干细胞活化以补充活性干细胞池[18,19]. 此外，肠道损伤后兼性干细胞可通过去分化以补充LGR5$^+$ ISCs活性干细胞池. 例如, 已分化的潘氏细胞通过激活Notch信号通路去分化补充活性干细胞池，促进肠道再生[20]. 已分化的肠道上皮细胞通过自噬提高哺乳动物雷帕霉素靶蛋白复合体1(mammalian target of rapamycin complex 1, mTORC1)活性去分化为活性干细胞，推动肠道上皮细胞再生和屏障恢复[21,22]. 目前尚未准确鉴定兼性干细胞的特异性表达标记，高自噬囊泡水平可能是多谱系兼性干细胞的潜在标志，细胞表现出DNA损伤抗性和细胞可塑性[22].

1.2 ISCs驱动幼龄动物肠道发育, 修复肠道稳态

在动物生长过程中，需要有足够的活性干细胞池应对内环境的变化，从而满足上皮更新需要，增加幼龄动物肠道上皮细胞表面积，促进肠道发育[23]. 研究表明，仔猪断奶后肠道活性干细胞和储备干细胞均减少，而SOX9$^+$细胞与Hopx$^+$细胞的体积比增加，表明储备干细胞补充活性干细胞池[11]. 幼龄动物肠道干细胞增殖驱动隐窝数量增加，与随后隐窝增生联合作用导致隐窝细胞不断向绒毛轴驱动，增加幼龄动物肠道上皮细胞表面积，促进小肠生长发育[23]. 仔猪结肠从出生到断奶阶段隐窝深度虽然持续增加，但断奶阶段隐窝分支减少[11]，表明断奶应激启动ISCs对肠道损伤修复的响应，但不能及时恢复肠道上皮健康. 小鼠的早期断奶通过减弱Wnt/β-catenin信号传导抑制ISCs的自我更新, 延缓ISCs分化为转运扩增细胞和潘氏细胞, 减弱ISCs驱动的肠上皮再生和隐窝裂变的活性，加速绒毛上皮细胞的凋亡, 从而表现为肠上皮萎缩[24].

在家禽生产中，雏鸡的首次饲喂时间易受到饲养管理的影响而延迟，这会限制雏鸡肠道隐窝深度和绒毛高度，改变ISCs的分化进程并破坏黏蛋白的产生；提早首次饲喂时间可提升肠上皮ISCs和祖细胞的增殖、分化, 或通过影响肠道菌群, 促进肠道成熟[25]. 综上所述, 活性干细胞的扩增推动隐窝裂变, 重新填充肠道组织受损的部位；随后ISCs分化命运的变化影响肠上皮中关键功能细胞的构成，对肠道健康和发育的影响极大.

1.3　干细胞增殖分化的调控机制

Wnt, 骨形态发生蛋白(bone morphogenetic protein, BMP), Notch信号通路在ISCs中表达活跃, 是参与ISCs增殖和分化调控的主要信号通路. Wnt信号不仅驱动ISCs增殖, 还影响Paneth细胞的成熟[6]. Wnt配体与ISCs表面卷曲蛋白和低密度脂蛋白受体相关蛋白(low density lipoprotein receptor-related protein, LRP)LRP5-LRP6结合, 抑制β-catenin泛素化, 诱导β-catenin的核易位与细胞核内淋巴增强子因子/T细胞因子(LEF/TCF)家族转录因子复合, 反式激活Wnt靶基因[26]. Wnt抑制剂Dickkopf阻断幼龄大鼠中的Wnt/β-catenin信号传导, 减少LGR5$^+$ ISCs, 从而阻止隐窝裂变[27]. 而注射Wnt激动剂R-spondin1可促进隐窝过度增殖[28].

BMP信号通路对ISCs的自我更新、复制和增殖起负调节作用, 其活性受到BMPRII, BMPRI, TGFβ, R-SMAD和SMAD4级联调节的严格控制[1]. 间充质细胞产生配体BMP2和BMP4, 激活具有丝氨酸/苏氨酸蛋白激酶活性的跨膜受体BMPRI和BMPRII复合物, 进一步通过SMAD蛋白的磷酸化调控靶基因表达[29]. 为减弱BMP信号对ISCs的抑制作用, BMP拮抗剂如Noggin, Gremlin-1和Gremlin-2在隐窝中的高度表达能够促进ISCs增殖[26].

Notch信号转导被证明参与调控肠道分化谱系, Notch信号传导的抑制增加杯状细胞的数量[30]. Hes1作为Notch信号通路靶基因, 激活后抑制转运扩增祖细胞中Atoh1的表达, 从而驱动向吸收谱系的分化; 而Hes1的缺失和Atoh1的表达促进ISCs向分泌谱系分化[1,20].

已有综述阐明Wnt, BMP, Notch信号通路调控肠道类器官细胞向不同谱系富集. 在肠道中, Wnt和Notch配体由潘氏细胞分泌, 其中Wnt的信号水平在上皮细胞表面并非均匀分布, 从隐窝到绒毛尖端的Wnt信号水平降低, 而BMP信号增加, 共同调节肠道干性. 此外还需要有表皮生长因子信号通路和R-spondin信号通路的共同作用, 调控机体肠道内ISCs自我更新和谱系分化平衡. 在类器官水平以关键通路为靶点调控细胞谱系定向富集, 如分化偏好向杯状细胞发展则有利于研究黏膜免疫和对病原体的反应. 然而在体内, 若靶向Wnt或Notch可能导致ISCs耗竭或诱发癌症. 因此, 了解微生物及代谢物影响ISCs功能及生态位可能有利于发现更为安全有效的调控上皮细胞命运、改善肠道发育的干预手段, 治疗疾病[29].

2　微生物调控肠道干细胞

动物出生后肠道微生物会随着时间的推移逐渐丰富并定植, 可通过细胞壁的直接作用、或通过不同的代谢产物间接作用(图1), 参与肠道发育过程中ISCs的增殖、分化[6,31]. 研究表明, 外源添加鼠李糖乳杆菌促进幼龄动物ISCs向潘氏细胞分化并缓解新生儿坏死性小肠结肠炎[6]. 植物乳杆菌WJL及其热灭活细菌细胞壁可通过作用于肠上皮的模式识别受体核苷酸结合寡聚化结构域蛋白2(nucleotide-binding oligomerization domain 2, NOD2), 缓解出生后营养不良对小肠隐窝细胞增殖的有害影响, 改善营养吸收, 从而促进宿主生长[32]. 同时, 唾液乳杆菌上清液中的代谢产物琥珀酸可调控ISCs活性[9]. 本小节将分别阐述微生物通过直接作用或间接通过代谢产物调控ISCs增殖、分化的研究进展.

2.1　微生物细胞壁及表面成分调控肠道干细胞发育

2.1.1　鞭毛蛋白

鞭毛是细菌的运动器官, 也是重要的毒力因子, 能够有效激活天然免疫应答[33,34]. 肠致病性大肠杆菌可引起幼儿腹泻, 其鞭毛是介导黏附的关键物质[35]. 肠腔中的鞭毛蛋白穿过黏液层到达上皮, 并在发生肠道损伤时到达固有层[36,37]. 鞭毛蛋白是宿主膜蛋白受体Toll样受体5(Toll-like receptor, TLR5)的特异性配体[38]. 有研究表明, 鞭毛蛋白与TLR5结合激活蛋白激酶磷酸酶-7(mitogen-activated protein kinase phosphatase-7, MKP-7)转录, 并抑制JNK信号通路活性, 从而减少回肠隐窝细胞凋亡, 保护小肠隐窝上皮细胞免受辐射损伤[39]. 在结肠中鞭毛蛋白可与ISCs的TLR5互作上调NADPH氧化酶1(NADPH oxidase 1, NOX1)表达, 释放活性氧(reactive oxygen species, ROS)增强表皮生长因子受体的激活, 促进结肠ISCs的增殖[40].

鞭毛蛋白也可能通过非TLR5介导的途径调控肠道发育. 例如, 研究表明鞭毛蛋白增加肠隐窝中肠道干细胞标志物OLFM4阳性细胞的数量, 恢复隐窝干细胞功能, 增加来源于LGR5$^+$ ISCs的M细胞密度的过程与TLR5无关[41]. 以及*Desulfovibrio vulgaris*鞭毛蛋白通过

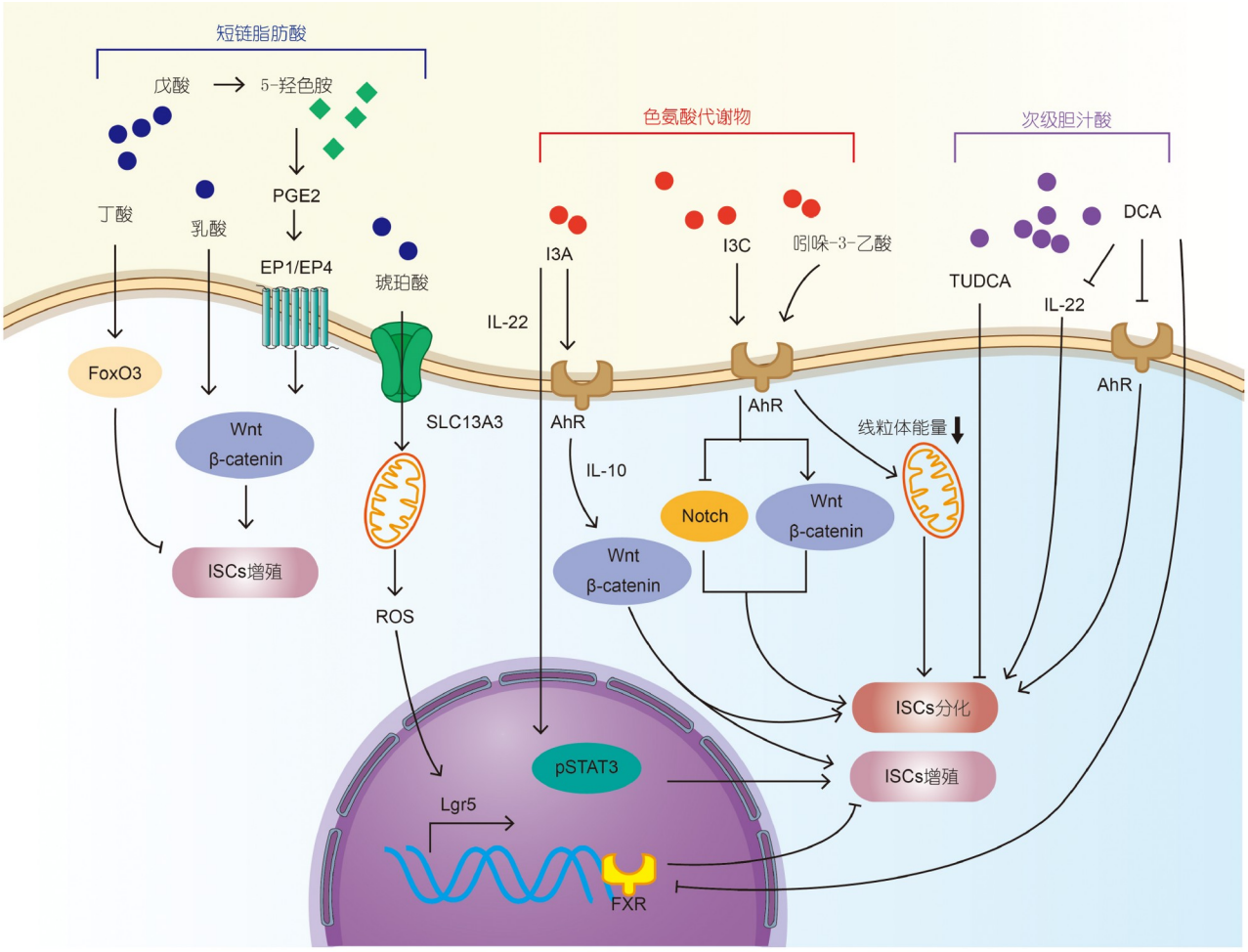

图 1 肠道微生物代谢物调控宿主肠道干细胞增殖分化机制
Figure 1 Mechanisms of gut microbiota metabolism-mediated host ISCs proliferation and differentiation

非TLR5介导的途径引起肠隐窝丢失, 与跨膜蛋白富含亮氨酸重复19(Leucine-rich repeat containing 19, LRRC19)相互作用诱导结肠缩短, 使DSS结肠炎恶化[42].

此外, 细菌鞭毛蛋白可能通过引起免疫反应, 抵抗肠道损伤. 研究表明鞭毛蛋白可诱导TLR5介导的IL-22和含CARD结构域的NOD样受体家族成员4(NOD-like receptor family CARD domain-containing protein 4, NLRC4)介导的IL-18的产生[43]. IL-22诱导STAT3蛋白磷酸化而与IL-18基因启动子结合, 通过IL-18促进类器官出芽并激活蛋白激酶B(protein Kinase B, Akt)-转录因子4(transcription factor 4, Tcf4)信号诱导LGR5^{+} ISCs扩增, 促进组织修复[44]. 这为研究者了解鞭毛蛋白的作用提供新的见解. 由此可见, 鞭毛蛋白作用于TLR5受体或通过非TLR5的途径, 诱导肠道上皮启动差异响应机制影响ISCs增殖, 使肠道炎症恶化或通过调控免疫反应促进组织修复.

2.1.2 肽聚糖

ISCs表达核苷酸结合寡聚化结构域样受体(nucleotide-binding oligomerization domain-like receptors, NLRs), 主要为NOD1和NOD2两型, 可识别细胞壁肽聚糖[45]. NOD1主要识别革兰氏阴性菌细胞壁肽聚糖的特征性组分二氨基庚二酸(diaminopimelic acid, DAP)[46,47]. DAP是免疫缺陷信号通路(immune deficiency, IMD)最有效的诱导物[48], 且IMD靶基因激活肽聚糖识别蛋白LC受体, 最终增加老年肠道中ISCs的增殖, 缩短果蝇寿命[49].

NOD2能够识别胞壁酰二肽(muramyl-dipeptide, MDP)结构. 有研究表明, MDP激活LGR5$^+$ ISCs的NOD2, 通过自噬相关16样蛋白1共同介导激活线粒体自噬, 清除ISCs中过量ROS, 保护ISCs免受氧化应激损伤, 并增加ISCs数量以维持肠道上皮再生[50]. 此外, 有研究表明肽聚糖经过胞壁酰胺酶降解产生MDP, 并且激活NOD2, 增加杯状细胞密度[51], 且DAP可能协同发挥作用. 因为研究表明MDP与DAP二者联合刺激激活NOD1和NOD2是上调杯状细胞数量并产生黏蛋白所必需的, 而单独刺激则不能发挥作用[52].

2.1.3　脂多糖

脂多糖(lipopolysaccharide, LPS)是革兰氏阴性菌细胞壁外侧的一种糖脂质, 通过脱落或细菌裂解释放. 研究发现, LGR5$^+$ ISCs细胞表达LPS配体TLR4, 在小肠中激活TLR4可通过p53上调凋亡调控因子(p53 up-regulated modulator of apoptosis, PUMA)通路、半胱天冬酶8或内质网应激诱导ISCs凋亡[53,54]. 研究表明, LPS激活TLR4促进PUMA通路表达, 使得ISCs增殖减少、凋亡增加, 该过程依赖于β干扰素TIR结构域衔接蛋白(TIR-domain containing adaptor inducing interferon-β, TRIF), 而不依赖于髓样分化因子88(Myeloid differentiation primary response gene 88, MyD88)和TNF-α[53]. 雏鸡腹腔注射LPS降低小肠杯状细胞密度以及隐窝内增殖细胞核抗原(proliferating cell nuclear antigen, PCNA)蛋白水平, 使肠上皮凋亡细胞增多, 增殖和凋亡平衡受损, 诱发黏膜炎症[55,56]. LPS处理回肠类器官降低LGR5和OLFM4的转录翻译水平, 减少肠道类器官出芽数量, 导致其干性丧失[57]. 在结肠中, 定植在近端结肠和盲肠隐窝的特异性核心微生物群产生的LPS能够通过TLR4/TNF-α/RIPK1/RIPK3信号通路介导结肠LGR5$^+$ ISCs或转运扩增细胞的坏死性凋亡, 从而抑制肠上皮增殖, 并促进ISCs向杯状细胞分化[54].

LPS在正常生理条件下抑制小肠及大肠ISCs增殖并促进凋亡; 在非正常生理条件如肠道炎症情况下, LPS则加重肠道损伤. 研究表明, 5 μg/mL高浓度LPS能够促进炎症性肠病结肠类器官的生长速度、增加隐窝数量, 提高Ki67, PCNA, Hopx和OLFM4表达, 上皮细胞异常增殖可能导致更加严重的肠道炎症[58].

此外, LPS可能在肠道发育中发挥有益作用. 机体对LPS损伤有一定程度的自发修复作用, LPS通过Krüppel样因子5(Krüppel-like factor 5, KLF5)转录因子直接激活储备干细胞, 并通过KLF5促进STAT5A的表达从而激活活性干细胞, 共同促进损伤后黏膜修复[59]. 有研究表明, LPS口服而非静脉注射将不会引发炎症[60], 低剂量LPS能够抑制促炎细胞因子的上调、激活巨噬细胞、调节免疫功能, 有利于治疗和预防疾病[61,62].

综上所述, 当肠道处于正常生理状态时, LPS抑制ISCs增殖促进凋亡; 在肠道炎症发生时, 则促进ISCs增殖使疾病恶化. 随着微生物来源的不同和LPS毒性成分脂质A结构的变化, LPS的毒性也随之不同[63]. 通过区分LPS的毒性差异、合理改变LPS处理方式和剂量, 可将LPS作为正向调控ISCs的有效治疗手段, 进一步发挥免疫改善作用.

2.2　微生物间接调控肠道干细胞发育

2.2.1　短链脂肪酸

肠道微生物代谢产物一方面可以作为宿主代谢的底物, 另一方面可以作为信号分子调控机体多种生理生化反应[64]. 动物肠道内有丰富的短链脂肪酸生成菌, 能够发酵膳食纤维产生以乙酸、丙酸、丁酸为主的短链脂肪酸(short-chain fatty acids, SCFAs)[65]. 研究发现, 仔猪断奶过程中结肠隐窝数量、隐窝深度、杯状细胞数量增加, 以及结肠隐窝中Ki67阳性细胞数量均与总SCFAs浓度呈正相关[66]. SCFAs充当组蛋白去乙酰化酶抑制剂使Notch靶基因Hes1转录激活, 促进ISCs向吸收谱系分化[67].

丁酸盐是肠道微生物代谢产物中最丰富的一种. 在正常生理状况下, 结肠细胞通过酰基辅酶A脱氢酶依赖性氧化磷酸化代谢丁酸盐作为其能量来源从而促进增殖, 同时避免隐窝底部利用葡萄糖的ISCs暴露在高浓度丁酸盐的环境[68]. 当肠道发生损伤时, 肠道上皮结构的破坏导致丁酸盐更容易进入隐窝, 通过增加转录因子FoxO3活性, 抑制ISCs增殖[68]. 戊酸可间接调控ISCs的增殖, 戊酸促进肠道5-羟色胺能神经元中血清素代谢相关基因色氨酸羟化酶-2(tryptophan hydroxylase 2, TPH2)的表达促进5-羟色胺产生, 通过5-羟色胺激活前列腺素E2(prostaglandins E2, PGE2)巨噬细胞亚群中PGE2的产生, 而PGE2与受体EP1/EP4结合, 增强ISCs中的Wnt/β-catenin信号传导, 促进ISCs的自我更新[69].

琥珀酸和乳酸作为代谢合成SCFAs的前体物质, 在盲肠和近端结肠中的浓度较高, 既能够为结肠细胞供能, 又具有信号传导功能[65,70]. 琥珀酸作为唾液乳杆菌的代谢产物, 经溶质载体家族13成员3(solute carrier family 13 member 3, SLC13A3)转运至ISCs内, 诱导高线粒体膜电位和ROS释放, 增强LGR5表达促进ISCs活性[9]. 以剂量50 mmol/L的琥珀酸饲喂蛋鸡或1000 μmol/L琥珀酸处理肠道类器官均能够增强LGR5和PCNA的表达[9]. 而另一项研究表明, 100 mmol/L高浓度琥珀酸注入大鼠远端结肠显著抑制结肠细胞增殖, 减少肠道隐窝大小[71]. 因此, 琥珀酸盐的生理功能可能取决于其浓度. 生理水平浓度的琥珀酸促进细胞再生及组织修复, 而高浓度琥珀酸诱导细胞凋亡[72], 其中的复杂机制还需要进一步研究. 乳酸能够增加隐窝细胞线粒体呼吸, 并与G蛋白偶联受体81(G-protein coupled receptor 81, GPR81)结合激活ISCs中的Wnt/β-catenin信号, 从而刺激ISCs增殖[73]. 综上, 肠道内丰富的SCFAs是调控ISCs功能的关键因素, 但浓度过高可能造成细胞毒性作用, 破坏肠道发育.

2.2.2 色氨酸代谢物

色氨酸代谢途径有三条——在肝脏中被吲哚胺2,3-双加氧酶(indoleamine 2,3-dioxygenase, IDO)或色氨酸2,3-双加氧酶(Tryptophan 2,3-dioxygenase, TDO)氧化生成犬尿氨酸、在中枢神经系统和肠道中由TPH合成血清素, 以及经肠道微生物代谢产生吲哚及其衍生物, 如吲哚-3-甲醛(Indole-3-carboxaldehyde, I3A)、吲哚-3-甲醇(Indole-3-carbinol, I3C)、吲哚-3-乳酸等. 色氨酸酶可催化色氨酸形成吲哚, 该酶存在于许多革兰氏阴性菌和革兰氏阳性菌中, 包括大肠杆菌、梭状芽胞杆菌和拟杆菌属[74]. 吲哚及其衍生物可激活芳香烃受体(aryl hydrocarbon receptor, AhR), 参与肠道上皮增殖、肠道稳态及肠道炎症的调控[75]. I3A可增加断奶仔猪肠道AhR和PCNA的表达[76], AhR诱导固有层淋巴细胞分泌IL-22, 进一步激活STAT3磷酸化, 促进ISCs增殖, 恢复受损肠黏膜[77]. 此外, I3A诱导AhR/IL-10/Wnt信号激活ISCs增殖和分化[78]. 并且同样能够通过AhR和IL-10促进杯状细胞分化, 而该过程与IL-22或Ⅰ型IFN无关[79]. 吲哚-3-乳酸在幼龄和成熟小鼠肠道中对细胞增殖的影响不同, 吲哚-3-乳酸促进幼龄小鼠回肠Ki67的表达, 而使成熟小鼠肠道Ki67表达降低[80]. I3C抑制肠道类器官的发育并以AhR依赖性方式促进小鼠肠道杯状细胞分化. I3C增加活性非磷酸化β-catenin的水平, 抑制Notch信号通路调控下游靶基因Hes1, 增加参与分泌谱系分化的Math1, 表明这种调节可能由Wnt和Notch信号通路介导[81]. 不同于I3A及I3C促进分泌谱系分化, 吲哚-3-乙酸抑制分泌谱系分化, 与通过AhR抑制ISCs线粒体呼吸从而限制分化所需能量有关[82].

2.2.3 次级胆汁酸

胆汁酸(bile acids, BAs)可分为初级和次级BAs. 初级BAs由肝脏中的胆固醇合成, 流入肠腔协助脂质消化吸收, 并在小肠后段及大肠经肠道微生物的作用部分被水解脱去7α羟基, 转化为次级BAs. 95%胆汁酸经肠壁重吸收由门静脉进入肝脏, 剩余5%随粪便排出[83]. 次级BAs可通过TGR5/R-spondin(Rspo)依赖性或FXR/Rspo非依赖性机制促进ISCs的增殖. 次级BAs通过激活肠道上皮TGR5诱导Rspo3表达, 增强Wnt/β-catenin信号通路, 进而促进ISCs自我更新和增殖[84]. 在人类和小鼠中, TGR5表达的缺失可导致肠道炎症, 抑制ISCs增殖和分化, 并破坏肠上皮稳态[85]. 另外, 次级BAs脱氧胆酸(deoxycholic acid, DCA)可能抑制FXR从而诱导$LGR5^+$ ISCs增殖[86]. 仔猪出生后14天内BAs的合成和代谢以及肝脏的重吸收能力得到增强. 猪去氧胆酸(hyodeoxycholic acid, HDCA)是猪的主要次级BAs, 可通过FXR抑制PI3K/AKT通路降低断奶仔猪肠道增殖标志物如PCNA和细胞周期蛋白D1的表达, 从而抑制肠道上皮细胞增殖[87].

多种次级BAs能够影响ISCs的分化功能, 且调控机制不相同. HDCA抑制十二指肠与回肠肠内分泌细胞的分化, 同时增加空肠内分泌N, CCK, D和肠嗜铬细胞分化[88]. 牛磺酸脱氧胆酸(Tauroursodeoxycholate, TUDCA)能够缓解内质网膜复合物亚基3缺失导致的内质网应激, 恢复肠道分泌谱系的分化, 减轻分泌功能障碍, 恢复类器官形成[30]. DCA通过下调AhR信号破坏ISCs的分化功能, 从而导致杯状细胞和MUC2减少[89]. 研究人员认为, 其可能的机制是DCA下调潘氏细胞IDO1的活性, 影响隐窝犬尿酸的旁分泌, 导致AhR配体不足[89]. 同时, 也有报道称DCA破坏产生IL-22的细胞如3型天然淋巴细胞并降低肠道IL-22水平, 导致ISCs分化为潘氏细胞和杯状细胞障碍[90].

目前在胆汁酸多种代谢物协同发挥作用的研究具有局限性, 不能明确是哪一种微生物代谢物的作用; 另一方面, 对于微生物代谢物次级BAs在生产中的应用转化尚未启动.

3　干预微生物调控肠道干细胞的营养手段

生命早期, 肠道隐窝中ISCs随着肠道发育而发生动态变化, 该过程对肠道微生物群高度敏感[6]. 通过直接或间接手段调控生态位中微生物因素, 无疑是干预幼龄动物肠道发育的主要方向(表1).

3.1　益生菌

益生菌是一类能够改善宿主健康的活微生物, 包括乳杆菌属、双歧杆菌属、芽孢杆菌属等. 已有大量研究报道饮食添加益生菌增加肠道内有益微生物的含量、调控肠道正常发育、保护肠道免受损伤[100]. 在生命早期塑造健康的乳酸菌群有利于肠道发育和黏膜屏障功能的维持[100]. 3日龄雏鸡灌胃罗伊氏乳杆菌22(*L. reuteri* 22)能够增加LGR5转录水平并激活Wnt/β-catenin信号通路, 促进PCNA的表达[101]; 添加枯草芽孢杆菌、地衣芽孢杆菌、粪肠球菌和酵母组成的复合益生菌与其作用类似, 均促进ISCs的增殖分化, 加速隐窝到绒毛轴的细胞更新速率, 促进肠道生长[101,102]. 此外, *L. reuteri* 22还抑制Notch信号通路, 诱导ISCs分化为杯状细胞, 以改善肠道先天黏膜免疫[101].

益生菌一方面促进正常机体肠道发育, 另一方面能够修复疾病状态下遭到破坏的肠上皮. 嗜酸乳杆菌能够抑制鼠伤寒沙门氏菌诱导的杯状细胞和潘氏细胞的过度扩增, 该作用可能与TLR2受体激活及抑制Wnt/β-catenin途径的过度激活有关[92], 同时通过调节Notch通路改善杯状细胞损失, 最终改善沙门氏菌诱导的肠

表 1　干预微生物调控肠道干细胞的营养手段

Table 1　Nutritional interventions for regulating intestinal stem cells through modulating microbial

营养调控	体内/体外模型	对微生物的影响	对ISCs的调控	参考文献
罗伊氏乳杆菌	5~7周龄C57BL/6小鼠	抑制小鼠柠檬酸杆菌定植	通过增加R-spondins的表达激活Wnt/β-catenin通路从而改善隐窝增生, 促进ISCs向潘氏细胞分化	[91]
嗜酸乳杆菌	6~7周龄SPF雌性C57BL/6小鼠	抑制鼠伤寒沙门氏菌的侵袭	改善潘氏细胞的过度扩增以及Notch通路激活导致的杯状细胞损失; 通过作用于TLR2改善对Wnt/β-catenin途径的过度激活	[92]
唾液乳杆菌和敏捷乳杆菌	280日龄母鸡	增加肠道益生菌的丰度, 如双歧杆菌、丁酸梭菌、干酪乳杆菌等; 降低病原微生物的丰度	通过抑菌作用降低隐窝LGR5水平防止ISCs过度分化, 维持ISCs的静止状态	[9,93]
唾液乳杆菌YL20	2日龄C57BL/6小鼠 19周龄小鼠小肠类器官	抑制阪崎肠杆菌与上皮细胞的黏附侵袭, 部分逆转潜在肠道致病菌肠杆菌和肠球菌的丰度增加以及双歧杆菌、拟杆菌门和乳酸杆菌丰度的降低	激活ISCs介导的肠上皮细胞增殖; 促进肠道类器官中上皮细胞的增殖, 挽救出芽受损的类器官, 防止LGR5的mRNA水平降低, 改善肠道干细胞功能, 增加杯状细胞数量	[94]
岩藻糖	4周龄C57BL/6小鼠	改变肠道细菌的组成和功能, 益生菌*Akkermansia*显着增加	*Akkermansia*和丙酸盐代谢的显着增加与ISCs的干性增加有关, 体现在增殖速率和分化细胞的数量的提高; 通过Wnt信号依赖性方式加速ISCs介导的肠上皮发育	[7]
低聚木糖	28日龄断奶仔猪	增加有益菌丰度, 降低有害菌丰度, 增加细菌多样性	增加回肠隐窝深度, 并且通过提高丁酸盐产量增加盲肠隐窝中杯状细胞数量	[95]
α-亚麻酸	6周龄雄性KM小鼠	增加瘤胃球菌科和普雷沃菌科丰度	瘤胃球菌科产生的SCFAs通过激活Wnt/β-catenin信号通路介导α-亚麻酸促进ISCs增殖	[96]
菊粉	成年C57BL/6小鼠	增加拟杆菌、厚壁菌和放线菌种的丰度	增加ISCs增殖、隐窝深度和结肠长度	[97]
棉子糖	7~8周龄SPF雌性BALB/c小鼠	促进罗伊氏乳杆菌的生长	罗伊氏乳杆菌促进棉子糖代谢成果糖并参与糖酵解, 促进ISCs增殖	[98]
碱性矿泉水	断奶仔猪腹泻易感模型	影响肠道微生物的多样性, 并提高有益微生物的数量	激活Wnt/β-catenin信号通路, 促进ISCs的增殖分化	[99]

上皮损伤[103]. 出生后外源补充鼠李糖乳杆菌可通过巨噬细胞极化调控肠道间充质生态位, 以促进ISCs向潘氏细胞分化, 缓解新生儿坏死性小肠结肠炎[6].

3.2 益生元

益生元不易被机体分解、吸收和利用, 而是一类能够被宿主微生物选择性利用的底物, 刺激有益菌生长, 抑制致病菌生长, 对宿主健康有益[104]. 目前研究较多的益生元包括低聚糖、多酚、黄酮、膳食纤维等. 低聚糖通过塑造肠道微生物群, 如提高机体肠道双歧杆菌和乳杆菌丰度, 发挥有益作用[105]. 唾液酸乳糖(salivary lactose, SL)是重要的乳寡糖, 6′-唾液酸乳糖(6′-SL)和3′-唾液酸乳糖(3′-SL)是唾液酸乳糖的主要形式. 研究发现, 仔猪出生后第3天至第38天补充6′-SL和3′-SL的混合物能够上调回肠中胶质细胞源性神经营养因子及其信号通路下游靶点环磷腺苷效应元件结合蛋白磷酸化, 提高Ki67和唾液酸转移酶ST8Sia Ⅳ转录和翻译水平, 从而促进新生仔猪肠道发育, 并预防早期断奶腹泻[106]. 2′-岩藻糖基乳糖(2′-fucosyllactose, 2′FL)能够增加仔猪回肠隐窝深度[107], 直接与TLR4结合[108], 提示其可能参与ISCs的调控.

植物提取物作为常见的益生元, 在调控幼龄动物肠道发育中也具有较大的应用价值. 通常, 植物提取物介导肠道菌群组成和代谢产物种类及丰度调控肠绒毛增长和发育, 降低肠上皮细胞凋亡, 促进细胞增殖[109~111]. 黄连素是一种关键的黄酮, 已被证明能够通过增加肠道基质细胞Wnt基因的表达, 促进$LGR5^+$ ISCs分化, 从而恢复结肠炎动物模型的肠道上皮完整性[112]. 膳食纤维类益生元棉子糖维持罗伊氏乳杆菌的生长, 进而将棉子糖代谢成果糖, 果糖增强并参与糖酵解以促进ISCs增殖[98].

在实际生产中, 益生元的过量摄入会导致肠道pH降低, 从而导致腹胀、腹泻的症状. 因此, 益生元应用过程中应确定使用剂量及与益生菌联合使用的方法, 从而达到最佳效果.

3.3 菌群移植

粪菌移植(fecal microbiota transplantation, FMT)是临床中主要用于肠道疾病治疗的菌群移植方法. FMT能够改变受体肠道微生物菌群结构, 改善肠道形态及屏障功能, 加强免疫功能, 提高抗病能力[113,114]. 同时, 母婴之间的微生物传递也是菌群移植的一种形式, 在此过程中新生儿获得母源肠道微生物群用于早期肠道菌系、屏障的建立, 继而影响婴儿的后续生长发育潜能[115]. 例如, 从母体移植粪便微生物群能够使剖宫产新生儿粪便微生物发育正常, 改善剖宫产新生儿肠道拟杆菌属的缺乏和双歧杆菌属定植的延迟[115]. 剖宫产婴儿双歧杆菌定植延迟的改善, 可能调节乙酸盐的产生和肠道酸性环境从而改善肠道健康[116]. 此外, 在畜牧饲养中普遍存在寄养模式. 约克夏和梅山母猪后代交叉寄养试验表明, 梅山母猪饲养的仔猪猪链球菌数较低, 抗炎细胞因子表达较高, 通过重塑菌群调控免疫状态从而影响生命早期肠道健康[117]. 但该研究并未分析母乳、粪便、皮肤、饲养环境的微生物群影响占比, 因此无法确定母体微生物来源及效应大小.

有研究发现, 健康成年猪粪便作为供体向受体仔猪进行FMT, 导致隐窝深度减少, 肠黏膜中杯状细胞的数量和MUC2表达增加, 这可能是通过激活TLR2, TLR4导致的[114]. FMT不仅有效传递供体粪便微生物, 还能够改变受体动物代谢特征, 调控肠道内代谢产物, 结合并激活相关受体以及下游通路, 从而调控ISCs及肠道发育[118]. 例如, 成年猪向仔猪FMT改变肠道微生物色氨酸代谢, 可能通过增加吲哚-3-乙酸激活AhR-IL22轴改善肠道生理功能, 转录组分析进一步表明FMT后Wnt和Notch信号通路的增加可能是保护肠道免受LPS诱导的ISCs及肠道发育损伤的因素[118].

需要注意的是, 以肥胖成年猪为供体的粪菌移植破坏仔猪肠道屏障, 增加受体仔猪血液促炎因子水平[119]. 这说明FMT并非有利无害, 未来需要建立粪便库并开展安全性研究, 来达到标准化[120].

4 总结与展望

肠道干细胞的增殖分化是影响肠道发育的关键因素, 平衡上皮细胞吸收和分泌谱系的分布对于肠道更新以及发育十分重要. 尤其是在生命早期, ISCs的增殖及分化命运将维持肠道健康及消化吸收功能. 然而幼龄动物肠道脆弱, 易受到损伤应激, 隐窝的破坏使ISCs环境暴露, 与肠道内容物和微生物接触对ISCs产生细胞毒性作用. 本文从肠道微生物角度入手, 探讨肠道微生物能够通过细胞壁及表面组分直接与宿主肠

道细胞模式识别受体结合，通过多种信号通路串联调控ISCs功能；还能够间接调控微生物代谢产物组成，影响肠道细胞能量底物利用水平或作为功能活性物质调控ISCs功能. 基于肠道微生物及代谢物调控ISCs命运，综述外源干预手段，以益生菌、益生元、菌群移植重塑肠道菌群组成改变肠道代谢特征.

微生物的结构和功能差异，一方面直接决定其表面物质或毒性因子的多样性[63]，另一方面深刻影响代谢物的代谢偏好[121]. 微生物通过直接作用和代谢产物的间接作用调控下游机制的研究较多，但对于上游发挥作用的特定细菌尚未明确，该调控作用可能依赖于多种微生物酶催化作用[122]. 然而，肠道微生物组成及多样性复杂，且大部分微生物目前未被成功分离培养，是目前微生物与宿主互作研究的主要制约因素. 未来需要明确特定微生物的贡献度，分离微生物并评估其益生特性. 饮食是改变微生物发酵底物，影响肠道微生物及其代谢物偏好的重要因素[74,123]，通过精准营养手段干预肠道微生物与宿主ISCs的互作在改善幼龄动物肠道稳态和生长发育方面具有较大的发展潜力和研究前景.

综上，深入研究微生物-代谢产物-ISCs功能之间的潜在机制，有助于开发基于特定微生物位点的干预手段. 将为改善幼龄动物肠道稳态，提高营养物质消化吸收从而促进生长提供新思路.

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Recent advances in the interaction between microbiota and intestinal stem cells regulating intestinal development in young animals

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Intestinal stem cells continuously migrate upwards along the crypt villus axis through proliferation and differentiation, so as to constitute the entire intestinal epithelium and serve as the structural basis for intestinal development during early life. Gut microbiota can regulate the fate and function of intestinal stem cells via their surface molecules or metabolites, thereby influencing the trajectory of intestinal development. This review examines how the intestinal stem cells and epithelial renewal drive intestinal development and restore intestinal homeostasis in young animals. This study focuses on elucidating the intricate regulatory mechanisms of the surface components of gut microbiota, short-chain fatty acids, tryptophan metabolites and secondary bile acids on the proliferation and differentiation of intestinal stem cells. Additionally, the stimulatory effects of nutrients, such as probiotics and prebiotics and microbial transplantation on the development of intestinal stem cells are thoroughly explored in this review, which providing a reference for studying the interactions between microbiota and intestinal development during early life.

gut development, stem cells, microorganisms, metabolites, nutritional regulation

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