Wnt信号通路在毛囊乳头细胞诱导毛囊形成及生长过程中作用的研究新进展

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摘要 大面积脱发治疗是目前面临的难题, 毛囊的体外培养与移植是很有潜力的治疗方案, 但临床上极少有成功的案例。毛囊形成、生长调控机制复杂, 体外培养难以模拟, 在当前研究中毛囊乳头细胞诱导毛囊形成是体外毛囊培养的主要方法。Wnt信号通路在毛囊乳头细胞诱导毛囊形成和周期再生的调控中起核心作用。该文就Wnt信号通路在毛囊乳头细胞诱导毛囊形成和周期生长过程中作用的最新研究, 以及近几年体外培养毛囊的新方法作一综述, 希望联合不同培养方法和调控因素的体外培养能为临床大面积脱发治疗提供新的思路和依据。

关键词 毛囊乳头细胞; Wnt信号通路; 体外诱导毛囊形成; 毛发周期生长; 三维球体联合培养法; 皮肤类器官毛囊培养

Recent Advances in the Role of Wnt Signaling Pathway in Hair Follicle Formation and Growth Induced by Dermal Papilla Cells

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Abstract Treatment of large area alopecia is a difficult problem at present. *In vitro* culture and transplantation of hair follicles is a potential treatment program, but there are few successful cases in clinical practice. The regulation mechanism of hair follicle formation and growth is very complex, and it is difficult to simulate *in vitro* culture. In the current research, hair follicle formation induced by hair follicle papilla cells is the main method of hair follicle culture *in vitro*, and Wnt signaling pathway plays a core role in the regulation of hair follicle formation and periodic regeneration induced by hair follicle papilla cells. This review summarizes the recent studies on the role of Wnt signaling pathway in follicle papilla cells induced follicle formation and growth cycle, and the new methods of hair follicle culture *in vitro* in recent years. It is hoped that *in vitro* culture combined with different culture methods and regulatory factors can provide new ideas and basis for clinical treatment of large area alopecia.

Keywords dermal papilla cells; Wnt signaling pathway; external hair follicle formation; hair cycle growth; 3D spherical joint culture method; hair follicle culture of organs like skin

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毛发移植是对原有毛发的重新分配, 不适用于较大面积毛发缺失患者[1]。针对大面积毛发缺失患者, 研究者们进行了一系列体外毛囊培养(hair follicle, HF)的实验, 但成功的极少。毛囊乳头细胞被证明在毛囊发育和周期生长过程中起十分重要的作用[2]。许多研究证实, 通过移植毛囊乳头细胞诱导出的毛发与原生部位的毛发性状相同[3-5]。目前研究中成功的主要是胚胎鼠、新生鼠和人的胚胎, 而尚未从成人头皮毛囊乳头细胞中诱导出功能完好的毛囊[6]。毛囊结构、形成过程涉及的信号分子通路较为复杂, 加上目前对诱导毛囊形成机制的研究不是很充分, 限制了体外培养毛囊的研究进展。毛囊发育生长受到精细的信号网络调控, 涉及多条信号通路, 包括Wnt、骨形成蛋白(bone morphogenetic protein, BMP)、转化生长因子-β(transforming growth factor-β, TGF-β)、SHH(sonic hedgehog)、丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)、Notch、成纤维细胞生长因子(fibroblast growth factor, FGF)、Jak-STAT、肿瘤坏死因子(tumor necrosis factor, TNF)、磷脂酰肌醇3激酶/蛋白激酶B(phosphatidylinositol 3 kinase-protein kinase B, PI3K-Akt)等[7-9]。其中, Wnt信号通路被视为是重要且不可或缺的, 其在毛囊发育和周期生长过程中具有核心作用[10]。近年来的研究表明[11], Wnt信号通路关键分子(经典Wnt信号通路) β-catenin蛋白是维持毛囊乳头细胞诱导活性和增殖功能的重要蛋白, 此外, Wnt/β-catenin途径被证明对干细胞多能分化、器官发育和组织再生修复有重要作用[12]。Wnt通路被认为是毛囊形态发生的重要调控因子, 其主要通过外胚叶发育不全蛋白/外胚叶发育不全蛋白受体/核因子κB(EDA/EDAR/NF-κB)信号通路发挥调控作用[13]。Wnt信号通路失调不仅会引起增殖和分化的紊乱, 甚至还会导致肿瘤的发生[14]。现将近几年对毛囊乳头细胞在诱导毛囊形成、生长过程中Wnt信号通路作用的研究综述如下。

1 毛囊的结构、发育及再生过程

毛囊由上皮细胞和间质细胞构成。毛干、内根鞘、伴随层、外根鞘和毛母质构成毛囊上皮; 真皮乳头和结缔组织鞘构成毛囊间质(图1)[15]。HF和真皮乳头(dermal papilla, DP)的细胞分别由胚胎基板(placode, Pc)和真皮凝结物(dermal condensation, DC)分化而来。其他HF细胞的出现在胚胎发生过程中受到严格的时间和空间控制。毛囊形态发生时期大致分为: 诱导阶段、器官发生阶段和细胞分化阶段。PAULS等[16]将毛囊分化形成分为8个阶段, NIVEDITA SAXENA等[17]根据毛囊前体细胞状态分期、各期形成的分子标记、重要作用信号分子将毛囊分化分为11个阶段(图1), 新的分类方法突出了早期毛囊分化过程和相关重要的分子信号, 其中Wnt、外胚叶发育不全蛋白(ectodysplasin A, EDA)、重组人成纤维细胞生长因子20(fibroblast growth factor 20, FGF20)、BMP信号在毛囊初始诱导时期十分重要。FGF20是DC形成直接需要的唯一已被鉴定的上皮信号, DC的聚集是通过FGF20介导的细胞迁移和聚集而不是增殖实现的。SHH、血小板衍生生长因子A(platelet-derived growth factor subunit A, PDGFA)、TGF-β信号在毛囊向真皮下方延伸时尤为重要。

成熟毛发呈周期性生长, 且毛囊周期分为生长期、退行期和静止期[18]。真皮乳头形成后, 负责毛囊形成后续步骤的信号传导, 其中包括毛囊干细胞分化为不同谱系细胞的一系列步骤。小鼠毛囊周期性循环需要位于外根鞘(outer root sheath, ORS)毛囊隆凸中的毛囊干细胞, 出生后, 毛囊继续生长产生毛发, 此生长期将一直持续到出生后14天; 随后毛囊进入退化期, 毛囊下方的2/3区域会退化, 剩余细胞在隆凸部下方形成毛芽, 直到毛囊底部的DP停留在隆凸毛芽的下方; 此后毛囊发育进入静止期, 随着隆凸和毛芽中干细胞被真皮乳头激活, 毛囊进入了新一轮的生长期, 隆凸干细胞能够分化形成ORS和促进毛囊再生的基质细胞, 基质细胞增殖并向上分化产生毛干和内根鞘(internal root sheath, IRS)。第一个循环的静止期只持续几天, 但之后的静止期可以持续3~4周以上(表1)。人类毛发生长期持续2~8年, 此阶段毛发生长活跃, 退行期持续2~3周, 休止期约为3个月, 结束于原有毛发脱落, 随后生出新毛发[19]。

2 Wnt信号通路及其激活过程

Wnt信号通路是一个复杂的调控网络, 它包括3个分支: 经典Wnt信号通路, 即Wnt/β-catenin信号通路; 非经典信号通路, 包括Wnt/PCP通路(planner cell polarity pathway)和Wnt/Ca^{2+}通路, 其中Wnt/Ca^{2+}通路由Wnt5a和Wnt11激活[20]。Wnt/β-catenin途径被

证明可以调控干细胞多能分化、器官发育和组织再生，对毛囊形态发生的启动也具有重要作用，有维持、诱导毛囊真皮乳头细胞(dermal papilla cell, DPC)再生、毛干生长，以及干细胞聚集、迁移、分化的作用[11]。其在功能上与Hippo、Notch和TGF-β等通路类似，也与这些发育调控相关的信号通路有着不同程度的关联。Wnt信号通路被拮抗时，胞质内的β-catenin与结直腺瘤性息肉蛋白–轴素–激活糖原合成激酶(APC-Axin-GSK-3β)结合，细胞因子1(CK1)和糖原合酶激酶3(GSK-3)使β-catenin氨基末端区域磷酸化后β-catenin开始降解，泛素连接酶E3(ubiquitin-ligase enzymes)中β-传导重复相容蛋白(β-transducin repeat-containing protein, β-Trcp)能识别并泛素化降解磷酸化后的β-catenin，*Wnt*靶基因被T细胞因子–类转导素剪切增强因子(TCF-TLE)/Groucho和组蛋白去乙酰化酶(histone deacetylase, HDAC)抑制；当Wnt信号途径激活时，Wnt配体与卷曲蛋白(Frizzled)、LRP5/LRP6共受体结合激活散乱蛋白(dishevelled, Dsh)，Dsh激活糖原合成激酶结合蛋白(GBP)，GBP与APC-Axin-GSK-3β结合，抑制其磷酸化，使其不能降解胞质内的β-catenin蛋白，胞质β-catenin入核，取代TLE而与转录因子淋巴增强因子(LEF)/TCF结合，招募两种组蛋白修饰因子CREB结合蛋白和Brg1及辅助激活因子Bsl9和Pygo，激活下游靶基因(*c-myc*、*cyclinD1*、*MMP3*等)的表达，其中*c-myc*和*cyclinD1*对细胞分裂和生长至关重要(图2)[20-22]。β-catenin蛋白是此信号通路的核心，于毛囊乳头细胞、毛母质细胞和外根鞘细胞中高表达，在毛囊干细胞激活及定向分化中起关键作用[23-24]。β-catenin在胞核内的聚集是Wnt/β-catenin信号通路激活的关键，葡萄糖诱导降解蛋白8同源物(glucose-induced degradation protein 8 homolog, Gid8)可通过增加β-catenin在胞核内停留时间而提高胞核内β-catenin水平[25]。局部表皮活性β-catenin的过表达会导致毛囊纤维化和毛囊周期性紊乱[26-28]，有研究显示，成年小鼠真皮成纤维细胞中β-catenin的上调可抑制新生毛囊的发生，但具体原因有待进一步分析[9]。Wnt信号通路受体内许多分泌蛋白的影响，Norrin是重组人卷曲蛋白受体4(frizzled class receptor 4, FZD4)的特异性配体，FZD4与LRP5/6相似，通过LRP5/6发挥作用；R-spondin蛋白(Rspo)通过结合LRP5/6和/或卷曲(Fz)受体发挥作用。Wnt抑制蛋白(Wnt inhibitory factor, WIF)和分泌卷曲相关蛋白(secreted frizzled-related protein, SFRP)直接与

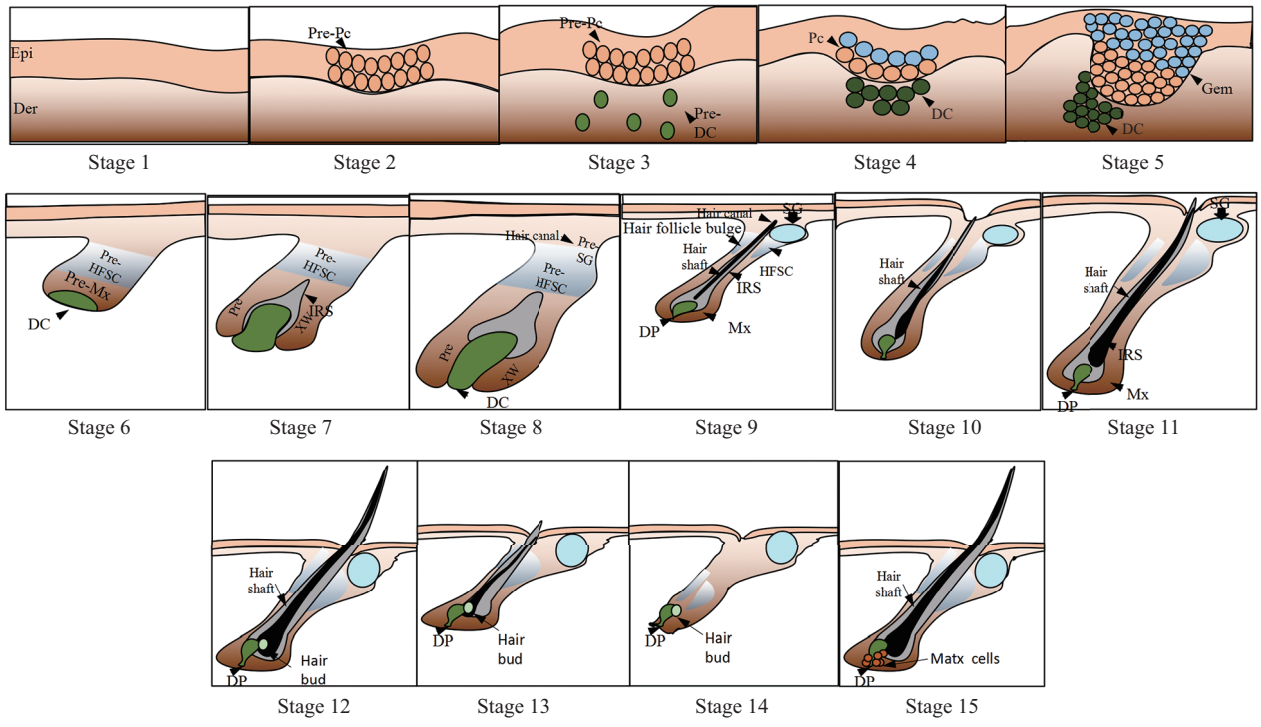

图1　毛囊发育生长形态图(根据参考文献[17-19]修改)

Fig.1　Hair follicle development and growth morphology (modified from the references [17-19])

表1 毛囊发育及生长时期形态(根据参考文献[17-19]修改)

Table 1 Hair follicle development and growth period morphology (modified from the references [17-19])

毛囊发育及生长时期 Hair follicle development and growth period	阶段分期 Phase in installment	毛囊发育生长状态 The state of hair follicle development
Hair follicles in the early developmental stage	Stage 1	Extensive unknown Wnt signaling activity in the upper dermis known as the “first dermal signaling” is key to HF induction The upper dermis transmits the “first Wnt epidermal signal” to the dermis, while the upper dermis transmits the “first Wnt dermal induced signal” to the epidermis
	Stage 2	The epidermis above the dermis appears placode precursors (pre-PC) The anterior substrate cells express Wnt10b signals
	Stage 3	Aggregatedpredermal condensation (pre-DC) is formed in the dermis below the epidermis where pre-PC is located, Pre-PC highly expresses Wnt10b signals
	Stage 4	Substrate formation in epithelial cells, pre-MX in basal layer, HFSC precursors in upper basal layer, and aggregation of DC in dermis. Pre-MX expresses Wnt10b signals
	Stage 5	Hypodermal germ appears in the epithelium, DC polarization occurs in the dermis, pre-MX expresses Wnt10b signals
Hair follicles in the late developmental stage	Stage 6	The downward-growing hairs consist of MX precursors at the leading edge and HFSC precursors above them, and the DC begins to be engulfed
	Stage 7	HFSC precursors remain in the upper part of the outer root sheath (ORS), and the internal root sheath (IRS) begins to form
	Stage 8	MX completely engulfs DP, internal root sheath (IRS) reaches the hair disc, hair stem generates, HFSC is located in the carina, and sebaceous glands (SG) begin to form
	Stage 9	The tip of the hair shaft leaves the inner root sheath and enters the hair Canal, and the DP becomes narrower
	Stage 10	Hair shaft further grows in dermis
	Stage 11	The hair stem extends out of the epidermis and the hair follicles elongate to reach the fat layer
Hair follicle cycle growth stage	Stage 12	After birth, hair follicles continue to grow and produce hair, which continues throughout life until 14 days after birth
	Stage 13	The lower two-thirds of the follicle degenerates and the remaining cells form hair buds just below the bulge until the DP at the bottom of the follicle stops just below the bulge
	Stage 14	Hair follicle development into the quiescent period, with bulging and hair bud stem cells are activated by the dermal papilla, hair follicles into a new round of growth
	Stage 15	Hair follicles enter the growing phase

分泌的Wnts和/或Fz结合; DKK(Dickkopf)和骨硬化蛋白(sclerostin, SOST)/WISE蛋白结合LRP5/6防止Fz-LRP6复合体形成; Shisa蛋白在ER中捕获Fz, 使Fz到达不了细胞表面[29]。Wnt蛋白的表达和分泌由基因*Wls*调控, 上皮细胞基因消融Wls抑制了毛基板的形成, 减少了基板标记物以及核β-catenin的表达, β-catenin表达上调使上皮基板形成, 但不能启动真皮乳头细胞凝集[30]。非经典Wnt/PCP通路的信号传导通路为: Wnt与Fz的结合激活了Dsh, Dsh通过Daam1信号, 激活Rho GTPase, 或者通过Rac GTPase, 进而激活JNK, 两种GTPase都会引起细胞骨架的变化。Wnt/Ca^{2+}通路的信号传导通路为: Wnt-Fz结合触发磷脂酶C(phospholipases C, PLC)激活, PLC随后水解磷脂酰肌醇4,5-双磷酸(phosphatidylinositol biphosphate, PIP2), 产生三磷酸肌醇(inositol triphosphate, IP3)和二酰基甘油(diacylglycerol, DAG), IP3导致细胞内钙的释放, 激活钙调蛋白激酶II(calcium-cam-dependent protein kinase II, CamKII)和蛋白激酶C(protein kinase C, PKC), CamKII激活T细胞核因子(nuclear factor of activated T cells, NF-AT)[31]。Wnt/β-catenin信号通路与EDA/EDAR/NF-κB信号通路是最早对毛囊形态发生起调控作用的两条通路[32], Wnt/β-catenin信号通路是NF-κB活化所必需的, *EDAR*是Wnt的直接靶基因。Wnt/β-catenin信号最初在初级毛囊原基中被激活, EDA/EDAR/NF-κB信号通路而后不断调节Wnt/β-catenin通路的活性直到毛囊基板发育的后期。Wnt10b和Wnt10a的局部表达维持需要NF-κB信号通路, 且Wnt10b是NF-κB的直接靶标[33]。

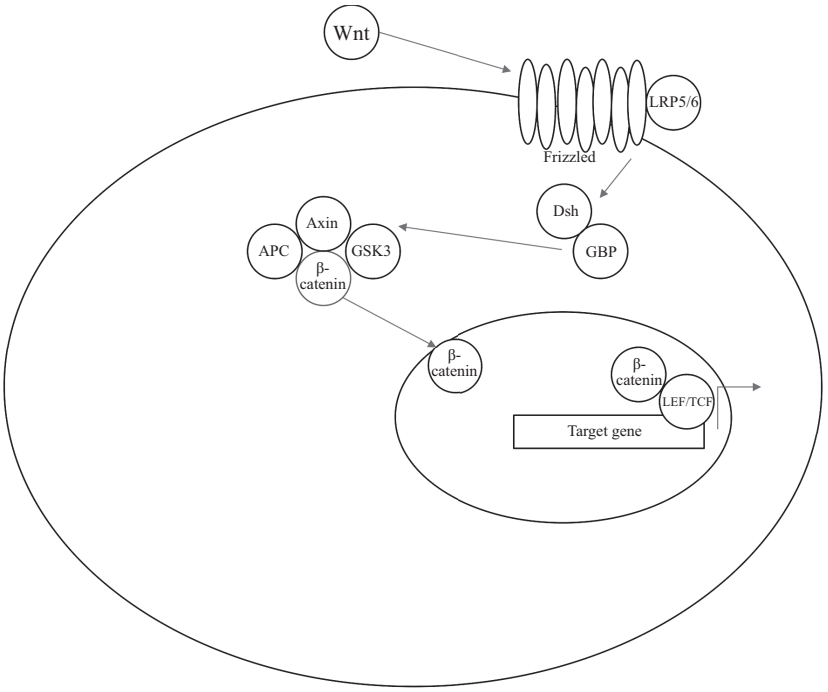

图2 Wnt/β-catenin信号通路激活模式图(根据参考文献[20-22]修改)

Fig.2 Schema of Wnt/β-catenin signaling pathway activation (modified from the references [20-22])

3 Wnt信号通路促进毛囊分化发育的相关影响分子

卷曲相关蛋白1(secreted frizzled-related protein 1, SFRP1)可通过抑制人毛囊球部Wnt配体分泌而抑制Wnt/β-catenin信号通路活性, 进而抑制毛干增长和毛干角蛋白表达。表皮缺失Fz6(Frizzled6)会使毛囊极性混乱导致生发方向随机[34]。在表皮层中敲除CR6交互作用因子1(CR6-interacting factor 1, *Crif1*)可引起Wnt/β-catenin信号降低, 抑制毛囊形态的发生, Crif1是一种线粒体内可调节线粒体氧化磷酸化多肽的合成蛋白[35]。研究发现, 12-*O*-十四烷酰佛波醇-13-乙酸酯能通过激活Wnt/β-catenin信号通路激活小鼠毛囊黑色素细胞, 使头发变黑[36]。Wnt、EDA/EDAR/NF-κB、TGF-β/SMAD、BMP和SHH通路已被发现与头发弯曲有关。毛囊干细胞(hair follicle stem cells, HFSCs)是位于毛囊隆突部具有自我更新能力和多向分化潜能的成体干细胞[10]。*LEF-1*敲除后毛囊变得短小, 出生后不能产生触须和体毛, 毛囊中细胞虽然能够分化形成黑色素细胞, 但不能产生黑色素。在隆突部和毛囊表皮中过表达LEF-1, 会导致毛芽样结构在表皮内形成[37]。毛发周期中*β-catenin*为无毛基因(*Hr*)的下游基因, *β-catenin*敲除的小鼠毛囊干细胞会分化为皮脂腺细胞, 来自隆突部的角质形成细胞祖细胞可分化为表皮和皮脂腺, 在小鼠和人类的经历第一次毛发周期后, 无毛基因突变会导致脱发[38]。*c-myc*是Wnt信号通路下游的靶基因, β-catenin参与从毛囊干细胞到短暂扩充细胞(transit amplifying cell, TA cell)的转化过程, 这表明β-catenin通过c-myc调节毛囊干细胞的分化[39]。

4 Wnt信号通路促进毛囊周期再生的调控分子

毛囊再生即毛囊由静止期重新进入到生长期。Wnt介导的毛发再生涉及维持去磷酸化β-catenin的稳定, 它与TCF/LEF相互作用并发生易位以促使生长促进基因的反式激活。在毛囊隆凸干细胞中敲除*β-catenin*, 会抑制毛囊生长, 如果敲除*Wls*, 则会出现毛发减少、表皮增厚的现象, 且毛囊停留在静止期[40]。毛囊干细胞(HFSCs)响应时空信号后分化, 有研究证明miRNA-29的表达抑制HFSCs谱系进展, 发现miR-29a/b1在小鼠毛发生长期或休止期中的持续过表达可抑制HFSCs及基质细胞的增殖、分化, 导致短毛发和脱发, 皮肤特异性低密度脂蛋白受体相关蛋白6重组蛋白(recombinant low density lipoprotein receptor related protein 6, Lrp6)或骨形态发生蛋白受体1A(bone morphogenetic

protein receptor type 1A, BMPR1A)的消融部分解释了短毛表型[41]。miR-218-5p和SFRP2在退行期和休止期表达程度相反, miR-218-5p促进了毛干的生长。分泌卷曲蛋白(SFRP)家族在哺乳动物中包含5个蛋白成员, 它们参与了Wnt/β-catenin通路以调控细胞凋亡和肿瘤发生[42]。*SFRP2*是miR-218-5p的直接靶标, miR-218-5p的表达可能会降低SFRP2的表达, 从而激活Wnt信号通路[43]。B细胞诱导成熟蛋白1(B lymphocyte induced maturation protein 1, Blimp1), 是一种转录抑制因子, 调节包括皮肤在内的多种组织中的细胞生长和分化, 真皮Wnt/β-catenin激活之前, 在真皮乳头细胞中受到动态调节, 小鼠皮肤成纤维细胞*Blimp1*消融延迟了HF的形成和出生后周期性生长[44]。有人利用离体培养的人毛囊证明非致死性内源性活性氧(ROS)水平的瞬时产生可有效促进体外培养毛囊进入生长期, 并且刺激了毛囊干细胞龛室的特异性活化过程, 包括诱导干细胞分化标志物细胞因子15(CK15), 总体细胞增殖和组织的持续生长, 其被证明与基因靶标周期蛋白D1(*cyclinD1*)的表达有关, 同时其抑制了Wnt信号拮抗剂、阻制剂Dickkopf相关蛋白1(dickkopf-related protein 1, Dkk1)和GSK-3的表达, 该观察结果表明, ROS信号是独立于任何外部信号而调节毛囊干细胞龛室的内在机制[45]。研究发现, 胚胎皮肤中富含三种分泌蛋白: 载脂蛋白-A1(apolipoprotein-A1)、半乳糖蛋白-1(galectin-1)和基膜聚糖(lumican), 它们对诱导形成新的毛囊十分重要, 改变了诱导毛囊真皮乳头细胞的基因表达, 激活了对再生过程很重要的胰岛素样生长因子(insulin-like growth factor, IGF)和Wnt信号[46]。同源异型基因C(homeotic C, *Hoxc*)基因的表达可重编程间充质DP细胞和改变上皮干细胞的再生潜力, 成年皮肤真皮中的*Hoxc*基因表达与区域性HF再生模式密切相关, 诱导*Hoxc*基因与活跃表观遗传区域的异位相互作用, 破坏*Hoxc*基因的区域特异性表达模式, 导致早熟HF再生, 单个*Hoxc*基因足以激活休眠的DP, 并通过规范Wnt信号促进区域性HF再生[47]。缺乏表皮生长因子受体(epidermal growth factor receptor, EGFR)的小鼠无法长出毛发, 但其机制目前还不完全清楚, 有研究表明小鼠*EGFR*基因消融导致基质细胞有丝分裂活性增加, 毛囊细胞凋亡, 以及形成毛发的上皮细胞谱系分化受损, EGFR对抑制增殖和保持干细胞未分化至关重要, *EGFR*敲除后毛囊中Wnt4、Wnt6、Wnt7b、Wnt10a、Wnt10b和Wnt16转录水平升高, β-catenin通路过度激活, 在缺乏EGFR的小鼠中Wnt拮抗剂SFRP1的过表达表明Wnt升高是毛囊缺陷的主要原因[48]。之前有人认为, 毛囊一旦进入生长期后, 毛囊干细胞中的Wnt信号即被抑制, 以减少其增殖, 促进其分化, 有研究发现Wnt4、Wnt7b和Ain2在生长期毛囊隆突部持续表达, 这表明生长期毛囊干细胞中的Wnt信号并没有被抑制, 但具体机制目前不清楚[38]。毛发移植术后常用到活化富血小板血浆(platelet rich plasma, PRP), PRP是通过激活Wnt信号通路促进DPCs的促毛发生长能力。PRP也激活了促DPCs增殖的MAPK、Akt信号通路, 使GSK-3下调[49]。

5 经典Wnt/β-catenin信号途径促进胚胎毛囊形成和周期再生

研究发现, Wntl、Wnt2、Wnt3、Wnt3a、Wnt7a、Wnt8a、Wnt8b、Wnt10a、Wntl0b等为Wntl类蛋白, 参与经典Wnt/β-catenin通路; Wnt4、Wnt5a、Wnt7b、Wnt11等为Wnt5a类蛋白, 参与非经典途径, 研究表明经典途径对诱导毛囊形成影响更大[23]。

Wnt1a有刺激毛囊形成和周期再生的作用; Wnt3a有刺激毛囊周期再生和生长期基因表达的作用; Wnt10a/b有刺激毛囊周期再生和生长的作用[30]。近年研究发现, 牛乳铁蛋白(bovine lactoferrin, BLF)显著诱导了Wnt3a、Wnt7a、LEF-1和β-catenin表达, 通过Erk/Akt和Wnt信号通路促进小鼠毛发生长, 并刺激DP细胞增殖[50]。SU等[51]研究证实了巨噬细胞胞外囊泡(macrophage cell extracellular vesicles, MAC-EVs)表面含量丰富的Wnt3a、Wnt7b与DP细胞相互作用时可激活Wnt/β-catenin信号通路, 其膜中Wnts的存在是DP细胞表面受体Frizzled和LRP5/6活化所必需的。

Wnt10b是近几年研究的热点内容。研究表明, Wnt10b可以通过Wnt/β-catenin信号通路促进獭兔DPCs的增殖, 诱导DPCs向G_1/S期过渡并上调DPCs中的β-catenin蛋白[52]。此外还发现, Wnt10b能显著促进毛干的伸长和DPCs的分化, 注射过表达Wnt10b(AdWnt10b)能促进隆凸内的干细胞增殖[53]。隆凸部是毛囊上部向外凸起形成的, 内含HFSCs。BMP信号通路负性调节基板的形成, BMP6和Wnt10b的平

衡调节着毛囊休止期和生长期的转变。当Wnt10b处于主导地位时，激活Wnt信号通路，抑制BMP信号通路，激活HFSCs，随着HFSCs的增殖，毛囊进入生长期；BMP6激活BMP信号通路，抑制了HFSCs的激活，使HFSCs处于静止状态[54]。

非典型的Wnt信号Wnt4增加生长期基因表达；Wnt5a抑制早期毛囊形成，也是SHH的靶点；非典型的Wnt信号被证明有维持干细胞的静止和抑制典型的Wnt信号的作用，Wnt7b增加生长期基因表达，促进创伤诱导的毛囊新生。最近证明，在表皮细胞中Wnt7b缺失会造成毛囊生长期变短并过早进入退化期，从而导致毛发变短[55]。

6 其他重要分子通路影响毛囊形成和周期再生

在整个毛囊发育的过程中，Wnt、SHH、BMP、Notch、TGF-β等信号通路在上皮细胞和间质细胞之间相互作用，共同调控毛囊发育命运[56]。Wnt通路在毛囊诱导过程中起重要作用，SHH参与形态发生和晚期分化，Notch信号决定干细胞命运，而BMP参与细胞分化，上述信号通路的过度激活或低水平激活，也在HF的周期性再生中发挥作用，影响了成年期的头发循环，任何一个与之相关信号分子的异常都可导致毛囊发育和周期再生障碍[57]。Wnt和SHH信号激活毛发进入生长期，而BMP和TGF-β信号为毛发生长的负性调控分子。

6.1 SHH信号通路

SHH信号通路能调节基板的增生及向下的生长，并主要通过激活SMO蛋白，进而激活神经胶质瘤相关家族锌指结构1(glioma-associated oncogene homolog 1, Gli1)发挥作用[56]。其与Wnt/β-catenin信号通路之间存在相互作用，Wnt/β-catenin可促进SHH表达，其通过LEF-1介导上皮型钙黏附蛋白下调，上皮SHH信号通路激活后可促进毛囊真皮乳头细胞成熟，并通过头蛋白(Noggin)维持其功能特性[56-58]。LEF-1是Wnt信号途径的必需分子，存在于胚胎毛囊分化激活Wnt/β-catenin通路的下游，β-catenin-LEF1复合物能调控毛干角蛋白基因表达，对毛干分化起重要作用[59]。Wnt通道激活时，LEF-1最初在毛芽处的干细胞中表达，而后在短暂增殖的毛母质细胞形成毛干时高表达[60]。SHH信号通路的激活在毛囊静止期向生长期的转变过程中也起着重要作用[64]。DING等[7]研究发现，DP通过下调隆凸部依赖性抑制作用诱导再生，DP可通过自动调节通路调节祖细胞中的SHH表达，DP中的SHH信号传导可以微调Wnt信号传导活动。

6.2 BMP/TGF-β信号通路

BMP对毛囊发育和周期再生也同样具有重要作用，主要通过BMP-Smads-SBE通路发挥作用。TGF-β与BMP信号通路相同，主要通过上述信号通路对毛发周期再生发挥负性调控作用[59]。研究表明，BMP信号通路负性调节毛囊基板的形成，BMP6和Wnt10b的平衡调节着毛囊休止期和生长期的转变[65]。当Wnt10b处于主导地位时，激活Wnt信号通路，抑制BMP信号通路，激活HFSCs，随着HFSCs的增殖，毛囊进入生长期；当BMP6处于主导地位时，激活BMP信号通路，抑制Wnt信号通路，从而抑制HFSCs的激活，HFSCs处于静止状态，毛囊处于静止状态。Noggin对Wnt信号通路可能起正反馈调节，Noggin是BMP信号的一种蛋白抑制剂，Noggin诱导胚胎HF的发生并且促进新HF的增长，*Noggin*缺失突变时，BMP信号明显上升，LEF-1、β-catenin两者的mRNA水平及蛋白水平均明显降低。活化BMP信号通路诱导毛囊发育进入退行期，抑制剂处理使毛囊由生长期至退行期可检测到BMP-4的表达[58]。

6.3 Notch信号通路

当Notch受体与配体结合时，激活毛囊干细胞然后促进毛囊从静息阶段向生长阶段的转变[67]。近年来有研究表明，毛囊细胞分化过程受间充质内Wnt5a和人类重组蛋白01(human recombinant protein 01, FoxN1)、Notch-CSl通路的调控，Wnt5a可促进FoxN1的表达，FoxN1在毛囊的分化中发挥重要的调控作用，特异性FoxN1信号传导可将色素从黑色素细胞传导至毛囊角质形成细胞[66]。

7 其他因素通过Wnt信号通路调节毛囊形成和周期再生

7.1 物理因素和植物提取物

有研究发现，通过特殊设计的皮肤拉伸装置，拉伸毛囊干细胞后会增殖，在适当的时间内给予适当的压力，头发才会再生[67]。巨噬细胞被拉伸后招募趋化因子，极化为M2表型，M2型巨噬细胞释放重组人肝细胞生长因子(HGF)和胰岛素样生长因子-1(IGF-1)等激活干细胞，促进毛发再生。莫罗尼

苷(morroniside)是山茱萸中含量丰富的一种物质, 研究发现莫罗尼苷能显著促进外根鞘细胞(outer root sheath cells, ORSCs)的增殖和迁移, 上调Wnt10b、β-catenin和LEF-1的表达[68]。

7.2 间充质干细胞

皮下脂肪组织通过调节毛囊周期对毛囊再生起重要作用, 毛发移植技术中毛囊部位携带一定量的脂肪组织有助于提高移植的成活率, 然而脂肪相关细胞重建HFs的作用目前尚不是十分清楚。研究证明, 由脂肪源性干细胞(adipose-derived stem cells, ASCs)提供的条件培养基, 可以促进DP细胞标志物和功能性碱性磷酸酶的活性, 外部ASCs壳和内部DP核(CSA-DPS)可重构ASCs中以PPARα信号为主的细胞排列和微环境位, 为胞类型的输入奠定了基础[69]。干细胞的相关作用一直是研究的热点, 有人研究人间充质干细胞(human mesenchymal stem cells, hMSCs)对斑秃(alopecia areata, AA)体外模型的影响, 用干扰素γ(interferon gamma, IFN-γ)预处理人皮肤乳头细胞, 诱导一个AA样的环境, 然后将hMSCs用到hDPCs中, 使得hMSC增强了hDPC的细胞活力, 激活了Wnt/β-catenin信号通路中的β-catenin和磷酸化GSK-3β, 并降低了IFN-γ诱导的hDPC中DKK-1的表达[70]。DKK分泌蛋白家族由DKK-1、DKK-2、DKK-3、DKK-4组成。有研究表明, DKK-1可使毛囊由生长期进入退行期, 并使毛发变短[71]。

7.3 纳米靶向药物

聚γ-谷氨酸(poly-γ-glutamic acid, γ-PGA)纳米粒子由于其控释性、低毒性和生物相容性等优点而受到人们的关注。4HGF是一种草药提取物混合物, 由发芽糙米中的桑黄、发芽大豆中的蛹虫草、何首乌、无花果和椰子油组成。将4HGF包入以聚乳酸(PGA)为基础的水凝胶纳米颗粒中, 以促进其渗透到毛囊(HFs)中, 与单独使用4HGF相比, PGA-4HGF持续释放, PGA-4HGF处理的C57BL/6N小鼠毛发长度增加, 诱导毛发生长期的早期启动, 并延长了毛发生长期的持续时间, 显著增加了真皮乳头细胞增殖, 通过诱导cyclinD1和CDK4蛋白水平显著增加β-catenin蛋白的总表达量[72]。

7.4 雄激素

雄激素性脱发是导致脱发最常见的原因。Wnt/β-catenin通路的激活与雄激素性脱发过程关系密切。成年小鼠毛囊隆凸部细胞中存在雄激素受体(androgen receptor, AR), AR抑制可增强Wnt/β-catenin的激活作用, 促进毛囊干细胞分化增殖[73]。雄激素损害真皮乳头诱导的毛囊干细胞(HFSC)的分化, 抑制了Wnt信号。研究者用双氢睾酮(dihydrotestosterone, DHT)刺激雄激素敏感的真皮乳头细胞(DPC), 证明DHT刺激单层培养DPCs, 下调DPCs的Wnt5a和Wnt-10bmRNA表达, 使Dkk-1上调, DPCs球形培养降低了Dkk-1水平, 增强了Wnt激动剂的表达, 对DPCs的诱导性有贡献。用非那雄胺(finasteride, FN)处理的DPCs能够显著增加其聚集和干细胞转录因子Nanog和Sox-2的表达, FN通过激活AKT、β-catenin和整合素-β1可上调干细胞调控蛋白[74]。

8 新体外毛囊培养法的相关应用

8.1 3D毛囊联合培养法新进展

有研究以新生小鼠表皮细胞和真皮细胞为研究对象, 使用一种皮肤等效物, 由含I型胶原蛋白的小鼠真皮细胞(mouse dermal cells, MDCs)和覆盖小鼠表皮细胞(mouse cells, MECs)组成, 发现3D皮肤等效培养试验可再生毛囊, 而在含有MEC的2D模型中预培养的MDCs则不能, 几个与毛发诱导相关的代表性基因在实验中比在2D模型中有更高的表达[75]。加入Wnt3a后, 再生的毛发数量增加。毛乳头(dermal papillae, DP)细胞是毛囊再生的诱导剂, 体外3D培养DP细胞已被证明能诱导毛囊再生[74]。X染色体失活特异转录本(*XIST*)基因的下调抑制了DP球对体内毛囊发育和再生的诱导作用[75]。YOSHIDA等[76]采用3D球体联合培养法, 证明*CHIR99021*与浮滴培养协同提高DP诱导毛囊形成的标记基因表达。试想如果Wnt信号通路激活剂、物理因素、干细胞、纳米靶向药物、毛囊支架、3D球体培养其中两种或两种以上因素或方法联合应用, 那么毛囊形成、周期再生的成功率会不会进一步增加?

8.2 皮肤类器官毛囊培养

最近研究人员优化了人多能干细胞形成表皮和真皮这两种成分的皮肤类器官培养条件, 逐步调节TGF-β和FGF信号通路, 以共诱导球形细胞聚集体中的人头部上皮细胞和神经脊细胞。在5个月中, 观察到出现了分层的表皮, 富含脂肪的真皮和由皮脂腺的色素性毛囊组成的类囊肿状皮肤类器官, 感觉神经元和雪旺氏细胞网络形成神经样束, 以类器官

毛囊中的默克尔细胞为靶标, 与人类突触相关相似的神经回路。用单细胞RNA测序比较胎儿标本与皮肤类器官的结果表明, 皮肤类器官与发育中期人类胎儿面部皮肤相同。此外, 当皮肤类器官被移植到裸鼠上时会形成平坦且有毛的皮肤, 但生长的头发较少并且缺少位于毛囊内和周围的免疫细胞, 其在皮肤中有多种作用[77]。

9 小结与展望

Wnt信号通路在毛囊形成和周期再生过程中有重要作用, 上述成果可能有助于体内外毛囊培养的研究。目前对于毛囊形成和周期生长中相关信号通路的研究仍不充分。毛囊体外培养周期较长, 可以进一步利用和研究相关分子, 抑制或促进相关信号通路, 加速这一过程。诱导出与移植受者有相同的毛囊周期及存活时间且功能完好的毛囊, 往往难以实现定时观察诱导新生毛囊发育、生长的状态, 从而根据需要给予不同刺激, 可能诱导出功能更加符合临床需求的毛囊。上述新方法和因素对毛囊培养的促进作用为我们体外培养毛囊带来了新思路, 3D球体、类器官、毛囊支架、物理、干细胞、纳米靶向药物等两种或两种以上的方法和因素的联合或结合培养可能会带来新的突破。皮肤类器官毛囊培养值得我们进一步深入探讨, 其免疫原性低, 但仍有多能干细胞致瘤性、培养周期长、毛发生成少等问题。通过在已建成的皮肤类器官种植及培养毛囊并大规模生产具有很大的研究意义, 相应的研究成果会对今后大面积脱发的防治产生重大影响。

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