hUC-MSCs肝样细胞分化机制的研究进展

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摘要 间充质干细胞(mesenchymal stem cells, MSCs)是来源于发育早期中胚层的一类多能干细胞, 广泛分布于全身结缔组织和器官间质中, 是一类具有自我更新、不断增殖和多向分化潜能的成体干细胞。随着组织工程和再生医学的研究和发展, MSCs成为一种治疗各种终末期肝脏疾病的潜在治疗手段。该文就人脐带间充质干细胞(human umbilicalcord mesenchymal stem cells, hUC-MSCs)的生物学特性、体外诱导分化为肝样细胞(hepatocyte-like cells, HLCs)及其可能分化机制的研究进展予以综述。

关键词 脐带间充质干细胞; 肝细胞样分化; 信号通路

Progress in the Mechanisms of hUC-MSCs Differentiation into Hepatocyte-like Cells

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Abstract Mesenchymal stem cells (MSCs) is a class of mesodermal multipotent stem cells, which are derived from the early developmental mesoderm. They widely distributed in connective tissues and organ stroma with characteristics of continuous self-renewal, proliferation and multipotential differentiation. With the progress of study in tissue engineering and regenerative medicine, MSCs have become a potential therapeutic tool for the treatment of various end-stage liver disease. Here, we reviewed the progress in biological characteristics of human umbilical cord mesenchymal stem cells (hUC-MSCs), their differential ability into hepatocyte-like cells, as well as its possible mechanisms.

Keywords umbilical cord mesenchymal stem cells; hepato-like differentiation; signal transduction

间充质干细胞(mesenchymal stem cells, MSCs)是来源于发育早期中胚层的一类多能干细胞, 广泛分布于骨髓(bone marrow, BM)、脂肪、血、脐带、牙髓等全身结缔组织和器官间质中, 是一类具有自我更新、不断增殖和多向分化潜能的成体干细胞, 不同的来源有其不同的特点。1966年, MSCs最早由Friedenstein等[1]在骨髓中发现, 其研究最为深入, 也是目前MSCs实验和临床研究的最主要来源。但骨髓来源的MSCs由于取材相对困难, 会给患者带来二次创伤及感染风险, 且随着患者自身年龄增长及疾病影响, 骨髓中MSCs的数量、体外扩增能力、分化能力等均出现明显的下降趋势[2], 因此临床应用受到一定限制。脐带血和外周血来源的MSCs数量少, 脂肪来源的MSCs由于周期动力学变化及异常

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核型演变, 可能导致恶性细胞的转化[3], 导致外周血和脂肪来源的MSCs的应用研究较少。人脐带间充质干细胞(human umbilical cord mesenchymal stem cells, hUC-MSCs)与其他来源的MSCs比较, 具有以下优点: (1)脐带中的干/祖细胞含量高且更原始, 具有更强的增殖分化能力[4]; (2)免疫原性低[4-5]; (3)易于分离, 纯度高; (4)扩增时培养体系较易统一, 便于质控; (5)可制成种子细胞冷冻, 复苏后细胞损失小[6]; (6)潜伏性病毒和病原微生物的感染及传播可控性强; (7)无侵入性采集[4]; (8)不存在伦理学问题[4,7]; (9)无致瘤性[4,8]; (10)多能性, 能向全层分化。1999年, Petersen等[9]首次发现骨髓间充质干细胞(bone marrow mesenchymal stem cells, BMSCs)具有分化为肝卵圆细胞的能力, 随后, 大量关于MSCs能转化为肝实质细胞的研究引起了广泛关注。2004年, Lee等[10]成功将人BMSCs诱导成肝样细胞, 并表达多种肝细胞基因和蛋白质。脂肪MSCs也可分化为肝样细胞, 其分泌的粒细胞集落刺激因子(granulocyte colony stimulating factor, G-CSF)、白细胞介素-6(interleukin-6, IL-6)、肝细胞生长因子(hepatocyte growth factor, HGF)等在肝衰竭的治疗中发挥了重要作用。其他来源于软骨、胎盘、关节滑膜、皮肤等MSCs均可在一定条件下分化为肝样细胞。然而, hUC-MSCs本身就表达成熟肝细胞的一些标志物, 如白蛋白(albumin, ALB)、细胞角蛋白-18(cell keratin-18, CK-18)、葡萄糖-6-磷酸酶(glucose-6-phosphatase, G-6-P)、磷酸烯醇式丙酮酸羧激酶(phosphoenolpyruvate carboxykinase, PEPCK)等[11], 表明hUC-MSCs本身更易于向肝样细胞分化, 或可直接代替肝细胞用于移植治疗非瘤性的终末期肝病和遗传代谢性肝脏疾病, 从而解决肝细胞来源困难及增殖能力有限的问题。因此, hUC-MSCs成为干细胞治疗研究与应用领域中理想的种子细胞, 并随之成为研究的热点[12], 在肝细胞定向分化研究中也已取得较大的研究进展。本文就hUC-MSCs的生物学特性、体外诱导分化为肝样细胞(hepatocyte-like cells, HLCs)及其可能分化机制的研究进展予以综述。

1　hUC-MSCs生物学特性

1.1　hUC-MSCs的形态特点

倒置显微镜下观察发现, hUC-MSCs呈贴壁生长, 形态为纺锤形及梭形, 似成纤维细胞, 呈平行排列或旋涡状生长。透射电镜观察显示, hUC-MSCs胞质丰富, 核大, 核仁明显, 常染色质多, 细胞器以粗面内质网和线粒体为主, 胞质内有较多游离的核糖体。

1.2　hUC-MSCs的分子标志物

hUC-MSCs表达多种分子标志物, 具有非特异性, 具体分子表达情况详见表1。

此外, hUC-MSCs还表达CK-3、CK-4、CK-12、CK-13、桥粒连接蛋白(desmoplakin)和紧密连接蛋白-1(zonula occludens-1)等[13]。逆转录聚合酶链反应(reverse-transcription polymerase chain reaction, RT-PCR)检测显示, hUC-MSCs表达未分化状态细胞标志物, 如FGFR-4(basic fibroblast growth factor receptor-4)、八聚体结合转录因子-4(octamer-binding transcription factor-4, Oct-4)、Nanog、肿瘤排斥抗原-1-81(tumour rejection antigen-1-81, Tra-1-81)等, 表明hUC-MSCs具有干细胞特性[8,14]。hUC-MSCs的鉴定应包括细胞的形态学特征和超微结构、细胞表面分子标志物的表达以及细胞的多向分化能力三个方面。目前, 国际干细胞治疗学会提出了鉴定人来源MSCs的三条基本标准[15]: (1)在标准培养条件下, MSCs具备对塑料底物的贴壁性; (2)MSCs群体阳性表达CD105、CD73、CD90等细胞标志物, 而不表达造血细胞标志物CD45、CD14、CD34等分子; (3)经体外诱导, MSCs能向成骨细胞、脂肪细胞及软骨细胞分化。

1.3　hUC-MSCs的免疫学特点

hUC-MSCs具有低免疫原性, 表达主要组织相容性复合体-I(major histocompatibility complex-I, MHC-I)类分子, 而不表达MHC-II类分子, 表达非经典HLA(human leukocyte antigen)分子(如HLA-E、HLA-G、HLA-F), 特别是HLA-G分子的表达, 它能够抑制NK细胞和T细胞的功能。通过分泌转化生长因子-β(transforming growth factor-β, TGF-β)、HGF、半乳糖凝集素-1及吲哚胺2,3-二加氧酶(indoleamine 2,3-dioxygenase, IDO)等细胞因子抑制T细胞的增殖[4,24]; 通过分泌IL-6、前列腺素E2(prostaglandins E2, PGE2)、IL-10和巨噬细胞集落刺激因子(macrophage colony-stimulating factor, M-CSF)等细胞因子抑制树突状细胞(dendritic cells, DC)的分化、增殖和成熟; 通过抑制美洲商陆丝裂原(pokeweed mitogen)或IL-4刺激的B细胞增殖[24]。hUC-MSCs不表达Fas配体及T细胞协同刺激分子CD80(B7-1)、CD86(B7-2)和

表1 hUC-MSCs分子标志物

Table 1 Molecular markers expressed by hUC-MSCs

分子标志物 Marker	可得性 Availability	流式检测文献来源 Flow cytometer (references)	RT-PCR文献来源 RT-PCR (references)	细胞免疫化学文献来源 Immunocytochemistr (references)
ASMA	+	NA	NA	[11,16]
CD3	–	[17]	NA	NA
CD10	+	[6,8]	[18]	[18]
CD13	+	[6,8,10-11,17,19]	NA	[18]
CD14	–	[6,8,10-11,17]	NA	[16]
CD19	–	[17]	NA	NA
CD29	+	[4,6,8,10-11,14,17,20]	[18]	NA
CD31	–	[17,21]	[18]	NA
CD34	–	[4,6,8,10-11,14,17,19-22]	[18]	[16]
CD44 (HCAM)	+	[4,6,8,10-11,14,17,20]	[18]	NA
CD45	–	[4,10-11,14,17,19-20,22]	[18]	[16]
CD49e	+	[11,17]	[18]	NA
CD51	+	[20]	[18]	NA
CD54 (ICAM-1)	+	[11]	[18]	[16]
CD56	–	NA	[18]	NA
CD73 (SH3)	+	[4,10-11,14,17,20,23]	[18]	NA
CD90 (thy1)	+	[6,8,10-11,14,17,19,22-23]	[18]	[13]
CD105 (SH2)	+	[4,6,8,10-11,14,17,19-23]	[18]	[13]
CD106 (VCAM-1)	+	[17]	NA	[16]
CD117 (C-Kit)	+/–	[6,8,11]	NA	NA
CD133	–	[10]	NA	NA
CD146	+	[6,8]	NA	NA
CD166	+	[4,10-11,17]	NA	NA
CK-7	–	NA	NA	[11,13]
CK-8	+	NA	[11]	[11,13]
CK-18	+	NA	[10-11]	[11,19,21]
CK-19	+	NA	[11]	[11,13]
HLA-DR (MHC-class II)	–	[4,11,14,17,19,23]	[18]	NA
HLA-DQ (MHC-class II)	–	[4,19]	[18]	NA
HLA-ABC (MHC-class I)	+	[4,11,14,17,23]	[18]	NA
OCT-4	+	[22]	[14,18]	[8]
Nanog	+	NA	[14]	NA
Tra-1-60	+	NA	[14]	[8,14]
SSEA	+	NA	[14]	[8,14]
SOX-2	+	NA	[14]	NA
Vimentin	+	NA	NA	[11]
Connexin 32	+	NA	[11]	[11]

通过流式细胞仪、RT-PCR、免疫细胞化学染色等检测得到上述结果, 不同文献可能结果不一致。+: 可检测到表达; –: 未检测到表达; NA: 未用该方法检测; ASMA: α-平滑肌; HCAM: 归巢关联细胞黏附分子; ICAM: 细胞间黏附分子; HLA: 人类白细胞抗原; MHC: 主要组织相容性复合体; SSEA: 特定阶段胚胎抗原; VCAM: 血管细胞黏附分子。

The results obtained by flow cytometry, RT-PCR, immunocytochemistry detection. There are contradictions between different reports. +: positive expression; –: negative expression; NA: not applicable; ASMA: α-smooth muscle actin; HCAM: homing associated cell adhesion molecule; ICAM: intercellular cell adhesion molecule; HLA: human leukocyte antigen; MHC: major histocompatibility complex; SSEA: stage specific embryonic antigen; VCAM: vascular cell adhesion molecule.

CD40(CD40L), 导致T细胞活化的第二信号功能丧失, 并通过诱导$CD4^+$、$CD25^+$调节性T细胞的形成抑制T淋巴细胞增生、发育与成熟, 减少细胞因子的分泌, 致使无法激活T细胞免疫应答, 最终不产生免疫

排斥反应, 从而诱导免疫耐受[25]。此外, Haiping等[23]发现, hUC-MSCs与T细胞共培养时抑制由DC细胞激活的T细胞的增殖, 而当用Transwell小室分离培养hUC-MSCs与T细胞时, 抑制作用明显减弱, 这一过程可能是通过*IDO*基因的表达发挥作用的。

目前, 虽然公认hUC-MSCs具有免疫抑制作用, 但在某些条件下hUC-MSCs可促进机体产生抗体, 提高机体免疫力。Ji等[17]将hUC-MSCs与已活化的B细胞体外共培养7 d, 共培养组高表达$CD138^+$ B细胞, 表明hUC-MSCs可促进活化的B细胞分化成浆细胞, 促进浆细胞产生免疫球蛋白, 并且这一过程是通过分泌PGE2介导发挥免疫调节作用的。有报道称, MSCs分泌低剂量肿瘤坏死因子-γ(tumor necrosis factor-γ, TNF-γ)时, MSCs可作为抗原递呈细胞, 促进体内$CD8^+$ T细胞的产生, 从而增强特异性免疫反应[26]。

2　hUC-MSCs诱导分化为肝样细胞的研究

2.1　细胞因子诱导法

hUC-MSCs的分化受介质的动态变化调控, 在不同条件下可以分化为不同胚层的细胞, 而特定的细胞因子则在hUC-MSCs的肝样分化中发挥重要作用, 例如碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)、HGF、抑瘤素M(costatin M, OSM)等。Campard等[11]在体外培养hUC-MSCs时先后加入表皮生长因子(epidermal growth factor, EGF)、bFGF、HGF、烟酰胺及OSM等诱导其肝样分化。HLCs呈扁平多角形态, 胞质中含颗粒状物质, RT-PCR及免疫细胞化学染色检测诱导后hUC-MSCs强表达甲胎蛋白(α-fetoprotein, AFP)、白蛋白、CK-18、CK-8、CK-19、G-6-P、PEPCK、酪氨酸氨基转移酶(tyrosine aminotransferase, TAT)、色氨酸2,3-二加氧酶(tryptophan 2,3-dioxygenase, TDO)及α1-抗胰蛋白酶(alpha-1 anti-trypsin, AAT)基因和蛋白质, 并具有成熟肝细胞的某些功能, 如储存糖原、合成尿素、G-6-P酶活性等。Guan等[22]采用类似诱导方式将hUC-MSCs诱导成HLCs, 也表达多种肝特异性蛋白, 具有成熟肝细胞的多种功能。Zhang等[27]研究表明, 在只有HGF和FGF-4的条件下同样能将hUC-MSCs诱导为肝样细胞, 诱导后细胞用免疫细胞化学染色、Western blot等检测到多种肝细胞特有标志物的表达, 并具有储存糖原和吞噬低密度脂蛋白能力。HGF和FGF-4通过与hUC-MSCs表达的HGF及FGF受体结合从而激活c-Met/HGF信号通路启动肝样分化这一过程[28]。大量研究表明, hUC-MSCs在各种细胞因子作用下可分化为肝样细胞, 为肝组织工程与肝脏疾病细胞治疗带来希望[12,22,27,29-31]。

迄今, 不同细胞因子组合可将不同种属及来源的MSCs诱导分化为形态和功能上与肝细胞类似的细胞, 但该方法存在诱导周期长(一般在3周以上)、分化效率低以及费用昂贵等缺点, 且诱导出来的肝样细胞并不具有肝细胞的所有功能。

2.2　共培养诱导法

自从发现MSCs具有多向分化能力起, 研究者们做了大量工作试图找到最有效、实用的诱导方法, 有学者采用BMSCs与肝细胞共培养的方法诱导肝样分化, 同样获得了成功。2006年, Lange等[32]通过逆转录病毒转导绿色荧光蛋白(green fluorescent protein, *GFP*)基因标记MSCs, 并用纤连蛋白包被培养皿, 建立与胎肝细胞共培养体系, 诱导2周后用流式细胞术分离GFP^+ MSCs, 结果显示, GFP^+ MSCs表达肝特异性基因。随后, Zhang等[33]做了类似实验, 他们将GFP^+标记的鼠MSCs与肝细胞共培养, 单纯HGF诱导作为对照组。与对照组相比, 共培养组强表达ALB、AFP、CK-18, 而HGF诱导组在第14 d时微弱表达肝细胞基因, 在第21 d时只有少量细胞表达肝细胞基因, 表明共培养的微环境更有利于BMSCs肝样分化, 共培养诱导效率显著高于细胞因子诱导。2013年, 李华等[34]在不添加外源诱导因子条件下, 将L02人肝细胞系与hUC-MSCs共培养, 通过RT-PCR分别于第7、14、21 d检测*AFP*、*ALB*、*CK-19* mRNA的表达。结果显示, 共培养组第7 d仅有*AFP* mRNA的表达, 第14 d时表达*ALB*、*CK-19* mRNA, 且随时间延长表达更明显, 阴性对照组在上述时间点均未检测到阳性表达, 表明无需额外添加外源诱导因子, hUC-MSCs可在人正常肝细胞共培养的微环境中, 向正常肝细胞分化。

共培养微环境可以促进hUC-MSCs肝样分化, 通过模拟人体内微环境, 促使hUC-MSCs在短时间内快速向肝样细胞分化, 相比细胞因子诱导, 共培养诱导效率较高, 若在体外扩大培养规模, 有望为肝细胞组织工程提供更为充足的肝细胞源。

2.3　基因转导诱导法

最近研究显示, 基因修饰也可能成为诱导hUC-MSCs肝样分化的方法之一。2011年, Huang等[35]首

次利用*p19*$^{Arf-/-}$鼠尾成纤维细胞(tail-tip fibroblasts, TTFs)从14种转录因子中筛选出3种转录因子进行组合, 即Gata4、肝细胞核因子-1α(hepatocyte nuclear factor-1α, HNF-1α)、Foxa3(forkhead box a3)能将TTFs直接转分化成诱导型肝样细胞(induced hepatocyte-like cells, iHep), iHep呈现上皮样细胞形态, 表达*ALB*、*AFP*、*TDO2*(tryptophan 2,3-dioxygenase 2)、*TTR*(transthyretin)、*AAT*、*CK-18*、*CK-19*等肝特异基因, 并具有储存糖原、合成尿素、吸收吲哚绿等成熟肝细胞的功能。将iHep移植到*Fah*$^{-/-}$小鼠体内后, 近一半小鼠肝功能恢复从而存活下来。Sayaka等[36]通过筛选12种转录因子认为, *HNF-4α*加上*Foxa1*、*Foxa2*或者*Foxa3*均能将鼠胚胎纤维母细胞和成体成纤维细胞诱导为肝样细胞, 且强表达*ALB*、*AFP*及上皮细胞标志物钙黏蛋白基因(*E-cadherin*), 同样具有成熟肝细胞的功能, 也能使*Fah*$^{-/-}$小鼠肝功能恢复。2014年, Huang等[37]又证实, *Foxa3*、*HNF-1α*、*HNF-4α*也能将人成纤维细胞直接转分化为人诱导型肝样细胞(human induced hepatocytes, hiHeps), hiHeps表达肝特异性基因, 并具有P450酶活性、胆汁药物清除等成熟肝细胞的功能。2016年, Xu等[38]将Foxa2及HNF-1α导入到BMSCs诱导为肝样细胞, 并高表达肝特异基因和蛋白质, 同时发挥成熟肝细胞功能, 如糖原储存、吲哚绿吸收及脂质聚积等。与前两种诱导方法相比, 基因转导法诱导效率高, 诱导时间短。此外, Simeonov等[39]采用转染修饰mRNA的方法诱导成纤维细胞直接转化成肝样细胞。这些方法虽不是以hUC-MSCs为基础获得有功能的肝细胞, 但为hUC-MSCs诱导肝样分化的研究提供了新的思路和潜在方法。

目前已研究的MSCs诱导方法有细胞因子诱导、MSCs与肝细胞共培养、直接转基因和转染mRNA的方法, 但都不甚成熟, 仍需进一步研究, 以提高其分化效率, 降低诱导分化成本。研究其分化的分子机制可为MSCs肝样分化效率和寻找新的分化方法提供思路, 因此, 我们对MSCs肝样分化的分子机制的研究进展作一总结。

3 hUC-MSCs肝样分化的可能分子机制研究

3.1 肝富集转录因子作用机制

肝细胞分化伴随着基因的转录水平控制, 在转录调控元件作用于肝细胞表达的研究中已经确定了一些肝脏转录因子——肝脏富集转录因子(liver enriched transcription factor, LETF), 包括HNF-1、HNF-3、HNF-4、HNF-6和CCAAT/增强子结合蛋白(enhancer-binding protein, C/EBP)家族, 调节肝细胞的发育和分化, 促进肝特异性基因的表达, 增强糖原合成。LETF家族在调节肝特异基因的表达中起重要作用, 尤其是HNF-4α。Odom等[40]通过染色质免疫共沉淀芯片(chromatin immunoprecipitation on chip, ChIP-chip)发现, HNF-1α、HNF-6及HNF-4α均能与肝特异性基因的启动子区结合, HNF-4α与42%肝基因的启动子区结合, 但只有6%的启动子区与HNF-1α结合, 而且大部分与HNF-1α、HNF-6结合的启动子区也都能与HNF-4α结合, 说明HNF-4α调控肝特异性基因的表达在转录水平上发挥重要作用。研究表明, HNF-4α通过结合肝特异性基因启动子区控制肝细胞形态和功能的分化以及肝上皮的产生, *HNF-4α*基因突变将会导致肝脏结构紊乱[41]。Takayama等[42]通过腺病毒转导*SOX-17*(sry-related HMG box-17)和造血表达同源异形盒(hematopoietically expressed homeo-box, *HEX*)基因到干细胞内促使形成肝样细胞, 但形成的肝样细胞缺乏成熟肝细胞功能, 当转导*HNF-4α*基因后, *HNF-4α*通过激活间充质–上皮转化(mesenchymal-to-epithelial transition, MET), 促进肝样细胞分化成熟。Hang等[19]通过质粒转染*HNF-4α*到hUC-MSCs中进行肝样分化诱导, RT-PCR检测发现, 诱导第21 d时, 转染*HNF-4α*后hUC-MSCs中*TAT*、*ALB*、*G6P*、*CYP3A4*(cytochrome P450 3A4)的表达明显高于对照组。Chen等[43]将*HNF-4α*基因转导入到人BMSCs中后同样得到类似结果, 可见HNF-4α主要在转录水平上调控肝特异基因的表达。

大量实验证明, HNF-4α在转录水平上调控肝特异性基因的表达与维持肝细胞正常形态结构上均具有重要作用[19,40-43], 但在目前大都数研究中, 诱导性的肝样细胞缺乏成熟肝细胞的所有功能, 而研究HNF-4α的具体作用机制能使我们高效诱导hUC-MSCs肝样分化, 重要的是, HNF-4α能促进肝样细胞成熟。

3.2 microRNA介导hUC-MSC肝样分化

miRNAs是一类非编码蛋白质的小分子RNA, 在机体内参与基因的表达调控, 其机制是通过和靶mRNA的3′非编码区结合位点结合引导沉默复合体(RNA induced silencing complex, RISC)而降解mRNA

或阻碍其翻译, 参与基因转录后调控[21,44], miRNAs在肝细胞增殖分化中发挥重要作用。miR122是肝脏特异性miRNA, 占成人总肝脏miRNAs的70%, 主要参与调节胆固醇和脂肪酸的代谢[45], 同时与丙型肝炎病毒的复制机制有关[46], 过表达miR122促进胎儿肝干/前体细胞向肝细胞分化[47]。Davoodian等[48]诱导脂肪MSCs肝样分化时发现miR122的表达上调, 他们将携带*miR122*基因的慢病毒感染脂肪MSCs进行肝样分化。RT-PCR检测发现, 转染组*miR122*表达上调, 肝特异性基因(如*ALB*、*AFP*、*CK-19*、*CK-18*及*HNF-4α*)的表达水平也均增加, 表明miR122可促进MSCs肝样分化。他们在另一项研究中则发现, *Let-7f* miRNA可负性调控MSCs肝样分化, 而当抑制*Let-7f* miRNA后, 上述肝特异性基因的表达均上调[49]。miR-148a可促进肝细胞发育过程中肝细胞成熟标志物的表达; miR-34a在肝脏再生的终末阶段表达上调, 并且通过下调抑制素-βB(inhibin-βB, INH-βB)及Met的表达抑制肝细胞增殖[50]; miR-30a主要表达于胆管原基及出生后胆道中, 但在肝细胞的增殖中也具有重要作用[21,51]。Cui等[52]在诱导hUC-MSCs肝样分化时用含miRNA抑制序列的病毒感染hUC-MSCs, miRNA芯片和RT-PCR检测肝样细胞内的miRNA表达谱变化, 结果显示, *miR-1246*、*miR-1290*、*miR-148a*、*miR-30a*、*miR-424*、*miR-542-5p*表达上调, *miR-17*、*miR-76*、*miR-146a*的表达则下调。表达上调的6种*miRNAs*中的任何一个失活均会阻止ALB的表达, 说明这6种miRNAs在肝向分化及维持肝特异基因的表达具有重要作用。然而, 过表达以上6种miRNAs任意一种或miR-122均不能启动肝样细胞分化。有趣的是, 过表达上述6种miRNA+miR-122组合时则可刺激hUC-MSCs诱导分化为功能性肝样细胞。该研究发现, 利用特异性的miRNA组合可以将hUC-MSCs直接诱导为在体内外均具有功能的肝样细胞, 表明多种特异性miRNA联合作用可能是hUC-MSCs分化为肝样细胞的机制之一。

近年来, 对miRNA的研究越来越多, 已成为研究的热点, 但miRNA作用于hUC-MSCs肝样分化分子机制报道却并不多, 目前还需更进一步研究。

3.3　Wnt/β-catenin信号通路对MSCs肝样分化的影响

Wnt/β-catenin信号通路调控干细胞的增殖、分化以及凋亡, 控制与细胞分化相关的生物化学过程[53], 参与动物胚胎形成的无数事件包括干细胞增殖以及神经嵴的特定分化[54]。当Wnt与Frizzled结合, 触发一系列信号级联反应导致胞质内非磷酸化的β-catenin聚集, 并易位于细胞核内与淋巴细胞增强因子/T细胞因子(lymphoid enhancer factor/T cell factor, LEF/TCL)的N端结合, 形成异二聚体激活靶基因, 如*c-myc*(cellular homologue of avian myelocytomatosis virus oncogene)、*cyclin D1*[55-58]、低密度脂蛋白受体相关蛋白5(low-density lipoprotein receptor-related protein 5, *LRP5*)、*Frizzled3*以及*p53*可上调Wnt/β-catenin信号通路, Wnt信号通路在决定干细胞的命运与分化中起了重要作用。Ke等[59]体外加入肝损伤组织提取液诱导BMSCs肝样分化, 逆向点分子杂交技术分别在诱导第0、7、11、21 d时检测6种关键基因*Wnt-1*、*Wnt-5a*、*Frizzled1*、*Disheveled*、糖原合酶激酶-3β(glycogen synthase kinase-3β, *GSK-3β*)及*β-catenin*的mRNA水平, Western blot测定Wnt-1、β-catenin蛋白质水平, RT-PCR分析肝特异性基因的表达情况, 结果在诱导第21 d, 6种关键基因与Wnt-1、β-catenin蛋白质水平均下降, 而肝特异性基因表达却逐渐增加, 表明在BMSCs肝样分化过程中Wnt/β-catenin信号通路是下调的。而当用Wnt/β-catenin通路特异性阻断剂Dickkopf-1(Dkk1)下调Wnt信号通路时, 发现BMSCs更早表达ALB, 从而证实阻断Wnt/β-catenin通路可促进BMSCs肝样分化。Yoshida等[60]体外研究脐血MSCs肝样分化时转染Fz8-siRNA(Fz8-small interference RNA)下调Wnt/β-catenin信号通路, 发现ALB、C/EBPα、P4501A1(CYP1A1)蛋白的表达增加, 这与Ke等[59]的研究结果一致。由此我们可以推断, 当Wnt/β-catenin信号通路抑制后同样可以促进hUC-MSCs肝样分化。然而如果在MSCs肝样分化过程中激活或上调Wnt/β-catenin信号通路可能会导致肿瘤表型的形成并表达肝癌相关蛋白[53]。

然而, Wnt/β-catenin信号通路抑制后究竟如何促进MSCs肝样分化具体作用机制尚不清楚, 目前仍需进一步研究。

3.4　Notch信号通路对MSCs肝样分化的影响

*Notch*基因编码一类在进化上高度保守的细胞表面受体, 参与调控细胞增殖和分化, 决定细胞命运, 影响干细胞/祖细胞自我更新及胚胎发育[61-62], 参与胆管上皮细胞与肝细胞的分化[63]。Tanimizu等[64]研究发现, Notch信号通路通过改变HNF的表达来调

控肝细胞的分化。妊娠中期肝门静脉附近胆管形成时, Jagged1主要在门静脉周围表达, *Jagged1*基因突变会使Notch信号通路异常, 导致肝内胆管发育缺陷, 引发Alagille综合征[65-66]。*Notch2*基因表达于大多数肝细胞中, 实际上, DIK^+肝前体细胞表达Notch胞内结构域将会抑制肝细胞分化, 减少ALB的表达。激活Notch信号通路上调HNF-1β的表达, 下调HNF-1α、HNF-4及C/EBPα的表达, 表明激活Notch信号通路主要促进胆管上皮的形成而抑制肝细胞分化。相反, 用特异性siRNA干扰*Notch2* mRNA及γ-分泌酶抑制剂L-685、L-458下调Notch信号通路将会促进肝细胞分化[67]。Ke等[67]用四氯化碳(CCl_4)和2-乙酰氨基芴(2-AFF)制造的肝损伤动物模型的肝萃取物作为诱导因子成功将BMSCs诱导为肝样细胞, RT-PCR及Western blot均检测到ALB的表达, 分化第21 d用反向斑点杂交实验检测发现Notch信号通路中的*Jagged1*、*Jagged2*、*Delta1*、*Delta3*、*Notch1*、*Notch2*、*Notch3*及*Presenilin1*基因的表达低于第0、7、14 d, 且分化第11 d时*Jagged2*、*Delta1*、*Delta3*、*Notch1*、*Notch2*、*Notch3*和*Presenilin1*的mRNA表达水平几乎降至到最低点, 但当加入Jagged1激活Notch信号通路后, BMSCs分化过程中ALB未被检测到, 表明上调Notch信号通路抑制MSCs肝样分化, 而下调Notch信号通路则可促进MSCs肝样分化。

3.5 MAPK信号通路对hUC-MSCs肝样分化的影响

丝裂原激活蛋白激酶(mitogen-activated protein kinases, MAPKs)是目前已报道的最重要的蛋白激酶类信号通路, 通过三级酶促级联反应(MAPKKK-MAPKK-MAPK)激活转录因子, 促进核内基因的转录。MAPK家族包括五大成员: 胞外信号调节蛋白激酶1/2(extracellular signal-regulated protein kinase1/2, ERK1/2)、p38MAPK、c-Jun N端激酶(c-Jun N-terminal kinases, JNK)、ERK5和ERK3/4[68]。Lu等[69]用FGF4和HGF细胞因子诱导BMSCs肝样分化, 诱导第7 d加入P38抑制剂(SB203580)、ERK1/2抑制剂(U0126)及MSK1(mitogen-and stress-activated kinase)抑制剂(H89)后, 结果大多数细胞呈现纤维样细胞融合形态, 并且*AFP*及*Foxa2*基因的表达显著减少, 表明阻断P38、ERK1/2及MSK1信号通路将抑制肝样分化, 且P38通路抑制效应最强。FGF4和HGF通过MAPK信号通路促进小鼠BMSCs分化为肝样细胞, 尤其是P38信号通路可显著促进小鼠BMSCs肝样分化。

此外, 研究报道, 丙戊酸(valproic acid, VPA)也能促进hUC-MSCs肝样分化。Chen等[70]将BMSCs与VPA共培养发现, VPA更能促使BMSCs表达AFP、CK-18、ALB等肝特异性蛋白质。随后, 研究者通过RT-PCR检测发现, VPA+FGF-4共同诱导时*FGFR-1IIIc*、*c-Met* mRNA的表达分别是对照组的2倍和5倍, 表明VPA处理后能够改变*FGFR-1IIIc*、*FGFR-2IIIc*及*c-Met*基因的表达状态, 增强BMSCs对HGF、FGF的敏感性, 从而激活FGFRs/FGF-4、c-Met/HGF信号通路促进肝细胞分化。而ERK/AKT信号通路是c-Met/HGF信号通路的下游靶基因, 说明VPA通过激活AKT/ERK信号通路增加hUC-MSCs表达内胚层基因, 包括*CXCR4*、*SOX17*、*Foxa1*、*Foxa2*、*c-Met*、*EOMES*及*HNF-1β*, 促使hUC-MSCs向肝细胞分化。当用抑制剂阻断AKT(protein kinase B)和ERK信号通路时, 发现VPA对hUC-MSCs肝向分化的调节作用减少, 证实VPA是通过AKT和ERK信号通路促进hUC-MSCs肝向分化的[31], 然而c-Met/HGF、ERK、AKT信号通路与hUC-MSCs肝向分化之间的相互关系还有待于更进一步研究。

实际上, hUC-MSCs肝样分化分子机制非常复杂, 可能受多个因素的调控, 除了可能与LETF、miRNA、Wnt/β-catenin信号通路、Notch信号通路及MAPK信号通路等生物化学因素有关外, 还可能受到细胞空间排列和生物物理因素的影响, 如通过力学–整合素信号通路调控hUC-MSCs肝样分化, hUC-MSCs通过整合素感受细胞外基质的力学条件决定分化时间及分化方向, 但其如何调控hUC-MSCs分化和细胞内基因表达的具体机制尚不清楚, 目前还在进一步研究中。图1为hUC-MSCs肝样分化可能分子机制。

4 问题与展望

hUC-MSCs在肝再生和肝疾病的细胞治疗中呈现出较多优势, 为终末期肝病的治疗提供了一个潜在的方法。但还有以下几个问题需要解决: 第一, 采用传统体外转化体系诱导hUC-MSCs的效率低, 转化扩增后的细胞数目还不够用于细胞移植手术[71]; 第二, hUC-MSCs移植在体内后转化生成有功能性的肝细胞较少, 移植4周后, 体内转化效率低于0.05%[71]; 第三, 尽管hUC-MSCs无致瘤性, 但安全问题仍然被人们所关心, 尤其是长期对免疫功能的影响和致瘤性风险, 甚至促进肿瘤的生长通过转变, 抑制抗肿瘤

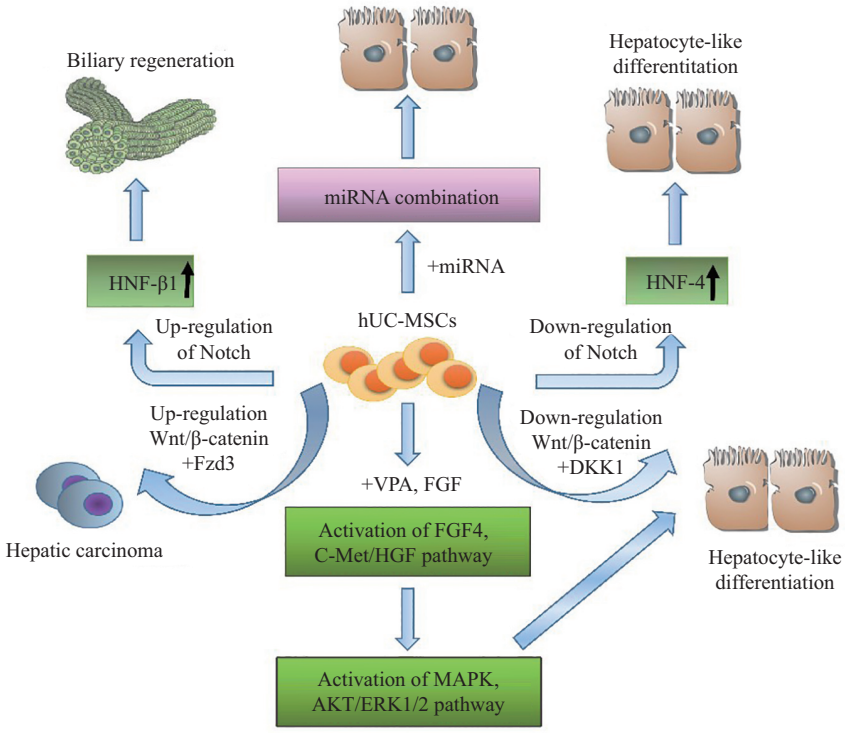

图1 hUC-MSCs诱导分化为肝样细胞可能机制

Fig.1 Possible mechanisms of hUC-MSCs differentiation into hepatocyte-like

免疫反应甚至直接对肿瘤的营养作用[72-73]; 第四, 有研究证明, MSCs在治疗慢性肝脏疾病时可能会加速纤维化进展[74]; 第五, 上述hUC-MSCs肝样分化机制只是部分研究结果, 其分化机制尚不完全明确。只有更好地了解和探索hUC-MSCs分化的分子机制, 才能进一步将hUC-MSCs应用于肝疾病的治疗, 服务于临床。

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