

调控肿瘤上皮-间质转化及肿瘤干细胞样特性的信号通路串话

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摘 要 上皮-间质转化(epithelial-mesenchymal transition, EMT)是上皮来源肿瘤细胞获得侵袭和转移能力的重要生物学过程。肿瘤干细胞样细胞(cancer stem-like cells, CSLCs)在肿瘤发生、侵袭、转移和复发中亦起着关键作用。近年发现, EMT与肿瘤干细胞样特性获得存在密切关联, 二者通过TGF- β 、Wnt/ β -catenin、Notch、Hedgehog、FGF、PI3k/Akt等多种信号通路及通路间的信号串话而交互作用, 共同影响着肿瘤发生、侵袭及转移, 了解调控EMT/CSLCs关键信号分子的功能及相互作用对于肿瘤靶向治疗具有重要意义。

关键词 上皮-间质转化; 肿瘤干细胞样细胞; 信号通路串话; 肿瘤发生; 肿瘤侵袭/转移

1 引言

“上皮-间质转化(epithelial-mesenchymal transition, EMT)”^[1]被认为是上皮来源肿瘤细胞获得侵袭和转移能力的重要生物学过程。上皮细胞在EMT过程中失去了细胞极性及与基底膜的连接, E-钙黏蛋白(E-cadherin)、紧密连接蛋白ZO-1(zonula occluden-1)等上皮标志物表达下调, 而波形蛋白(vimentin)、纤连蛋白(fibronectin)、N-钙黏蛋白(N-cadherin)等间质标志物表达上调, 为其脱离肿瘤主体发生侵袭和转移创造了条件^[2]。近来研究发现, EMT过程除了参与肿瘤的侵袭、转移, 还参与了与肿瘤干细胞样细胞(cancer stem-like cells, CSLCs)或称肿瘤起始细胞(tumor-initiating cells, TICs)^[3]类似性质的肿瘤自我更新、肿瘤发生^[4], 并增强了肿瘤耐药性^[5]。在永生化人类乳腺上皮细胞(human mammary epithelial cell with SV40 LT/st, HMLE)中分离出的间质细胞亚群(mesenchymal subpopulation, MSP)细胞, 同时表达CSLCs标志CD44^{high}/CD24^{low}及EMT转录因子TWIST, 且乳腺微球体(mammospheres)形成能力较强^[6-7]。此外, 经EMT诱导因子TGF- β 诱导的侧群细胞(side population cells, SP)亦表现出间质特性及侵袭能力增强^[8]。

上述研究似乎支持肿瘤EMT过程与CSLCs特性获得通过某些分子学或细胞学行为而相互影响、共同促进了肿瘤的恶性程度。近年来多项研究发现, 肿瘤EMT与CSLCs特性获得通过转化生长因

子- β (transforming growth factor-beta, TGF- β)、果蝇无翅基因同源小鼠乳房肿瘤病毒整合位点家族成员(wingless-type MMTV integration site family members, Wnt)、果蝇翅缘缺刻基因(*Notch*)、刺猬因子(Hedgehog, Hh)、成纤维细胞生长因子(fibroblast growth factor, FGF)、磷脂酰肌醇3激酶(phosphoinositide 3 kinase, PI3K)等多种信号通路及通路间的信号串话而交互作用, 共同影响着肿瘤发生、侵袭及转移。

因此, 本文对近几年关于调节EMT及CSLCs特性的信号通路及信号串话研究展开综合论述, 尝试阐明EMT/CSLCs特性信号调控网络及信号间的协同或拮抗作用, 探讨调控EMT/CSLCs特性的关键信号分子的功能及相互作用, 为构建基于EMT/CSLCs特性关键信号分子的早期诊断及靶向治疗手段提供研究思路。

2 维持EMT与肿瘤干细胞样特性的信号通路及信号串话

2.1 TGF- β 信号网络对于EMT/CSLCs特性的调控

TGF- β 家族是由多种组织分泌的、广泛分布的

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一大类生长因子超家族,广泛参与细胞增殖、分化和细胞外基质分泌、免疫调节、血管形成、胚胎发育、创伤愈合、骨重建等生理过程。

TGF- β 家族可通过Smad通路^[9]及非Smad通路^[10]多种信号网络诱导EMT发生,并在CSLCs自我更新及未分化状态维持中发挥关键作用。采用TGF- β 诱导乳腺癌细胞可通过激活Ras/MAPK通路发生EMT及获得乳腺CSLCs表型CD44^{high}/CD24^{low}^[10]。TGF- β 1可上调EMT转录因子(SNAI1/Snail、SNAI2/Slug、TWIST)而促进EMT发生及肿瘤起始。经他莫西芬及TGF- β 1诱导的HMLE细胞(永生化人类乳腺上皮细胞,无致瘤性)异位表达转录因子TWIST或SNAI1进而发生EMT,细胞显示间质表型的同时也获得了CD44^{high}/CD24^{low}表型;与对照组相比, HMLE细胞的软琼脂集落直径增加,乳腺微球体形成能力增强且自我更新能力增强,皮下注射入裸鼠的肿瘤发生率增加^[6]。此外, TGF- β 还可诱导乳腺癌细胞MCF-10CA1a表达转录激活因子3(activating transcription factor 3, ATF3),并上调SNAI1、SNAI2及TWIST1从而加强细胞运动性^[11]。

2.1.1 TGF- β -Notch信号串话 TGF- β 通路及Notch通路下游靶分子HEY1(Hairy亚基因家族)、JAG1/Jagged-1(Notch1配体)形成串话,共同调控肿瘤EMT及CSLCs特性。采用RNA干扰沉默HEY1或JAG1、化学失活Notch-1均可阻断TGF- β 诱导的EMT^[12]。而Notch通路靶分子HEY1及JAG1参与多种肿瘤CSLCs的特性维持:预先采用 γ -分泌酶抑制剂RO4929097干预NOD/SCID/IL2 γ R^{-/-}(NOG, 非肥胖糖尿病/重症联合免疫缺陷/IL2 γ R阴性)小鼠,再将黑色素瘤细胞注射入该小鼠体内,经实时定量PCR检测小鼠Notch通路下游因子HEY1及黑色素瘤干细胞标志物CD166、CD271及JARID1B的mRNA表达水平,干预组上述指标与未干预组相比均显著降低,且小鼠移植成瘤率降低,表明Notch通路抑制(尤其是HEY1抑制)与黑色素瘤干细胞特性丧失存在密切关联^[13]。采用RNA干扰技术敲除人脑微血管内皮细胞的JAG1基因可使动物移植瘤及体外培养的多形性恶性胶质瘤CSLCs自我更新能力丧失、肿瘤生长停滞,表明Notch/JAG1信号通路活化对于维持多形性恶性胶质瘤CSLCs特性具有重要作用^[14]。

2.1.2 TGF- β -Wnt信号串话 Scheel等^[7]发现,与HMLE²⁴⁺细胞(表型为CD24⁺的HMLE亚群,具有上

皮细胞特性)相比, HTWIST细胞(过表达TWIST)及MSP细胞(经HMLE分离的间质亚群)TGF- β 信号显著活化而骨形态发生蛋白(bone morphogenetic protein, BMP)信号抑制, Wnt经典信号拮抗剂-分泌蛋白DKK1(Dickkopf-1)分泌下调,细胞外经典及非经典Wnt配体拮抗剂-分泌型卷曲相关蛋白1(secreted frizzled-related protein1, sFRP1/FrzoA)基因表达亦下调。他们采用TOP/FOP双荧光素酶报告系统检测MSP细胞的 β 连环蛋白(β -catenin)/T细胞因子(T cell factor, TCF)/淋巴增强子(lymphoid enhancer factor, LEF)通路活性,发现该通路的保守靶基因Axin2比HMLE²⁴⁺细胞对照组上调了15~30倍。MSP细胞Wnt5A配体上调从而活化自分泌非经典Wnt/ β -catenin信号通路,其下游通路涉及的蛋白激酶C(protein kinase C, PKC)、C-Jun蛋白氨基末端激酶(C-Jun N-terminal kinase, JNK)活化,进而激活TGF- β /JNK通路(非Smad信号通路),与Wnt经典及非经典通路形成串话共同促进EMT及癌干细胞特性形成。

2.1.3 TGF- β -PI3k/Akt-miR-200b/Suz12/E-cadherin信号串话 乳腺癌细胞的Akt1基因敲除后可促进TGF- β 诱导的EMT及干细胞样表型表达, Akt1/Akt2比值、微小RNA-200(miR-200)丰度与mRNA编码的E-cadherin丰度呈正相关,证实TGF- β 与PI3K/Akt通路存在串话,并受Akt/miR-200/E-cadherin轴调控^[15]。进一步的研究显示, miR-200b低表达可导致miR-200b的直接靶基因、多梳蛋白抑制复合物2(polycomb repressive complex 2, PRC2)的亚基Suz12高表达,进而导致多梳蛋白介导的E-cadherin基因抑制,最终促进多种乳腺癌细胞系形成CSLCs,并促进肿瘤生长及侵袭能力,表明miR-200b/Suz12/E-cadherin通路对于乳腺癌CSLCs特性维持具有重要意义^[16]。

2.1.4 TGF- β -PI3K/Akt/mTOR信号串话 TGF- β 可通过Smads通路转录性调节EMT发生,也可通过激活PI3K/Akt/哺乳动物雷帕霉素靶分子(mTOR)通路翻译性调节细胞蛋白合成,使细胞在EMT过程中体积增大,并进一步促进细胞的侵袭/转移特性。使用雷帕霉素可显著抑制该病理过程^[17]。最近研究发现, TGF- β 在调控上皮细胞发生EMT的同时可迅速诱导mTOR复合物2(mTORC2)激酶活化,并调节EMT相关细胞骨架改变及基因表达;而使mTORC2失活则可抑制肿瘤细胞在活体内播散,显示mTORC2通路可能是TGF- β 信号的关键下游分支,

参与了细胞转移及侵袭的过程,可作为抑制EMT及肿瘤侵袭、转移的敏感靶点^[18]。

研究表明, mTOR亦参与了CSLCs相关的肿瘤发生。mTOR通过正向调节信号传递与转录激活因子3(signal transducers and activators of transduction 3, STAT3)通路,可促进乳腺癌MCF7细胞系中的侧群细胞在体外形成细胞群落及小鼠体内肿瘤发生,表明该侧群细胞富集了CSLCs^[19]。将敲除*PTEN*基因的人前列腺上皮细胞移植入免疫缺陷小鼠,发现小鼠体内mTORC2活性增强并促进了肿瘤起始。值得注意的是, mTORC2活性对于维持正常前列腺上皮细胞的功能不是必需的^[20],因此研究靶向mTORC2的治疗策略对于肿瘤的治疗具有较大意义。

2.1.5 TGF- β -SOX4/SOX2信号串话 Y染色体性别决定区相关高迁移率族蛋白盒(SRY-related high-mobility group box, *SOX*)基因是指具有一个HMG-box基序保守区,且编码的蛋白质与SRY/Sry(sex-determining region of Y chromosome)基因产物在该保守区具有60%以上氨基酸序列相似性的基因, *SOX*基因在个体发育、维持干细胞特性中发挥重要作用^[21]。*SOX*基因的突变和异常表达与多种肿瘤的发生及进展有关^[22],如*SOX4*被认为是人类乳腺癌致癌基因-人类表皮生长因子受体2(human epidermal growth factor receptor 2, HER2/neu/C-erbB2)的调节者^[23]。

*SOX4*作为TGF- β 信号通路的直接靶基因,参与TGF- β 诱导的肿瘤EMT过程及CSLCs特性获得。采用siRNA敲除*SOX2*、*SOX4*可显著抑制神经胶质瘤起始细胞形成微球体及自我更新能力,并降低CD133⁺细胞群分布,表明TGF- β -*SOX4*/*SOX2*信号串话对于维持神经胶质瘤起始细胞特性具有重要作用^[24]。采用RNA干扰技术敲除*SOX4*或*SOX4*靶基因神经纤毛蛋白-1(neuropilin-1, *NRP-1*)、信号素3C(semaphorin 3C, *SEMA3C*)可显著抑制肝细胞癌细胞系体外迁移活性,肝细胞癌细胞表现出间质-上皮转化(MET)样表型,导致裸鼠移植瘤肿瘤发生及肝内转移,证实*SOX4*在肝细胞癌EMT过程及侵袭、转移中发挥着关键作用。

2.2 FGF信号通路对于EMT/CSLCs特性的调控

FGF家族的生理作用有促进血管生长、促进创伤愈合与组织修复、促进组织再生及参与神经再生等^[25]。研究发现,多种癌瘤通过激活FGF信号参与EMT及CSLCs特性获得,表现出促进肿瘤增殖、肿

瘤血管发生、肿瘤侵袭/转移、肿瘤抗化学药物及抗放射治疗等作用^[26-29]。

2.2.1 FGF-MAPK/ERK信号串话 间质标志物N-cadherin可与FGF受体(FGFR)相互作用,防止FGFR配体诱导的细胞内摄作用而增加FGFR稳定性,促进FGF信号激活; N-cadherin与FGF-2协同启动转移信号级联反应,持续性激活MAPK/ERK通路,促进*MMP-9*基因转录及细胞侵袭,二者协同促进乳腺癌转移、侵袭及癌细胞外蛋白酶分泌^[27]。但也有报道,FGF-1可通过MAPK/ERK通路逆转TGF- β 1诱导肺癌细胞A549细胞系发生的EMT,恢复其上皮形态^[30]。

在脑瘤CSLCs^[31]、乳腺癌CSLCs^[32]体外培养过程中, FGF作为一种促生长因子,可增强CSLCs的自我更新能力、促进CSLCs微球体形成,并发现其机制与激活FGF-ERK通路相关^[33]。

2.2.2 FGF/uPAR-Wnt/ β -catenin信号串话 膀胱癌细胞株NBT-II通过自分泌FGF-1或FGF-2信号降低E-cadherin在细胞膜的表达,进而获得EMT及侵袭活性,同时发现尿激酶型纤维蛋白酶原活性因子受体(urokinase type plasminogen activator receptor, uPAR)是FGF/FGFR信号的早期靶蛋白, E-cadherin及白细胞共同抗原相关蛋白酪氨酸磷酸酶(leukocyte antigen related protein tyrosine phosphatase, LAR-PTP)则是FGF/FGFR信号的晚期靶基因^[26]。

uPAR信号可单独或通过EMT机制共同诱导乳腺癌细胞CSLCs特性形成^[34]。最近发现,在受辐射的髓母细胞瘤中由uPAR介导的Wnt/ β -Catenin信号增强,显示uPAR可以作为一种潜在的癌干细胞特性激活物^[35]。

2.2.3 FGF-Wnt/FZD4信号串话 通过二聚体化学诱导剂(chemical inducers of dimerization, CID)诱导前列腺定位表达可诱导纤维细胞生长因子受体1(inducible FGFR1, iFGFR1)的小鼠发生与EMT高度同步的前列腺癌,并增强Wnt受体FZD4的表达^[28];采用RNA干扰技术沉默前列腺癌细胞*FZD4*基因或*ETS*相关基因(ETS related gene, *ERG*)均可导致活化整合素 β 1(β 1 integrin)及E-cadherin表达增加,证实FZD4是人前列腺癌细胞*ERG*癌基因诱导的Wnt信号分子及EMT的介质^[36]。 β 1 integrin是细胞黏附分子家族的重要成员之一,主要介导细胞与细胞外基质(ECM)之间的相互黏附及双向信号传导,在恶性肿瘤组织中可表达降低、缺失^[37]或过表达^[38],而基因

沉默 $\beta 1$ *integrin*可抑制TGF- β 介导的p38 MAPK活化及EMT过程^[39]。

FGF可促进体外培养的人非小细胞肺癌患者恶性胸腔积液分离的原代肺癌细胞、人非小细胞肺癌NCI-H460细胞的微球体及贴壁细胞生长,并同时检测到上述细胞可高表达FZD4及CSLCs细胞标志物乙醛脱氢酶(aldehyde dehydrogenase, ALDH)、8聚体结合转录因子4(octamer-binding transcription factor 4, OCT4)、同源域蛋白(Nanog homeobox protein, Nanog)、STAT3及Notch3,上述细胞移植入NOD/SCID小鼠可有效导致肿瘤生成^[40],从而表明FGF-Wnt/FZD4信号串话对于人非小细胞肺癌CSLCs特性获得具有重要作用。

2.2.4 FGF/FGFR/Tbx3通路 采用雌激素或FGF9预处理乳腺癌细胞株及新鲜乳腺癌组织分离的乳腺癌细胞,可通过旁分泌FGF/FGFR/Tbx3信号通路诱导乳腺癌CSLCs扩增,使用他莫昔芬或FGFR小分子抑制物可有效阻止雌激素诱导的CSLCs扩增;抑制*FGFR*或*Tbx3*基因表达可消除乳腺微球体形成^[29]。

*Tbx3*基因是*T-box(Tbx)*基因家族的成员之一,这一家族属于发育调控相关转录因子基因家族。*Tbx3*在头颈部鳞状细胞癌(HNSCC)中过表达,并促进HNSCC表现出EMT表型。采用RNA干扰技术沉默*Tbx3*基因可抑制HNSCC的侵袭能力^[41]。

2.3 Wnt信号通路对于EMT/CSLCs特性的调控

目前,认为Wnt通路的组成主要包括细胞外因子(Wnt)、跨膜受体卷曲蛋白(Frizzled, FZD/Frz)、胞质 β -连锁蛋白(β -catenin)及核内TCF等一系列蛋白。Wnt信号通路不仅在胚胎发育过程中起着至关重要的作用,并与肿瘤、CSLCs的发生发展具有密切关系。经顺铂(cisplatin, CDDP)筛选的人非小细胞肺癌A549细胞高表达Wnt/ β -catenin信号及肺癌CSLCs标志物OCT4,基因敲除 β -catenin可导致Wnt靶基因细胞周期蛋白D1(*cyclin D1*)下调,并抑制A549细胞的增殖、克隆形成、迁移及耐药能力, OTC4表达降低^[42]。

2.3.1 Wnt/ β -catenin/TCF通路 经典Wnt/ β -catenin信号通路可激活SNAIL1参与EMT过程, SNAIL1亦可与 β -catenin相互作用促进Wnt靶基因表达^[43]。Wnt/ β -catenin信号通路在维持人类皮肤恶性鳞状细胞癌CSLCs表型中发挥关键作用,敲除 β -catenin基因导

致CSLCs消除、移植瘤消失^[44]。作为肝癌等CSLCs标志的EpCAM⁺细胞亦显示Wnt/ β -catenin信号通路激活^[45]。Wnt/ β -catenin信号通路特异性下游效应因子TCF/LEF突变导致小鼠小肠隐窝干细胞增殖能力丢失,表明TCF4在小肠干细胞自我更新中发挥了重要作用, β -catenin/TCF4复合物是一种促癌因子^[46]。

2.3.2 Wnt5A-PKC信号串话 人黑色素瘤细胞株UACC1273经重组Wnt5A处理后,可通过非经典通路抑制*KISS-1*(转移抑制因子)及*CD44*(可引导转移瘤归巢)基因表达,同时上调SNAIL1及vimentin、下调E-cadherin诱发EMT从而获得运动性,此EMT过程由蛋白激酶C(PKC)介导而非依赖经典的Wnt/ β -catenin信号通路^[47]。

在共同表达肿瘤排斥抗原-1-60(TRA-1-60)、CD151及CD166三种干细胞标志物的前列腺癌CSLCs中发现PKC/NF- κ B信号高表达,证实持续的PKC/NF- κ B信号激活有助于维持前列腺CSLCs特性^[48]。

2.4 Notch信号对于EMT/CSLCs特性的调控

Notch信号通路是进化中高度保守的信号转导通路,其调控细胞增殖、分化和凋亡的功能涉及几乎所有组织和器官。完整的Notch信号通路包括跨膜蛋白受体(Ptch)、跨膜蛋白(Smo)、转录因子Gli及下游靶基因。

在具有CD44⁺/CD24⁻表面标志的转移性乳腺癌CSLCs中发现Notch通路异常活化,被认为是乳腺癌发生的早期事件^[49]。临床前研究表明,Notch信号可保护乳腺癌免受药物诱导的凋亡,抑制Notch信号可阻碍乳腺CSLCs存活^[50]。Notch-2水平在多种脑瘤CSLCs中表达增高,在髓母细胞瘤的Notch信号被阻断后可使正常小脑颗粒神经元前体细胞的分化通路被激活,肿瘤失去了琼脂克隆形成能力及异种移植成瘤能力,CD133⁺细胞群减少了约50%,SP细胞群消失,表明Notch信号在髓母细胞瘤起始中发挥了关键作用^[51]。

2.4.1 Notch-SNAIL1信号轴及Jagged-Notch-SNAIL2信号轴 体外过表达Notch-1的永生化上皮细胞SNAIL1表达增强, E-cadherin表达抑制,接触抑制丢失,发生EMT并伴随致癌性转化^[52]。参与调控肿瘤发生的SNAIL2在Notch-1介导的E-cadherin抑制中发挥着关键作用,可导致 β -catenin活化及抵抗失巢凋亡;同时发现,Notch-1靶基因*HEY*可作为人类乳腺癌Jagged-Notch-SNAIL2信号轴活化的潜在标志物^[53]。

2.4.2 Notch-HER2信号串话 在HER2⁺乳腺癌细胞株培养分离的CSLCs中, Notch-1信号活化可上调HER2表达水平; 而特异性沉默*Notch-1*基因表达则导致HER2表达减少、裸鼠移植瘤乳腺微球体形成率降低^[54]。而与HER2⁺乳腺癌细胞相比, HER2⁺乳腺癌细胞中Notch-1、Notch-3过表达更加明显, Notch信号被基因敲除后导致细胞失去抵抗放疗的能力而致死, 这一现象在CD44⁺细胞中同样比CD44⁻细胞更明显, 表明抑制Notch信号可引起HER2⁺乳腺癌CSLCs染色体损伤, 并有利于增加癌细胞对于放疗等治疗手段的敏感性^[55]。

2.5 Hh信号通路对EMT/CSLCs特性的调控

Hh是在生物体内广泛存在的一种高度保守的基因。Hh可以通过Patched(Ptc)和Smoothened(Smo)两种受体发挥信号传递作用, 核内因子包括转录因子Ci/Gli、丝氨酸/苏氨酸蛋白激酶Fused(Fu)、Fu抑制剂(SuFu)、类运动蛋白Costal-2(Cos-2)、蛋白激酶A(PKA)等。Hh信号在细胞分化、胚胎发育、器官形成、损伤修复和肿瘤发生中都有重要的生理和病理意义。

Hh信号通路可在多种癌瘤生成中被激活或表达增高^[56], 并与干细胞、EMT标志物表达有关^[57]。Hh信号可促进CD44⁺伴CSLCs特性的侵袭性前列腺癌发生^[58]。Hh与慢性髓性白血病(chronic myelogenous leukemia, CML)耐药性和白血病复发都有关联, 可通过Smo受体增加CML-CSLCs数量^[59]。此外, EMT及Hh信号的激活可加强低分化肝癌的耐药性及侵袭性^[60]。Hh信号与Wnt、Notch、EGF/FGF、TGF- β /活化素(Activin)/Nodal/BMP等信号通路均存在串话, 共同参与组成了干细胞及致癌信号网络^[61]。

2.5.1 Hh/Gli-SNAI1-Wnt/ β -catenin信号串话调控EMT Hh家族成员结合膜表面受体后可使Gli转录因子稳定及核积聚, Gli可结合并上调SNAI1进而启动EMT^[62]; Gli1还可通过SNAI1及E-cadherin调节核内 β -catenin信号表达与Wnt通路串话调控EMT^[63]。

2.5.2 Hh/Gli-Nanog信号轴调控CSLCs特性 有学者认为, Gli1是Hh信号及致癌性启动的关键感受器, 可作为Hh信号通路激活的标志基因, 可通过调控胚胎干细胞样基因表达促使CSLCs重编程进而促进其侵袭、增殖及转移, 靶向Gli1、自调整Gli1-Nanog(维持恶性胶质瘤和癌干细胞存活及扩增的关键分子)模块为结肠癌的治疗提供了新的思路^[64]。

2.6 PTEN/PI3K/Akt通路对于EMT/CSLCs特性的调控

第10号染色体缺失的磷酸酶和张力蛋白同源物基因(phosphatase and tensin homolog deleted on chromosome ten, *PTEN*)是一种抑癌基因, 可拮抗PI3K/AKT信号。在多种类型的癌症中, *PTEN*基因突变或缺失。PI3K是一种胞内磷脂酰肌醇激酶, 具有磷脂酰肌醇激酶及丝氨酸/苏氨酸激酶的活性, 可活化蛋白激酶B(PKB, 或称Akt), 后者能直接磷酸化多种转录因子进而促进细胞生长、代谢、增殖和存活信号。近年来发现, PTEN/PI3K/Akt通路参与调控EMT/CSLCs特性的获得^[65-66]。

2.6.1 PTEN/PI3K/Akt/糖原合成酶激酶-3 β (glycogen synthase kinase-3 β , GSK-3 β)-Wnt/ β -catenin信号串话 内源性GSK-3 β 是PI3K/Akt信号通路的关键下游蛋白, 可以磷酸化 β -catenin进而促进其降解。PI3K及Wnt在肿瘤细胞中常被激活, 二者作为抑制因子可直接抑制GSK-3 β , 从而激活SNAI1-EMT级联反应, 活化 β -catenin、抑制E-cadherin, 进而促进肿瘤发生、启动EMT过程^[65-66]。Perry等^[67]在*PTEN*基因敲除并同时活化 β -catenin的小鼠模型中发现长期造血干细胞(long-term HSCs, LT-HSCs)扩增并无进一步分化, 表明PTEN/PI3K/Akt与Wnt/ β -catenin通路存在密切联系, 二者共同参与维持细胞干性。

2.6.2 PTEN/PI3K/Akt-RAS/MAPK信号串话 新近研究发现, PTEN丢失及RAS/MAPK信号通路激活可促进前列腺CSLCs起始的EMT及肿瘤转移^[68]。在转移性乳腺癌细胞系及侵袭性乳腺癌患者肿瘤样本中发现TWIST1发生磷酸化, 并证实TWIST1磷酸化是由Akt/蛋白激酶B介导的, Akt/PKB通过调节转录靶基因TGF- β 2、增强TGF- β 受体信号, 进而维持PI3K/Akt信号过度活化, 最终促进了EMT及乳腺癌转移^[69]。

3 现存问题及研究展望

在基因失调或致癌因子的作用下, 生命体正常的生理信号通路平衡被打破, 多通路、多分子共同参与形成了肿瘤EMT、CSLCs特性获得信号通路及信号串话调控网络(图1), 导致或促进了肿瘤的起始、侵袭、转移及复发。目前, 国内外研究发现了多种参与恶性肿瘤EMT/CSLCs特性获得的信号通路及关键分子, 并采取某些手段干预信号调控以达

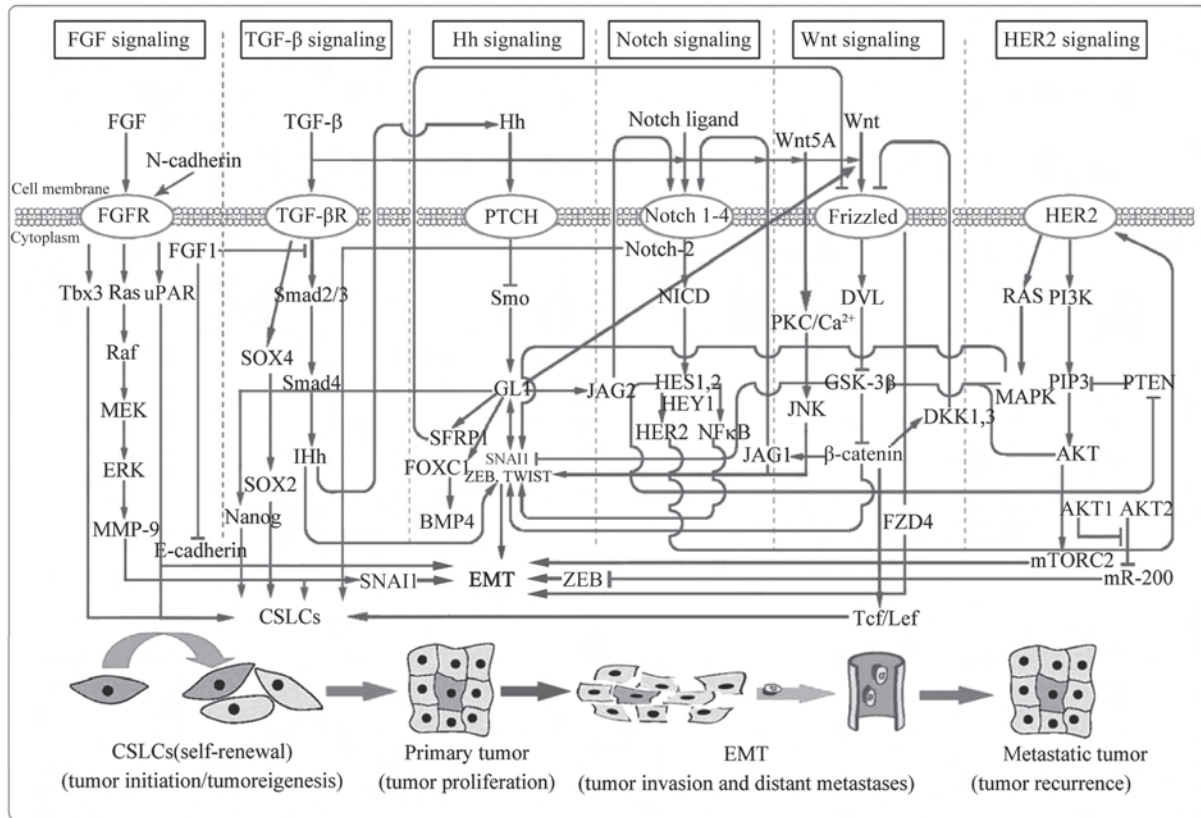


图1 肿瘤EMT、CSLCs特性获得信号通路串话调控网络(根据参考文献[70]修改)

Fig.1 Signaling crosstalk in the regulation of epithelial-mesenchymal transition and cancer stem-like cells properties acquisition (modified from reference [70])

到治疗肿瘤的目的。但大多数信号通路广泛参与维持生命体的正常生长发育、生理功能及内环境稳态等, 如何在不影响正常生理功能的前提下差别性地抑制信号分子引发的肿瘤相关恶性级联反应、使通路恢复正常的生理信号转导, 是构建安全、有效的肿瘤个体化治疗的关键策略之一。探讨EMT/CSLCs特性获得等肿瘤特异性信号串话对肿瘤生物学行为的调控、从整体上实现对生命体内信号传导平衡的恢复或将成为肿瘤领域的研究重点。

因此, 通过建立体外和体内恶性肿瘤EMT模型, 模拟并干预肿瘤微环境, 筛选、鉴定肿瘤EMT/CSLCs分子标志谱, 通过体内外RNA干扰及DNA测序明确肿瘤细胞EMT/CSLCs特性获得的关键靶基因及突变位点, 研究肿瘤的表现遗传因素(DNA甲基化、组蛋白修饰及非编码RNA)对EMT/CSLCs的影响, 筛选干预癌细胞EMT和靶向CSLCs的小分子药物及RNA干扰靶点, 对信号通路涉及的下游因子及其引发的级联反应进行研究, 明确EMT与CSLCs的

内在关系及相互作用机制, 阐明恶性肿瘤基因表达调控网络, 进而构建基于EMT/CSLCs特性获得关键信号分子的早期诊断及靶向治疗手段, 对于减少肿瘤的发生、侵袭、转移、耐药及复发, 改善肿瘤患者预后、延长生存率、提高生存质量具有重要意义。

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Signaling Crosstalk in the Regulation of Epithelial-Mesenchymal Transition and Cancer Stem-Like Cells Properties Acquisition

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Abstract Epithelial-mesenchymal transition (EMT) is an important biological process that epithelial tumor cells can obtain the ability of invasion and metastasis. Cancer stem-like cells (CSLCs)/tumor-initiating cells (TICs) play a key role in tumorigenesis, tumor invasion, metastasis and recurrence. In recent years, it was found that EMT had a close correlation with the acquisition of CSLCs properties, they promote tumorigenesis, tumor invasion and metastasis by complicated interaction through signaling crosstalk between TGF- β , Wnt/ β -catenin, Notch, Hedgehog, FGF, PI3k/Akt and other signaling pathways. Understanding the functions and interactions of key molecules within the context of EMT/CSLCs signaling is critical to design targeted therapeutics.

Key words epithelial-mesenchymal transition(EMT); cancer stem-like cells(CSLCs); signaling crosstalk; tumorigenesis; tumor invasion/metastasis

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