乳腺癌干细胞调控及靶向治疗研究进展

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摘要 乳腺癌干细胞是乳腺肿瘤内具有自我更新能力以及多向分化潜能的细胞, 乳腺癌的发生、发展、转移、复发与干细胞的高致瘤性、高侵袭转移性、治疗抵抗能力密切相关。深入研究乳腺癌干细胞相关细胞因子及微环境因素的调控对乳腺癌的临床靶向治疗具有重要指导意义。该文就近年来乳腺癌干细胞调控相关信号转导通路、转录因子、表观遗传调控因子以及微环境因素进行综述, 探讨乳腺癌干细胞及其相关信号因子作为乳腺癌治疗靶点的潜在价值, 为临床靶向治疗乳腺癌提供新方向。

关键词 乳腺癌干细胞; 乳腺癌; 信号通路; 靶向治疗

Progress on Regulation and Targeted Therapy of Breast Cancer Stem Cells

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Abstract Breast cancer stem cells are cells with self-renewal capabilities and the potential for multidirectional differentiation within breast tumors. The occurrence, development, metastasis, and recurrence of breast cancer are closely associated with the high tumorigenicity, high invasive metastatic ability, and treatment resistance of stem cells. In-depth research on the regulation of breast cancer stem cells-related cytokines and microenvironmental factors is of significant guiding importance for clinical targeted therapy of breast cancer. This article reviews recent advances in the regulation of breast cancer stem cells, including signaling pathways, transcription factors, epigenetic regulatory factors, and microenvironmental factors. It explores the potential value of breast cancer stem cells and their associated signaling factors as therapeutic targets for breast cancer, providing new directions for clinical targeted therapy of breast cancer.

Keywords breast cancer stem cells; breast cancer; signaling pathways; targeted therapy

乳腺癌是最常见的癌症之一, 约占全球新发女性癌症患者比例的30%, 2020年度GLOBOCAN统计数据显示乳腺癌已成为发病率第一的癌症, 其致死率仅次于肺癌, 预测至2025年, 全球乳腺癌相关死亡人数将高达76万, 呈显著上升趋势[1]。乳腺癌是一种异质性恶性肿瘤, 在组织病理学上可分为导管癌、小叶癌、管状癌和乳头状癌; 在分子分型上可分为Luminal A亚型(ER^+/PR^+, $HER2^-$)、Luminal B亚型(ER^+/PR^+, $HER2^+$)、HER2阳性($ER^-/PR^-/HER2^+$)和三阴性($ER^-/PR^-/HER2^-$)。

研究表明, 包括乳腺癌在内的大多数实体癌都是干细胞疾病[2]。乳腺癌干细胞(breast cancer stem cell, BCSC)是首个被分离鉴定的实体瘤干细胞。2003年, AI HAJJ等[3]通过荧光激活细胞分选技术

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(fluorescence activated cell sorting, FACS), 从乳腺癌组织中分离到表面分子标记为$CD44^+/CD24^{-/low}/Lin^-$的细胞, 该细胞能在非肥胖性糖尿病/严重联合免疫缺陷(non-obese diabetic/severe combined immune deficiency, NOD/SCID)小鼠体内形成肿瘤, 并且在小鼠瘤体中发现的同种$CD44^+/CD24^{-/low}/Lin^-$细胞亚群, 也可在小鼠体内成瘤, 而接种无此表型的肿瘤细胞则不能形成肿瘤, 有力证明了成熟的分化完全的小鼠乳腺中存在具有分化潜能和自我更新能力的BCSC, 或癌症起始细胞。目前, 用于鉴定与分选BCSC表型的方法主要包括: 表面标志物分选法、侧群细胞分选法、醛脱氢酶活性测定分选法、肿瘤细胞球体培养法等[4-5]。肿瘤干细胞具备与成体干细胞相似的增殖特性, 其增殖潜能强大但增殖速度缓慢。多数细胞处于细胞周期的G_0/G_1期[6], 同时表达大量耐药基因, 因此对于化疗、放疗以及内分泌治疗等常规手段并不敏感[7]。这使得传统的癌症治疗方法难以特异性地针对肿瘤干细胞, 因此, BCSC被认为是肿瘤发生与发展的基础。

目前, 干细胞生物学的发展逐步成熟, 对BCSC的深入研究为改善患者预后、提高患者生存率提供了新的方向, 为乳腺癌的根治提供了希望。本文主要综述BCSC调控乳腺癌发生与发展的相关分子机制, 进一步阐述靶向BCSC在乳腺癌治疗中的新方法, 为乳腺癌的治疗提供新思路。

1 调节乳腺癌干细胞的信号通路

细胞自我更新能力的失调是肿瘤发生、发展的重要因素。在乳腺中, Notch、Wnt/β-catenin和Hedgehog等通路参与调节干细胞的自我更新与分化之间的平衡, 在器官发生以及维持成体组织中发挥重要作用[8]。

1.1 Notch信号通路

Notch信号转导通路由受体、配体和DNA结合蛋白等三部分组成。Notch跨膜受体(Notch1~4)被相邻细胞提供的配体[DLL1/3/4(delta-like ligand 1/3/4)、JAG1/2(Jagged ligand 1/2)]激活, 并经历γ分泌酶介导的一系列切割后, Notch胞内结构域(Notch intracellular domain, NICD)与Notch蛋白分离并易位到细胞核, 激活下游转录因子如Hes和Hey等。小分子化合物ASR490可下调NICD及其下游效应物Hey1和Hes1的表达, 进而诱导BCSC中溶酶体与自噬体的融合来抑制干细胞生长, 且对正常细胞无毒副作用, 可作为靶向药物抑制乳腺肿瘤生长[9]。Notch信号通路的异常激活还会促进BCSC产生化疗/放疗抵抗[10], 研究表明, 上皮–间质转化(epithelial-mesenchymal transition, EMT)也可赋予癌细胞迁移、侵袭、自我更新和耐药的能力, 其特征是细胞黏附分子中E-钙黏蛋白(E-cadherin, CDH1)表达水平降低, 波形蛋白(vimentin, VIM)、N-钙黏蛋白(N-cadherin, CDH2)和纤连蛋白表达水平升高[11], 而CDH2和Notch2在三阴性乳腺癌(triple negative breast cancer, TNBC)细胞中共表达, 其表达量与干细胞相关基因*SOX2*和*NANOG*的表达量呈正比[12], 表明CDH2与Notch2的协同作用可能成为BCSC靶向治疗的研究方向之一。此外, LAWAL等[13]通过生物信息学研究发现, INFG/STAT1/Notch3的相互作用在癌症相关成纤维细胞(cancer-associated fibroblast, CAF)与BCSC之间构建了关键的分子连接, 这一连接与TNBC细胞的耐药性、侵袭性以及致瘤能力密切相关。下调STAT1的表达可以显著降低细胞致瘤性及CAF的转化潜能。同时, 研究表明γ-山竹黄酮(gamma mangostin, gMG)具有类似的作用, 并能改善阿霉素(doxorubicin, DOX)的耐药性。沉默*Notch4*可通过下调Cdc42的表达来抑制肿瘤转移, 但这种处理会导致*NANOG*的表达上调, 从而增强细胞的致瘤能力[14]。因此, Notch4可能并不适合作为治疗靶点。这也表明乳腺癌治疗需要综合考虑多个分子通路的影响, 以确保更有效的治疗效果。

1.2 Wnt/β-catenin信号通路

Wnt/β-catenin通路参与包括乳腺在内许多器官的发育。Wnt蛋白与细胞表面的卷曲受体蛋白家族(frizzled)以及低密度脂蛋白受体(low-density lipoprotein receptor-related protein, LRP)结合, 导致细胞质中β-catenin的稳定和积累, 随后β-catenin蛋白易位至细胞核与淋巴增强因子1/T-细胞因子(1ymphoid enhancer factor 1/T cell factor, LEF/TCF)家族转录因子结合, 激活典型的Wnt信号转导途径。GRP78/LRP5/β-catenin信号转导是一种促进BCSC发生的新途径, 其中GRP78是一种在多种恶性肿瘤中过度表达的内质网应激蛋白, GRP78靶向剂头孢塞利(cefoselis)可通过上述信号通路直接作用于BCSC抑制其增殖以及致瘤能力[15]。研究表明多种癌细胞中Wnt通路负调控因子的基因发生突变和缺失, β-catenin大量聚集并与转录因子形成复合物, 激

活下游靶基因, 推动细胞周期发展或异常蛋白质产生, 诱导EMT过程使细胞发生癌变[16]。盐霉素(salinomycin, SL)和DOX通过纳米递送系统可有效渗透到3D培养的BCSC微球中, 下调Wnt/β-catenin信号通路相关基因(*β-catenin*、*LRP6*、*LEF1*和*TCF12*)以及干细胞相关基因(*OCT4*、*NANOG*和*Hes1*)的表达, 有效逆转EMT过程, 这可作为乳腺癌治疗的新方法[17]。与之相似, 绿原酸(chlorogenic acid, CA)能够通过靶向*LRP6*抑制EMT和乳腺癌的侵袭, 可作为乳腺癌治疗的候选药物之一[18]。

1.3 Hedgehog信号通路

Hedgehog信号以空间、时间和浓度依赖的方式广泛参与细胞增殖、迁移和分化的调节。Hedgehog配体通过跨膜受体Ptch将信号转导至跨膜蛋白SMO(smoothened)上, 激活一系列下游蛋白, 从而调控靶基因的表达。研究发现, Ptch和下游蛋白Gli1和Gli2的表达在正常乳腺干细胞和BCSC中上调, 在细胞分化过程中下调[19], 表明Hedgehog信号蛋白可能是包括BCSC在内的许多类型癌症干细胞的潜在治疗靶点。小分子GANT61是胶质瘤相关癌基因同源物蛋白(glioma-associated oncogene homolog, GLI)的转录抑制剂, 可阻碍Hedgehog信号转导从而抑制TNBC中BCSC的增殖[20]。此外, 从茄属植物中分离的甾体生物碱澳洲茄胺与GLI1锌指结构域之间具有高亲和力, 可以显著下调MCF-7细胞中GLI1与肿瘤干细胞标记物CD44和ALDH的表达, 是一种新的靶向BCSC抗癌候选药物[21]。酸浆苦素A(physalin A)同样为从茄科植物中分离得到的化合物, 可作用于Hedgehog通路, 通过下调GLI与G蛋白偶联受体SMO的表达水平从而抑制BCSC相关多能性基因(*OCT4*、*CD44*、*SOX2*、*c-Myc*、*NANOG*)的表达[22]。另有研究发现, 姜黄素(curcumin)可下调TNBC细胞中CDH1、VIM和Hedgehog通路中Gli1、Gli2、SMO的表达, 进而抑制癌细胞增殖、侵袭以及迁移[23]。此外, ETS(E-twenty six)转录因子家族中的ETV4(ETS translocation variant 4)一方面可通过转录促进趋化因子受体CXCR4(C-X-C chemokine receptor 4)的表达激活Hedgehog信号转导, 另一方面可通过增强糖酵解活性, 促进BCSC干性。这表明靶向ETV4可能是破坏癌细胞代谢过程和抑制肿瘤干性的有效方法, 为治疗癌症提供新的方向[24]。

此外, NF-κB、BMP-2、PI3K/AKT/mTOR等多条信号通路与BCSC的EMT过程、耐药性、细胞干性紧密相关[25], 上述信号通路的抑制剂可能是乳腺癌的靶向治疗药物(图1)。

2 调节乳腺癌干细胞的转录因子

BCSC自我更新能力及多能性的维持取决于多个细胞因子对其信号通路的调控[26]。OCT4是一种POU结构域转录因子, 在小鼠胚胎发育期间的所有多能干细胞及未分化的胚胎干细胞中特异性表达, 与细胞的可塑性和分化能力直接相关[27]。TNBC细胞中趋化因子配体16(recombinant chemokine c-c-motif ligand 16, CCL16)通过与细胞表面趋化因子受体2(recombinant chemokine c-c-motif receptor 2, CCR2)特异性结合激活p-AKT/GSK3β信号通路, 导致β-catenin发生核易位并与*OCT4*启动子结合, 从而诱导OCT4的表达。因此, CCL16可能是乳腺癌症治疗的有效靶点[28]。信号转导和转录激活因子3(signal transducer and activator of transcription 3, STAT3)与*CCL16*启动子结合可促进CCL16表达, 与干细胞的自我更新能力直接相关[29]。一些STAT3共调控因子还会影响BCSC的增殖。例如, 孕激素与乳腺癌的化疗耐药性和不良预后有关[30-31]。有研究发现, 使用特定的孕激素反义寡核苷酸可抑制STAT3的激活并降低STAT3相关致癌蛋白的表达水平[32], 可能在乳腺癌症细胞中也有类似的作用。

NANOG是能够独立于LIF-STAT3途径维持干细胞多能性的关键因子[33]。沉默NANOG可以抑制乳腺癌细胞的干细胞特征, 包括降低$CD44^+/CD24^-$细胞比例、抑制干细胞相关基因及分化标志物的表达、抑制微球体形成等[34], 还可降低蛋白激酶Cε(protein kinase Cε, PKCε)表达水平进而有效抑制乳腺癌细胞的侵袭[35]。*SOX2*/*OCT4*与*NANOG*启动子的相互作用会促进*NANOG*的转录[36]。SOX2是维持细胞干性的主要调节因子[37], 在内质网应激分子假性激酶3(tribbles pseudokinase 3, TRIB3)高表达的BCSC中起着重要作用。TRBI3通过抑制AKT-FOXO1相互作用, 促进*SOX2*的转录表达。因此, 通过干扰TRIB3-AKT相互作用可直接靶向BCSC治疗癌症。研究表明, 敲除*TRIB3*可降低MDA-MB-231细胞中SOX2和c-Myc的蛋白表达水平[38]。在30%~50%的高级别乳腺癌中, c-Myc表达失调, 其表达量与BCSC标记物CD44、CD24和ALDH的表达量呈正相

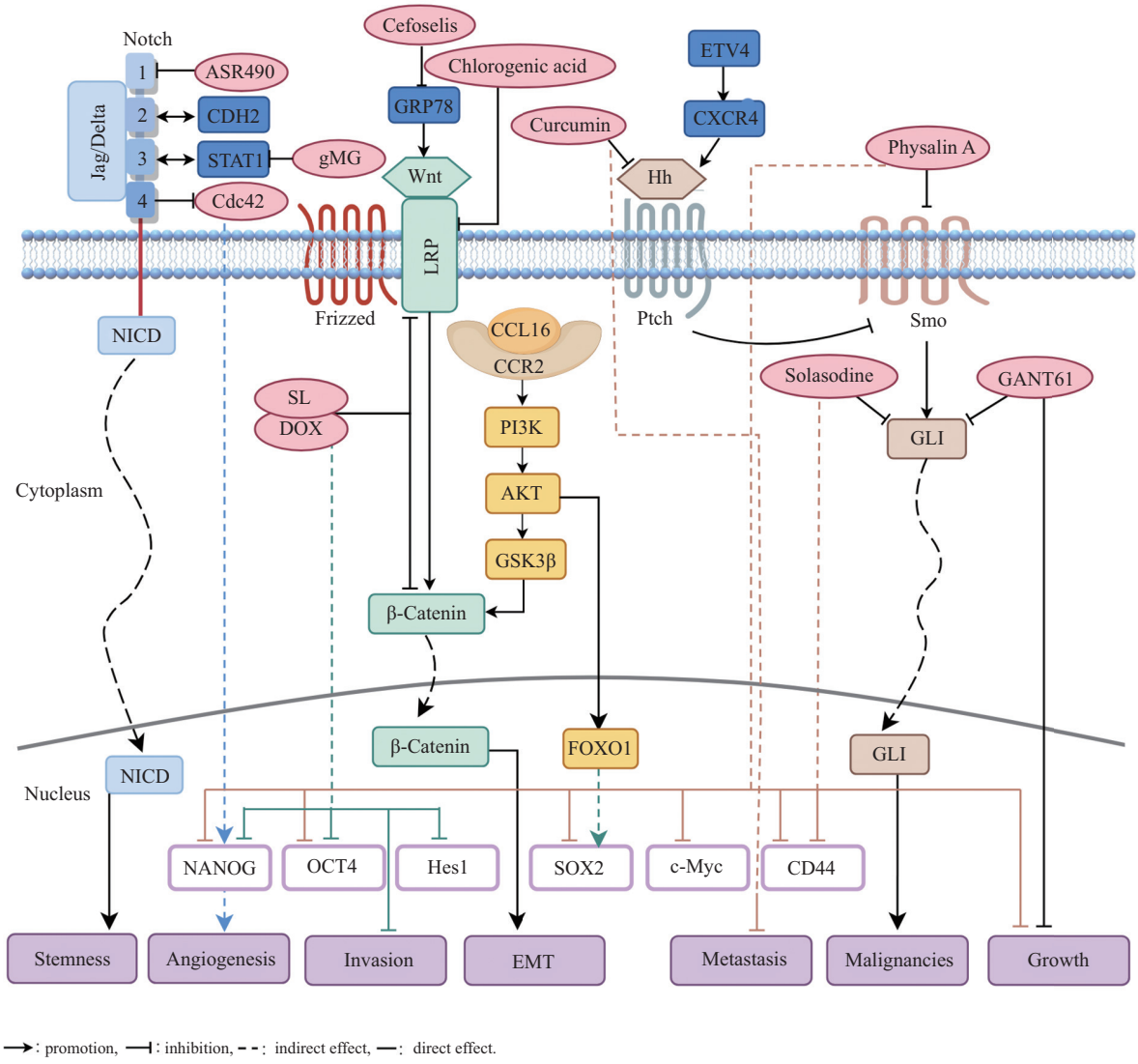

图1 调节乳腺癌干细胞(BCSC)的关键信号通路(根据参考文献[25]修改, 并采用Figdraw绘制)

Fig.1 Critical signaling pathways involved in regulating BCSC (breast cancer stem cell) (modified from the reference [25], and illustrated by Figdraw)

关[39]。作用于c-Myc的小分子最近已成为临床候选药物, 如抑制剂361(MYCi361)和975(MYCi975)可破坏c-Myc-MAX(MYC associated factor X, MAX)的相互作用, 降低MYC蛋白的稳定性, 从而抑制Myc依赖性癌细胞的增殖[40]。

3 乳腺癌干细胞的表观遗传学调控因子

表观遗传是指非DNA序列改变而导致的表型或基因表达变化的遗传现象, 其调控机制涵盖了DNA甲基化、组蛋白翻译后修饰和非编码RNA等多个方面。表观遗传学修饰通过调节基因的表达方式、表达时间和表达位置, 决定分化过程中细胞的命运[41]。因此, 表观遗传失调严重影响肿瘤的发生、发展以及治疗期间癌细胞与免疫系统之间的相互作用。

DNA甲基化与BCSC的干性维持与免疫逃逸有关。SULTAN等[42]研究表明, 由于抗原处理和呈递基因(transporter associated with antigen processing, *TAP*)启动子超甲基化, 4T1细胞的*TAP1*和*TAP2*以及共刺激分子CD80的表达量减少, 免疫活性小鼠中的BCSC对T细胞介导的攻击的敏感性降低, 因此, 脱甲基剂可用于促进抗原呈递, 提高肿瘤对T细胞或免疫检查点抑制剂的敏感性[43]。此外, 核转录因子FOXO3a是FOXO蛋白家族的一种转录因子, 与多种癌症的发生、发展和不良预后有关[44]。DNA甲基转移酶1(DNA methyltransferase 1, DNMT1)介

导的*FOXO3a*启动子的超甲基化可导致乳腺癌细胞中FOXO3a表达下调, 从而增强BCSC干细胞特性与致瘤能力[45], 这一发现为乳腺癌的治疗提供了潜在的靶点。类端粒沉默干扰体1(disruptor of telomeric silencing 1-like, DOT1L)是一种高度保守的组蛋白甲基转移酶, 可介导基因启动子区域的组蛋白H3K79(H3Lys-79)的甲基化。TCGA数据分析显示, DOT1L在乳腺癌组织中的表达量显著高于正常乳腺组织, 在TNBC中的表达量高于侵袭性较低的管腔型乳腺癌[46]。因此, TNBC的高转移率与H3K79高度甲基化相关[47]。 PsA(psammaplin A, PsA)是一类于海绵生物中被发现的酚类化合物, 具有抑制DNMT活性的特性, SUB BYUN等[48]采用硒硫键替代二硫键合成了新的PsA结构类似物, 该类物质抑制DOT1L介导的H3K79甲基化并在TNBC细胞中表现出抗肿瘤活性, 其还可通过调控EMT标记物, 包括上调CDH1或者下调CDH2和VIM的表达, 显著抑制TNBC的侵袭和迁移。此外, 组蛋白甲基转移酶SUV4-20H2, 是一种作用于组蛋白H4的甲基转移酶。靶向SUV420H2的miRNA-29a通过减弱组蛋白的三甲基化从而下调结缔组织生长因子和早期生长反应蛋白1(early growth response-1, EGR1)的表达, 促进TNBC细胞的EMT过程, 进而促进BCSC的迁移和侵袭[49-50]。

多种非编码RNA在乳腺癌干细胞(BCSC)中表达异常, 且参与调节细胞的生存和凋亡等关键生物学过程[51]。其中, 微小RNA(microRNA, miRNA)如miR-155、miR-140、miR-21、miR-22、miR-24、miR-208a、miR-10b、miR-27a、miR-99a、miR-29b、miR-34、miR-221/222、miR-142、miR-520b、Let-7和miR-30, 在维持BCSC干性与细胞耐药性方面发挥关键作用[52-55]。同时, 如miR-200家族、miR-128、miR-600、Let-7c、miR-30、miR-34和miR-489, 可能具有肿瘤抑制作用, 其过表达能够降低BCSC干性或逆转耐药性[56-57]。这些miRNA通过不同的机制在调节BCSC的自我更新、介导肿瘤转移和耐药性方面发挥着重要作用。长链非编码RNA(long non-coding RNA, lncRNA)如HOTAIR、ROR和00617均能通过EMT信号通路调节BCSC干性[58], 是靶向BCSC治疗的潜在生物标志物和治疗靶点。

4 调节乳腺干细胞的微环境因素

BCSC不仅起源于过度激活的正常干细胞或发生突变的祖细胞, 也是肿瘤微环境(tumor microenvironment, TME)诱导癌细胞分化的结果[59]。低氧是乳腺癌微环境的关键特征之一。缺氧诱导因子1(hypoxia inducible factor-1, HIF-1)是由HIF-1α和HIF-1β亚基组成的异二聚体, 它参与了细胞的干性与代谢重编程、肿瘤的侵袭与转移、肿瘤免疫逃避等关键过程中基因的表达, 其异常表达往往与患者死亡率密切相关[60]。研究表明, HIF-1能够诱导激活核纤层蛋白A识别因子(nuclear prelamin A recognition factor, NARF), NARF进而将组蛋白去甲基化酶KDM6A(lysine demethylase 6A)募集至OCT4结合位点, 导致组蛋白H3(H3K27me3)去甲基化, 从而促进OCT4介导的*NANOG*、*SOX2*和*KLF4*基因的表达[61]。与之相似, 紫杉醇诱导HIF-1依赖性S100钙结合蛋白A10(S100 calcium binding protein A10, S100A10)的表达。S100A10和膜联蛋白A2形成复合物, 并与组蛋白伴侣SPT6(suppressor of Ty 6 homolog)、KDM6A相互作用, 促进上述多能性基因的转录, 维持干细胞基因组稳定[62]。靶向HIF-1/KDM6A所介导的表观遗传学通路可能是乳腺癌治疗的新方法。雌激素受体β(estrogen receptor beta, ERβ)是一种核受体, 在正常乳腺和乳腺癌细胞中均有表达。研究发现, 截短ERβ1配体结合结构域(ligand binding domain, LBD)可以增强TNBC细胞系的紫杉醇耐药性以及细胞干性, 而敲低HIF1/2α则能够减弱此种干性。进一步研究揭示, ERβ4敲低的细胞系对紫杉醇敏感。因此, 针对化疗耐药的TNBC患者, 联合使用紫杉醇、ERβ1激活剂以及ERβ4抑制剂可能会取得更好的疗效[63]。与之相似, 钙网蛋白以HIF-1依赖的方式介导Wnt/β-catenin信号转导促进BCSC富集, 可能是乳腺癌治疗的靶点之一[64]。组蛋白赖氨酸甲基转移酶SETD5是乙酰转移酶EP300(E1A binding protein p300)与HIF-1α的上游效应因子, 缺氧条件下, SETD5的敲低可显著降低细胞核中HIF-1α、己糖激酶-2(hexokinase 2, HK2)和磷酸果糖激酶-2(phosphofructokinase-2, PFK2)的表达水平, 表明SETD5通过与EP300/HIF-1α结合, 在乳腺癌干细胞中调节糖酵解的过程中扮演着至关重要的角色[65], SETD5可能是潜在的治疗靶点。

肿瘤发生过程中会招募免疫细胞导致炎性因子富集, 形成肿瘤干细胞赖以生存的炎性微环境。肿瘤相关巨噬细胞(tumor-associated macrophage,

TAM)通过分泌表皮生长因子, 激活细胞内STAT3/SOX2信号通路进而维持BCSC干性, 促进肿瘤的生长与转移[66]。在乳腺癌TME中, TAM作为数量最多的免疫细胞, 常表现出促肿瘤的M2表型。肿瘤相关的脂肪细胞(cancer-associated adipocyte, CAA)可以分泌大量的白细胞介素-6(interleukin 6, IL-6), 通过激活STAT3诱导巨噬细胞M2极化, 进而促进乳腺肿瘤EMT及增殖转移。此外, CAAs可以上调巨噬细胞中PD-L1的表达间接发挥免疫抑制作用。CAA、TME与癌细胞之间的相互作用为乳腺癌治疗提供了新方向[67]。TME由许多不同的类型的细胞和细胞外基质(extracellular matrixc, ECM)组成, 与乳腺肿瘤的发生密切相关[68]。研究发现, ε-肌聚糖[epsilon (ε)-sarcoglycan, SGCE]与E3泛素化连接酶c-CBL(casitas B-lineage lymphoma)相互结合, 通过抑制表皮生长因子受体(epithelial growth factor receptor, EGFR)的溶酶体降解, 从而稳定EGFR蛋白水平, 这一作用不仅调控ECM的沉积和重塑, 且在ε-肌聚糖含量上升时与BCSC的增殖能力和耐药性呈正相关。因此, ε-肌聚糖的含量可能成为乳腺癌的诊断、预后生物标志物[69]。

5 结语

综上可见, 除了内在遗传和表观遗传学变异外, 外源性因素如肿瘤微环境, 也可调节BCSC的异质性和可塑性, 并进一步影响乳腺癌的治疗及预后。目前, 大多数乳腺癌治疗方法并未采用针对BCSC的靶向策略, 导致治疗失败或癌症复发, 而针对BCSC的新一代特异性抗肿瘤药物有望大幅提高肿瘤的治疗效果。已有临床实验证明, γ分泌酶抑制剂(gamma-secretase inhibitor, GSI) MK-0752与多西他赛联合使用可显著减少干细胞的数量, 并降低乳腺肿瘤中乳腺球的形成率[70]。此外, Wnt5a模拟肽Foxy-5对于转移性结肠癌、乳腺癌和肺癌均具有抑制作用[71]。尽管已取得了一些临床试验成果, 但目前只有少数与其他药物联合的抑制剂进入了临床试验阶段, 对此已有学者进行了较为全面的总结, 可查看参考文献[11]。

此外, BCSC在干细胞样和分化状态之间的可塑性表明, 靶向治疗不能仅仅局限于这一小群体, 而应该考虑采用联合治疗策略, 以涵盖更为广泛的肿瘤细胞群体。因此, 乳腺癌的靶向治疗仍然具有挑战, 探索能够作为BCSC治疗靶点、预后观测的生物标志物将是未来研究的重点。

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