肿瘤干细胞与细胞衰老的关系研究进展

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摘要 肿瘤干细胞(cancer stem cells, CSCs)是肿瘤细胞中的一部分具有致癌性、自我更新能力并能产生异质性肿瘤细胞的细胞。CSCs异常的分化以及无限的增殖, 在肿瘤的发生、发展中起着极为重要的作用, CSCs的异常分化甚至会促进癌症的复发。目前, 越来越多的研究表明, CSCs与衰老之间关系密切, 衰老肿瘤细胞可转分化形成CSCs, CSCs本身也可发生衰老变化, 而这些衰老的CSCs与肿瘤的发生、发展均具有密切关系, 在肿瘤的复发和转移中扮演重要角色。该文就CSCs的特征、CSCs与细胞衰老的关系及CSCs衰老调控机制研究方面作一综述, 为临床从衰老角度探讨肿瘤治疗新方法提供新思路。

关键词 肿瘤干细胞; 衰老; 调控机制; 肿瘤微环境

Research Progress in the Relationship between Cancer Stem Cells and Cellular Senescence

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Abstract CSCs (cancer stem cells) are a subset of cancer cells that are carcinogenicity, self-renewal and can produce. The abnormal differentiation and unlimited proliferation of CSCs play an extremely important role in the occurrence and development of tumors, and the abnormal differentiation of CSCs can even promote cancer recurrence. At present, an increasing number of studies have shown a close relationship between CSCs and senescence. Senescence cancer cells can transdifferentiate into CSCs, which can also undergo senescence changes. These senescence CSCs are closely related to the occurrence and development of tumors and play an important role in tumor recurrence and metastasis. This article provides a review on the characteristics of CSCs, the relationship between CSCs and cellular senescence, and the research on the senescence regulation mechanism of CSCs, providing new ideas for exploring new methods of tumor treatment from the perspective of senescence in clinical practice.

Keywords cancer stem cells; senescence; regulation mechanism; tumor microenvironment

肿瘤干细胞(cancer stem cells, CSCs)是一类存在于肿瘤组织内具有自我更新能力并能产生异质性肿瘤细胞的特殊细胞, 具有分化成瘤能力, 可促进肿瘤生长[1]。CSCs是肿瘤复发的潜在动力, 可以促进肿瘤的复发和转移, 最早由LAPIDOT等[2]于1994年在急性髓系白血病(acute myeloid leukemia, AML)的研究中发现, 同时还发现具有免疫表型$CD^+/CD38^-$的CSCs细胞。CSCs与正常干细胞有许多共同的特

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征, 如相对静止、活跃的DNA修复系统, 较强的增殖与化疗耐药性[3-5], 干性标记乙醛脱氢酶(acetaldehyde dehydrogenase, ALDH)的表达[6]以及悬浮培养液中的球体形成能力[7]。随后, 在许多肿瘤中都发现了CSCs的踪迹及相应的标志物[8-9]。

近年来, CSCs的生成与衰老之间的联系引起了人们的广泛关注。首先, 在肿瘤发展的起始、转移和复发阶段均发现逐渐加强的肿瘤细胞衰老迹象[10-11], 而这些阶段也是CSCs产生的主要阶段。其次, CSCs可直接出现细胞衰老表现, 并且衰老对CSCs的形成和化疗耐受方面也发挥调控作用[12]。由于CSCs是导致肿瘤发生、侵袭和复发的关键细胞类型, 因此深入探讨衰老与CSCs之间的关系以及CSCs衰老的调控机制可进一步了解肿瘤的发生机制, 进而为从细胞衰老角度探寻清除或抑制CSCs的治疗新方法提供研究切入点。

1 肿瘤干细胞定义及特征

肿瘤干细胞是肿瘤细胞内的一个小亚群, 是一部分具有无限增殖、自我更新能力的异质性细胞, 主要分布在低氧、低pH的环境中[13], 对周围环境的适应性较强[14]。CSCs具有更为活跃的细胞代谢功能、更有效的DNA修复系统(DNA检查点激酶)和持续的干性特征以及代谢重编程能力(可塑性)[15]。与正常干细胞相比, 致瘤性与高乙醛脱氢酶活性是CSCs区别于正常干细胞的特性[16], 在ALDHs家族中, ALDH1主要与CSCs的干性和化疗耐药有关[17]。目前肿瘤干细胞的表面标志物有CD44、CD133和CD24以及ALDH等[18-20]。

此外, CSCs还具较强的耐药性, 这与其处于静止状态密切相关, 静止期是一种细胞不活跃分裂、非永久性的细胞休眠, 大部分癌细胞在该阶段会被清除, 但一部分处于静止状态的CSCs会在后续癌症的发生发展中起到一定的作用[21]。如在阿霉素诱导乳腺癌的研究中发现, 在阿霉素处理后, 乳腺癌细胞的数量减少, 但小鼠体内处于休眠状态的CSCs数量并没有明显变化[22]。此外, 用替莫唑胺(Temozolomide, TMZ)对胶质瘤患者进行化疗时, 在胶质瘤中不断发现增多的胶质瘤干细胞(glioma stem cells, GSCs), 且实验证明这是分化肿瘤细胞和GSCs之间表型相互转化的结果[23]。总之, CSCs在肿瘤的发生发展以及后续的治疗中具有重要作用, 是参与肿瘤发生、转移、复发等的重要细胞类型。

2 细胞衰老

细胞衰老是一种永久性的细胞周期停滞状态, 细胞在不同压力刺激下均会发生衰老变化[24]。对于大部分有分裂能力的细胞而言, 都会经历衰老的过程, 即使是肿瘤细胞也会在一定刺激下出现衰老表现[25]。衰老的细胞在生化形态、代谢和分泌[26]等方面会发生相应的变化, 如衰老细胞的体积会变大, 比增殖细胞具有更平滑的形状; 出现与衰老相关的异染色质焦点[27]与DNA损伤焦点[28]、衰老相关信号通路的激活[29]、大量炎性因子的分泌[又被称为衰老分泌表型(senescence-associated secretory phenotype, SASP)]和衰老相关的调控基因如*p53*、*p21*以及*p16*等表达水平升高等[30]。此外, 细胞衰老的重要特征还包括细胞代谢和表观遗传重编程[31]。研究发现, 肿瘤组织内也存在不同类型的细胞衰老[24], 在阿霉素治疗乳腺癌的实验中, 肿瘤内衰老相关半乳糖苷酶(senescence-associated-β-galactosidase, SA-β-gal)染色阳性细胞数量明显增多[32]。

由于衰老本身是一种细胞退出增殖周期的增殖停滞状态, 因此衰老最早被认为是一种肿瘤抑制机制, 也衍生出了多种以诱导肿瘤细胞衰老为目的的肿瘤衰老诱导方法。但随着研究的逐渐深入, 研究者发现肿瘤与衰老之间关系复杂, 细胞衰老对于肿瘤而言, 也是一把双刃剑。年龄是影响肿瘤患病率的主要因素之一, 随着年龄增长, 机体衰老水平提升, 肿瘤发生率递增, 在乳腺癌的报道中, 老年患者血清培养会促进癌细胞的增殖[33], 提示器官或组织的衰老环境可能促进肿瘤发生。更重要的是, 近年来在临床以及药物诱导的研究中发现, 衰老与CSCs存在密切联系, 肿瘤细胞衰老后会表达*Oct4*等干性基因[34]。如在乳腺癌临床研究中发现, p16阳性的衰老乳腺癌细胞通常也会表达*Nanog*等干性基因, 同样在卵巢癌报道中, 用二氯化钴(cobalt chloride, $CoCl_2$)处理后卵巢癌细胞SA-β-gal活性增强, 同时伴随着*CD133*等干性基因的表达量增加[35-36]。因此, 深入研究CSCs与衰老之间的关系对于我们深入认识肿瘤发生发展, 探索更有效的肿瘤治疗方法具有重要意义。

3 肿瘤干细胞衰老

3.1 肿瘤干细胞衰老表现

近年来研究发现, CSCs与衰老关系密切, CSCs

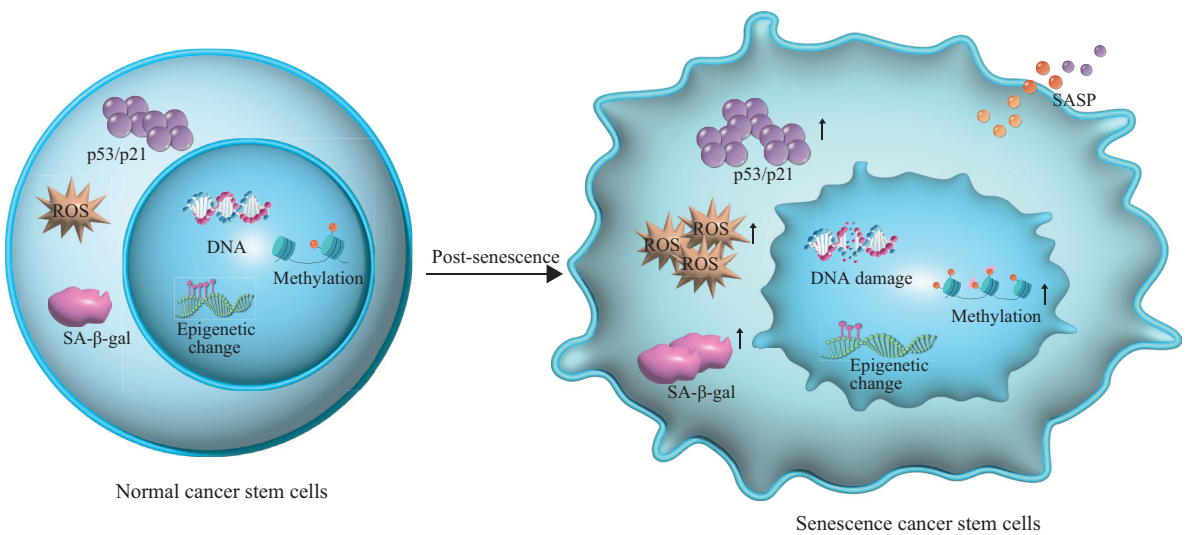
本身也会发生衰老转变并出现衰老细胞的相关表型(图1)。在肝癌的研究中, 阿霉素可诱导肝癌干细胞(liver cancer stem cells, LCSCs)出现衰老变化, LCSCs细胞出现扁平和扩大形态, SA-β-gal染色阳性细胞数量增加, 同时, 衰老的LCSCs中 *p16*、*p21*、*p53*基因上调, *Sox2*、*KLF4*等干性基因被激活; 且上清液中SASP的主要成分IL-6和TGF-β1表达水平增加[37], 以上结果都提示阿霉素可诱导LCSCs衰老。同样在用依托泊苷(etoposide, ETO)处理卵巢畸胎瘤细胞系PA-1细胞(一种CSCs样细胞)时, PA-1细胞出现体积变大、扁平状形态等变化, SA-β-gal染色阳性细胞数增多, 同时细胞中干性基因*Oct4*和衰老相关基因*p21*表达水平增加[39], 该实验表明ETO可诱导PA-1出现衰老变化。

多倍体巨细胞癌细胞(polyploid giant cell cancer cells, PGCCs)是一种肿瘤干细胞样细胞[36]。研究发现, 紫杉醇可诱导卵巢癌细胞产生PGCCs, 这些卵巢癌PGCCs出现衰老表型, 同时细胞中Oct4、Nanog、Sox2等干细胞转录因子表达水平上升[40]。此外, 卵巢癌的其他研究也发现当这些多倍体巨癌细胞进一步失去分裂能力时, 会表现出衰老特征, 如细胞体积变大、呈扁平形状, 细胞中SA-β-gal阳性表达等[41]。

3.2 衰老微环境与肿瘤干细胞

肿瘤微环境(tumor microenvironment, TME)变化通常被认为是肿瘤发生的重要原因, TME影响CSCs变化的重要因素是缺氧和血管周围环境。而研究发现, 衰老微环境与CSCs之间也存在着密切联系(表1), 在结直肠癌的研究报道中, 肿瘤细胞分泌的磷脂酶D2(phospholipase D2, PLD2)能够诱导其周围组织中成纤维细胞的衰老和相关SASP因子的表达, 且研究还发现衰老的肿瘤细胞分泌的SASP因子促进了结直肠癌CSCs的*Oct4*、*Nanog*、*Sox2*等干性基因的表达[42], 证实了衰老微环境对CSCs发生和维持具有重要作用。在骨髓瘤实验中, 将阿霉素诱导的衰老骨髓瘤细胞(multiple myeloma, MM)培养液作为条件培养液, 用该溶液去培养MM时发现骨髓瘤干细胞样细胞(cancer stem-like cells, CSLCs)数量增加, 实验表明CSLCs的出现与衰老细胞表达的SASP有密切联系[43]。而移植衰老细胞也可以促进免疫缺陷小鼠的肿瘤发生, 并促进癌前细胞向恶性干细胞样细胞的转分化[44], 进一步表明, 衰老微环境对CSCs产生具有重要作用。

据报道, 肿瘤坏死因子(tumor necrosis factor, TNF)、IL-6等SASP因子在诱导CSCs上发挥一定作用[45], 不断积累的衰老细胞可通过释放大量SASP因子导致肿瘤炎症环境形成, 进而形成利于CSCs发生和维持的环境[46]。在卵巢癌实验中, 紫杉醇处理后SA-β-gal染色呈阳性的衰老卵巢癌细胞数增多, 该



衰老CSCs细胞体积变大, p53、p21等衰老相关蛋白表达量增加, 细胞内活性氧(ROS)水平增加, 染色体DNA损伤增加(如图中箭头所示), 表观遗传方面发生甲基化增加等变化。

Senescence CSCs have larger cell volumes, increased expression of senescence related proteins such as p53 and p21, increased levels of intracellular ROS (reactive oxygen species), increased chromosomal DNA damage (as shown by the arrows in the image), and increased epigenetic methylation.

图1 衰老CSCs的形态及特征(根据参考文献[38]修改)

Fig.1 Morphology and characteristics of senescent CSCs (modified from reference [38])

研究发现, 衰老肿瘤细胞分泌的IL-6以富集靶向标志物GPR77$^+$/CD10$^+$的方式激活血管内皮生长因子(vascular endothelial growth factor, VEGF)生成从而维持CSCs的干性[47]。其他实验也证实积累的衰老细胞通过旁分泌促进人胚胎上皮肾细胞的致瘤性, 肿瘤组织中干细胞样细胞数量增多, 并认为这是随个体年龄增长肿瘤发生率增加的原因之一[44]。在癌基因诱导衰老(oncogenes induce senescence, OIS)的原代小鼠研究中发现, 在NF-κB、IL-1等SASP因子表达增多的同时, *CD34*、*CD44*、*Hmga2*等一些干性基因表达水平也增加, 在敲低NF-κB后, 细胞中*CD34*、*Nestin*等干性基因表达下降, 这直接说明了在一定程度上SASP可调节细胞中干性基因的表达[48]。化疗诱导的肿瘤细胞衰老及其产生的SASP因子也会改变CSCs的微环境, 进而继续驱动肿瘤的发展。如在铂诱导的卵巢癌中, 衰老细胞分泌的IL-6水平增加, 从而促进了CSCs的富集[49]。

3.3 细胞衰老与肿瘤干细胞产生之间的关系

衰老是细胞对应激状态的自然内在反应, 细胞衰老避免了潜在的恶性细胞以不可逆的方式增殖, 因此衰老被认为是肿瘤的抑制机制[50]。研究发现, 在面对环境变化、化疗药物等压力条件时, 部分肿瘤干细胞可退出增殖周期进入衰老状态, 但也有部分细胞进入静止/休眠状态, 肿瘤干细胞进入衰老或者静止状态与细胞内不同分子或信号通路的激活有关[51]。骨发生蛋白(bone morphogenetic protein, BMP)和TGF-β信号通路优先促进前列腺癌干细胞衰老[52], 而核受体亚家族2F组成员-1基因(nuclear receptor subfamily 2 group F member 1 gene, *NR2F1*)等则优先驱动乳腺癌起始细胞进入静止/休眠状态[53]。此外, KOBAYASHI等[52]发现个别肿瘤干细胞在出现衰老表型的同时, 也可表现出静止表型, 提示衰老肿瘤干细胞存在向其他状态转变的可能, 而这种转变参与了肿瘤的转移、复发等过程。

值得注意的是, 研究发现, 细胞衰老可增强肿瘤细胞的干性重编程能力[12]。随着年龄增长, 在老年大鼠胃黏膜中CSCs标志物CD133、LRG5和ALDH1等的表达水平呈现增加趋势[54]。另一项研究发现, 在阿霉素诱导的衰老淋巴瘤细胞中干性基因(如*Sca-1*等)表达量增加, 使用p53抑制剂后, 部分衰老的淋巴瘤细胞可以重新进入细胞周期, 这些从衰老屏障释放的淋巴瘤细胞干性基因表达量继续增加, 并表现出更强的肿瘤生成能力, 该研究首次证明了细胞衰老可以自发地诱导肿瘤细胞的干性重编程, 进而产生CSCs, 提示CSCs可直接从衰老肿瘤细胞转变而来[12]。此外研究还发现, 在阿霉素处理结肠癌的实验中, 衰老的结肠癌细胞形态扁平、SA-β-gal活性升高, p53及p21活性升高, 且这些

表1 衰老微环境对肿瘤干细胞的作用

Table 1 The role of aging microenvironment on cancer stem cells

处理因素/起始变化 Handling factors/initial changes	衰老细胞类型 Aging cell type	微环境因素变化 Changes in microenvironmental factors	肿瘤干细胞变化 Tumor stem cell changes
Phospholipase	Fibroblasts	Increased levels of macrophage MIF (migration inhibitory factor), HGF (hepatocyte growth factor), and chemotactic factor	Increased expression of *Oct4*, *Naong*, *Sox2* dry genes [42]
Adriamycin induction	Multiple myeloma cells	Interferon γ induced increase in protein 10 and chemokine (C-X-C motif) ligand 10	Promote the emergence, maintenance, and migration of tumor stem cell like cells [43]
–	Fibroblasts	Elevated levels of IL-6, IL-8, and IL-27	*Oct4*, *Naong* stem cell gene expression increases, enhancing tumorigenicity of tumor stem cell-like cells [44]
Paclitaxel	Ovarian cancer cells	Elevated levels of IL-6, VEGF	Increased expression of *Oct4*, *Naong*, *Sox2* dry genes [47]
Oncogene induction	Epithelial cells	Elevated levels of NF-κB, IL-1, p38	Increased expression of *CD34*, *CD44*, *Hmga2*, *Nestin* dry genes [48]
Platinum	Ovarian cancer cells	Elevated levels of IL-6	Promote the enrichment and formation of ovarian cancer stem cells, and upregulate ALDH [49]

–: 无处理。

–: no treatment.

细胞开始表达干细胞因子*Nanog*以及*ALDH*等干性基因[55]。在结肠癌的另一研究中, 用5-氟尿嘧啶及阿霉素诱导后, 也发现癌细胞中SA-β-gal活性增加, *Nanog*干细胞因子表达水平增多, 且CD24、CD44, CD133等CSCs的标志物比例显著增加[56]。这些研究均提示, 细胞衰老可通过促进干细胞重编程诱导CSCs产生。

4 肿瘤干细胞衰老的调控机制

4.1 表观遗传调控

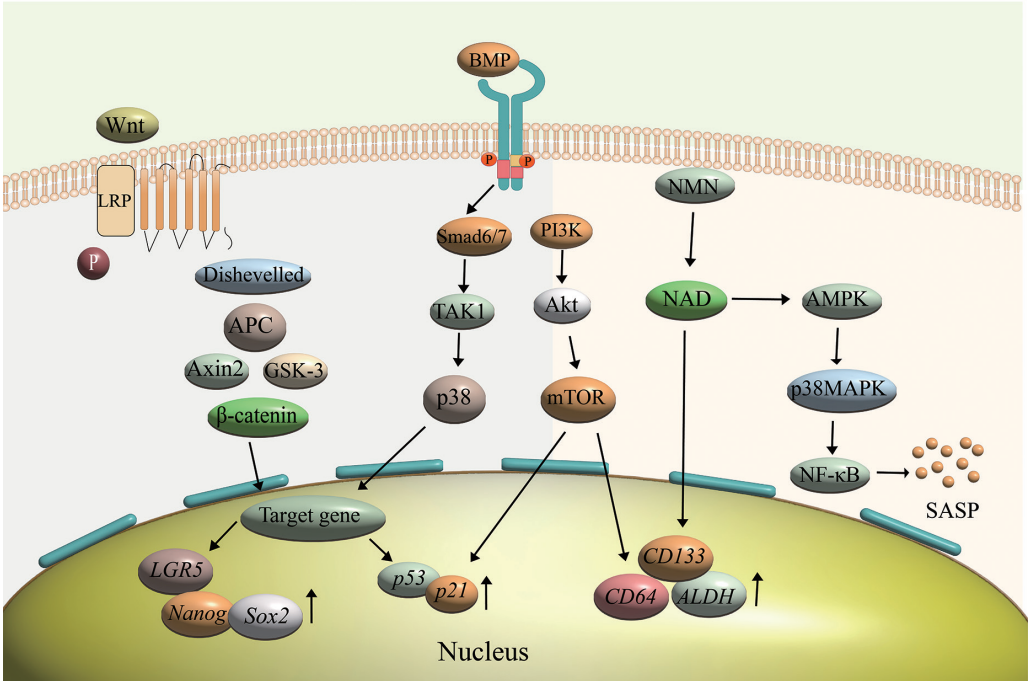
表观遗传调控对维持正常的干细胞是至关重要的, 在DNA甲基化、组蛋白修饰等表观遗传的调控下, 干细胞可以在特定细胞中分化产生, 但异常的表观遗传变化也会导致正常的干细胞转化为CSCs[57]。如在肺癌的报道中发现通过敲除DNA甲基转移酶1(DNA methyltransferase 1, DNMT1), 可消除IL-6介导的衰老相关基因*p53*和*p21*高甲基化, 从而降低肺癌CSCs的富集趋势[58]。这些研究均表明, 一小部分衰老的肿瘤细胞可通过表观遗传改变进而促进CSCs的形成。

4.2 DNA损伤反应(DNA damage response, DDR)

DNA损伤反应对于维持基因的稳定性具有重要作用, 也是调控肿瘤细胞和细胞衰老的重要机制之一[59]。研究发现, DDR也参与了CSCs的衰老调控, 在ETO诱导PA-1(畸胎瘤细胞系)衰老的实验中, 大部分的PA-1细胞都会表达γH2AX(DDR的特异性标志物)[60], 该课题组在后续的研究中发现随着DDR水平的增加, PA-1细胞中Oct4和p21会共表达[61], 此外, CHITIKOVA等[62]还发现在DDR过程中衰老的细胞会表达*Nanog*等干细胞基因。此外, DDR还会影响组织干细胞的正常功能, 进而在衰老细胞群体中促进CSCs发生[63-64]。以上研究都提示DDR的激活参与了CSCs衰老调控。

4.3 调控CSC衰老的信号通路

相关研究提示, 多条信号通路在CSCs衰老调控中发挥重要作用(图2)。在阿霉素处理致LCSCs衰老的实验中, CTNNB1、AXIN2等Wnt信号通路关键分子表达量显著增加, 表明在实验中阿霉素处理激活了该通路, 随后使用IWR-1抑制Wnt/β-catenin信号通路转导后发现, LCSCs衰老状态无明显变化, 但其干性基因*LGR5*、



Wnt信号通路、TGF-β信号通路可通过激活p38等胞内分子, 促进*p21*等衰老相关基因表达, 导致细胞衰老。NAD-Sirt通路可通过激活NF-κB等途径促进SASP因子的表达, 导致肿瘤干细胞衰老, 同时, 肿瘤干细胞标志物*CD133*、*ALDH*等表达量增多。

Wnt signaling pathway, TGF-β signaling pathway can promote the expression of senescence related genes such as *p21* by activating intracellular molecules such as p38, leading to cellular senescence. The NAD-Sirt pathway can promote the expression of SASP factors by activating NF-κB and other pathways, leading to senescent of tumor stem cells. At the same time, the expression of tumor stem cell markers such as *CD133* and *ALDH* increases.

图2 调控CSCs衰老的信号通路

Fig.2 Signal pathways regulating the senescence of CSCs

*Nanog*等的表达水平下降, 表明Wnt/β-catenin信号通路可促进衰老CSCs的干性基因表达[37]。PI3K/mTOR信号通路对CSCs干性维持、增殖及分化等能力调控发挥重要作用。在前列腺癌[65]和卵巢癌[66]的研究中均发现PI3K/mTOR信号通路对CSCs干性调节发挥关键调节作用, 可上调*CD117*、*ALDH*等干性基因表达水平。值得注意的是, PI3K/mTOR信号通路也参与了细胞衰老的调节, 激活该信号通路可诱导胰腺癌细胞衰老, 上调胰腺癌细胞干性基因表达水平, 促进胰腺癌的发生发展[67]。此外, TGF-β信号通路可通过调节核糖体稳定参与细胞代谢水平调控[68], 进而调控肿瘤细胞衰老[69]。研究发现, TGF-β信号通路在调控CSCs衰老过程中也发挥了一定的作用。在前列腺癌研究中, 骨基质细胞分泌的BMP7可以通过激活p38丝裂原活化蛋白激酶和促进细胞周期抑制因子p21的表达和转移, 抑制基因*N-myc*下游调节基因1(*N-myc* downstream regulator gene 1, *NDRG1*)的表达, 诱导前列腺癌CSCs衰老[52]。

NAD-Sirt1信号通路可作为多种癌症的治疗靶点, 烟酰胺磷酸核糖转移酶(nicotinamide phosphoribosyl transferase, NAMPT)是烟酰胺腺嘌呤二核苷酸(nicotinamide adenine dinucleotide, NAD)补救途径的限速酶, 通过NF-κB途径调节SASP因子的表达[70]。在上皮性卵巢癌(epithelial ovarian cancer, EOC)的报道中, 用顺铂诱导EOC细胞衰老后, SA-β-gal阳性细胞数增多, IL-6、IL-8等SASP因子及干性标志物CD133、ALDH等表达量增多, 然而当顺铂与NAMPT抑制剂FK866合用后, 发现顺铂处理后EOC细胞的生长趋势显著降低, 且细胞中IL-6、IL-8, ALDH等表达均受到抑制, 表明NAMPT通过调节SASP因子促进CSCs衰老, 同时也说明NAMPT调节的SASP在顺铂诱导的EOC细胞衰老进而产生CSCs中发挥关键介导作用[71]。

值得关注的是, 诸多CSCs干性维持信号通路在其衰老过程中也发挥调控作用, 而一些衰老调控的信号通路也可调控干性基因表达, 这些信号通路在多个节点上存在交叉。例如, 在肝癌CSCs中, mTOR信号通路的激活在促进其衰老变化的同时, 也激活了*CD44*、*CD64*等干性基因的表达[72]。而淋巴瘤研究发现, CSCs干性维持的关键信号通路Wnt信号通路被激活后, 促进了衰老相关基因*p21*的表达[73]。这些均说明, 对于CSCs而言, 衰老调控与干性调控信号通路之间存在密切联系, 对这一领域的深入研究与探讨有望为进一步明确CSCs衰老调控机制提供重要依据。

5 总结与展望

随着研究的不断深入, 越来越多的研究人员注意到细胞衰老与肿瘤之间存在密切关系, 细胞衰老一方面可以抑制肿瘤细胞的过度增殖, 另一方面也可能促进肿瘤细胞的干性转分化和CSCs的产生。由于CSCs在肿瘤的发生、转移和复发中的独特作用, 因此持续探讨CSCs与衰老之间的关联将有助于更深入认识CSCs生物学特征, 进而开发靶向CSCs的治疗新靶点, 为消除CSCs开发更有效的方法。此外, 研究表明在一些实体瘤中, CSCs对常规的肿瘤治疗方法具有耐受性, 而从衰老角度切入, 有望开发出针对这些耐药CSCs的独特治疗方法, 为肿瘤治疗新方法的开发提供新思路。但CSCs与细胞衰老之间的关系仍然是复杂的, 其有效清除方法和清除时间仍需要进一步的实验来探索和阐明。

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