钙敏感受体影响肿瘤发生和发展的机制

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摘要 钙敏感受体(calcium sensing receptor, CaSR)是体内广泛表达的一种跨膜受体, 主要功能是通过调节甲状旁腺激素的分泌维持机体的钙稳态。此外, 钙敏感受体还参与多种生理病理过程的精细调控。越来越多的研究表明, 钙敏感受体与肿瘤的发生发展密切相关。由于钙敏感受体的功能多样性和组织特异性, 在不同的肿瘤类型中, 钙敏感受体表现出促癌和抑癌两种截然相反的作用: 在前列腺癌、肾癌中表现为促癌作用; 在结肠癌、成神经细胞瘤、甲状旁腺癌中表现为抑癌作用。对钙敏感受体在肿瘤中的作用机制研究, 有利于以钙敏感受体为靶点的抗肿瘤药物筛选和研发以及肿瘤的精准治疗。基于此, 该文总结了钙敏感受体在不同肿瘤中的作用及其机制, 有望为相关研究提供理论借鉴和参考。

关键词 钙敏感受体; 肿瘤; 作用机制

Mechanisms of Calcium Sensing Receptor Regulation on Tumorigenesis and Development

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Abstract As a widely-expressed transmembrane receptor, calcium sensing receptor (CaSR) plays an important role in maintaining body calcium homeostasis by regulating parathyroid hormone secretion. In addition, CaSR is implicated in various physiological and pathological processes. Increasing evidences indicate that CaSR is closely related to tumor progression. Due to the functional diversity and tissue specificity, CaSR may function as either tumor promoter or tumor suppressor in different types of tumors. CaSR acts as a tumor promoter in prostate cancer and renal cell carcinoma. While in colon cancer, neuroblastoma and parathyroid carcinoma, CaSR is a tumor suppressor. Studies on the role of CaSR in different tumors are beneficial to research and development of new anti-tumor drugs, as well as tumor therapy targeting CaSR. Herein, we summarized the function and underlying mechanism of CaSR in different types of tumors, aiming to provide theoretical basis for future research.

Keywords calcium sensing receptor; tumor; function mechanism

钙敏感受体(calcium sensing receptor, CaSR)属于C族G蛋白偶联受体, 由1 078个氨基酸组成, 在细胞膜上以严格的同源二聚体形式发挥功能[1]。CaSR的每个亚基主要分为3部分: 与配体结合并负责调节受体二聚化的胞外N-端结构域、七次跨膜疏水螺旋和参与下游信号传递的胞内C-端结构域[2]。CaSR可以被多价阳离子(如Ca^{2+}和Mg^{2+})、聚胺类化合物、多肽类和氨基糖苷类抗生素激活, 并通过偶联β-arrestin和多种G蛋白如Gq/11、Gi/o、Gs、G12/13介导信号的传递[3]。

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由于CaSR的激动剂以及其偶联的G蛋白种类繁多, 其所介导的下游信号通路和生理学功能也多种多样, 并且CaSR还表现出明显的组织功能特异性。在甲状旁腺、肾脏、肠道和骨骼中, CaSR有着很高的表达, 是维持机体钙稳态的重要调控因子[4]。在这些组织中, CaSR可以感知环境中的钙浓度, 通过调整甲状旁腺激素的分泌、维生素D的合成以及钙离子的吸收和释放来维持机体的钙稳态[5]。CaSR的基因突变会引起钙离子代谢的严重失衡, 从而导致疾病的发生, 引发高/低血钙、原发性甲状旁腺机能亢进、继发性甲状旁腺机能亢进和继发性甲状旁腺功能减退等疾病[6]。CaSR在心血管系统、呼吸道以及神经系统中也有一定的表达, 主要参与基因表达、激素分泌、炎症反应和细胞的增殖、运动、分化、凋亡等过程的调控[7-9]。CaSR的功能破坏可以诱发血压失控、血管钙化、哮喘和老年痴呆[10-11]。

对细胞命运的调控功能决定了CaSR在肿瘤发生发展过程中的关键作用。由于CaSR具有组织功能特异性, 导致其在不同的肿瘤中表现出不一样甚至完全相反的作用。CaSR在肿瘤细胞中的作用可以分为两大类: 一类是在高血钙类肿瘤如前列腺癌、卵巢癌、肾癌和睾丸间质瘤中表现出的促癌因子作用; 另一类是在非高血钙类肿瘤如结肠癌、成神经细胞瘤、甲状旁腺癌、胃癌和胰腺癌中表现出的抑癌因子作用。除了上述肿瘤外, CaSR对乳腺癌同样有着重要的调控作用, 有研究发现, CaSR可以促进乳腺癌的发生发展[12], 也有研究证明, CaSR对乳腺癌细胞生长有抑制作用[13]。对于这种矛盾的现象, 研究人员还没有一个明确的解释, 相关机制还有待进一步探究。由于在肿瘤进展过程中的重要作用, CaSR可以作为肿瘤治疗的有效靶点和检测标志。阐明CaSR在不同癌症中详细的作用机制, 有利于以CaSR为靶点进行相关的肿瘤精准治疗药物的研发。

1 作为促癌因子的钙敏感受体

1.1 钙敏感受体与前列腺癌

前列腺癌是常见的男性癌症之一, 日常饮食中摄入过量的钙离子会增加前列腺癌的患病风险[14]。在前列腺癌小鼠模型中, 给小鼠喂养高钙饲料会导致细胞增殖、微创、组织炎症以及前列腺癌标志物的表达, 最终促进前列腺上皮内瘤的形成, 而增加小鼠饮食中维生素D的含量可以明显抑制钙的促肿瘤发生效应。在体外培养的前列腺癌细胞系PC-3中, 胞外钙离子刺激能够诱导细胞增殖、促进钙池调控的钙离子内流(store-operated calcium entry, SOCE)、上调经典瞬时受体电位通道蛋白TRPC6(transient receptor potential canonical 6)和CaSR的表达, 钙离子的这些效应同样都能被维生素D所抑制[15]。研究发现, 维生素D的缺失是导致前列腺癌恶化的重要因素[16], 这暗示维生素D极有可能通过抑制钙的促癌效应降低前列腺癌的发生、发展和恶化。此外, 对前列腺癌患者的研究表明, *CaSR*的基因突变和表达增加与致死性前列腺癌的发病率密切相关[17-18]。骨转移是前列腺癌恶化和致死的重要原因, 胞外钙离子能够通过上调CaSR的表达进而增强癌细胞的增殖和骨转移能力, 因此骨转移的列腺癌细胞比原位前列腺癌细胞CaSR表达量更高[19]。 CaSR通过激活蛋白激酶B(protein kinase B, PKB)信号通路增加细胞的附着能力, 促进癌细胞的骨转移[20]。CaSR还能与锌敏感受体ZnR相互作用, 通过调节Zn^{2+}依赖的丝裂原活化蛋白激酶(mitogen-activated protein kinase, MAPK)信号通路促进前列腺肿瘤的生长, 同时上调钙离子结合蛋白S100A4的表达, 促进前列腺癌细胞的迁移[21]。这些研究表明, CaSR在前列腺癌的发展和恶化过程中起着重要的作用, 甚至是骨转移引发病人死亡的关键因素。

1.2 钙敏感受体与肾癌

肾癌是泌尿系统中常见的肿瘤, CaSR主要促进肾癌细胞的骨转移过程。肾癌的骨转移是其恶化的一个重要特征, 由于骨转移的肿瘤细胞对传统的化疗和放疗不敏感, 发生骨转移的病人常常表现出不良的生存状态, 骨转移的肾癌细胞还会引起骨质溶解并导致骨痛、病理性骨折、高血钙、脊髓和神经根压迫等骨骼并发症[22]。研究表明, 在骨转移的肾癌细胞中CaSR的表达量明显偏高, 因此CaSR可以作为检测肾癌骨转移的一个重要指标。此外, 过表达CaSR可以导致肾癌细胞在小鼠体内具备更高的骨转移能力[23]。在骨转移或者过表达CaSR的肾癌细胞中, 胞外钙离子通过激活PKB、MAPK、桩蛋白等信号通路以及下调肿瘤抑制因子磷酸酶(phosphate and tension homology deleted on chromsome ten, *PTEN*)基因的转录促进肿瘤细胞的增殖和迁移[24]。这些研究表明, 抑制CaSR的活性和表达能够对肾癌发展和恶化起到有效的缓解作用。

1.3 钙敏感受体在其他癌症中的促癌作用

恶性肿瘤体液性高血钙症(syndrome of humoral hypercalcemia of malignancy, HHM)是由未发生大范围骨转移的肿瘤细胞分泌甲状旁腺激素相关蛋白(parathyroid hormone-related protein, PTHrP)引起的, 在大鼠睾丸间质瘤转移模型中, 激活CaSR可以促进睾丸间质瘤细胞分泌PTHrP, 加重PTHrP依赖的HHM, 而*CaSR*基因的失活突变体可以抑制这一效应[25]。卵巢癌是女性中致死性很高的一类癌症, *CaSR*的单核苷酸多态性突变与卵巢癌的易感性相关[26]。黑色素瘤是一种严重的皮肤癌症, CaSR的反向变构调节剂NPS-2143能够通过磷脂酰肌醇激酶PI3K通路介导黑色素瘤细胞的凋亡[27]。

2 作为抑癌因子的钙敏感受体

2.1 钙敏感受体与结肠癌

结肠癌是世界第三大常见癌症, 与前列腺癌相反, 饮食中钙离子摄入不足会增加患结肠癌的风险。另外, CaSR胞内结构域的非同义突变也与结肠癌患病率相关[28-29]。与正常结肠粘膜细胞相比, 结肠癌细胞中CaSR表达量明显降低, CaSR启动子区的超甲基化、组蛋白H3第9位赖氨酸的去乙酰化以及miR135b和miR146b的表达都可以导致CaSR表达降低[30-32]。结肠癌患者的组织样本研究显示, CaSR的表达与促凋亡蛋白量和患者良好的生存状态正相关, 而与促增殖因子表达量以及肿瘤的致死率负相关[33]。饮食中增加钙离子的摄入量, 不仅能降低结肠癌的患病风险, 还能改善结肠癌病人的生存状态, 但是这种改善效果仅对结肠直肠癌组织中表达CaSR的病人有效[34]。

β-连环蛋白(β-catenin)能够通过调控细胞周期关键蛋白的表达促进细胞增殖[35]。在结肠癌细胞中, 激活CaSR能够降低细胞内β-catenin的蛋白水平和核转运过程, 从而抑制细胞的增殖[36-37]。结肠癌细胞系HT29和Caco2-15过表达CaSR能够抑制肿瘤细胞的增殖, 促进细胞的凋亡[38]。此外, 维生素D摄入不足也会增加结肠癌的患病风险。研究表明, 维生素D的代谢产物1,25-二羟维生素D可以增加CaSR启动子的转录活性同时激活CaSR, 通过下调胸苷酸合成酶和抗凋亡蛋白survivin的表达, 抑制细胞的增殖和分化, 最终增强抗结肠癌药物的作用效果[39-40]。

CaSR还与结肠癌细胞的迁移侵袭、上皮−间充质转化(epithelial-mesenchymal transition, EMT)以及肿瘤干细胞特性相关。人源结肠癌细胞中有10%~20%细胞不表达CaSR, 这些肿瘤细胞有着非常高的恶性程度和耐药性, 其EMT相关蛋白和致瘤miRNAs的表达明显增高, 而抑癌因子的表达则明显降低。当这些细胞过表达CaSR时, 结肠癌细胞的迁移侵袭、EMT能力以及肿瘤干细胞特性明显降低, 抑癌因子的表达也明显升高[41]。

综上, CaSR对结肠癌的发生、发展、转移以及恶化过程有明显的抑制作用, 因此可以作为结肠癌治疗的有效药物靶点和诊断标志物。同时, 维生素D可以通过CaSR信号通路起到预防和治疗结肠癌的作用。

2.2 钙敏感受体与成神经细胞瘤

成神经细胞瘤是交感神经系统中的恶性肿瘤, 常发于儿童时期。分化程度低的恶性成神经细胞瘤几乎不表达CaSR。成神经细胞瘤中的CaSR受基因和表观遗传调控, CaSR启动子区突变和超甲基化都可以降低成神经细胞瘤中CaSR的表达[42-43]。此外, 基因多态性研究发现, *CaSR*基因的失活突变也与成神经细胞瘤的恶性程度、原癌基因(*v-myc* avian myelocytomatosis viral oncogene neuroblastoma derived nomolog, *MYCN*)增强型表达和不良临床结果正相关[44]。在*MYCN*增强型表达的成神经细胞瘤细胞系中过表达CaSR可以降低肿瘤细胞的增殖和致瘤性[42]。使用钙离子或正向变构调节剂Cinacalcet激活成神经细胞瘤中的CaSR能够明显引起肿瘤细胞的凋亡, 但是钙离子诱导的凋亡过程依赖于细胞外调节蛋白激酶(extracellular regulated protein kinases, ERK)信号通路, 而Cinacalcet诱导的细胞凋亡则依赖于内质网应激[42,45]。Cinacalcet还可以促进成神经细胞瘤的分化和肿瘤睾丸抗原(cancer-testis antigen, CTA)的表达, CTA是一种表达于正常睾丸、胎盘和多种肿瘤组织的抗原, 可以作为一个有效的肿瘤免疫治疗的靶点[46]。这些研究表明, CaSR能够抑制成神经细胞瘤的生长和恶化, 并且可能在该肿瘤的免疫治疗过程中起到重要的作用。

2.3 钙敏感受体与甲状旁腺癌

甲状旁腺癌是一种不常见的恶性肿瘤。同结肠直肠癌和成神经细胞瘤一样, 在甲状旁腺癌细胞中, CaSR表达水平比正常甲状旁腺细胞低, 但不同的是, 甲状旁腺癌中*CaSR*基因启动子区并没有发生明显

的甲基化修饰[47]。是否同样存在组蛋白的去乙酰化修饰、相关miRNA的表达和基因的缺失, 以及这些因素是否与CaSR表达降低相关则需要进一步确认。

除了降低CaSR的表达, 甲状旁腺癌细胞还通过高表达孤儿受体GPR64, 与CaSR形成异源二聚体, 降低CaSR下游的信号通路[48]。Cinacalcet可以抑制尿毒症小鼠的甲状旁腺增生[49]。在甲状旁腺肿瘤细胞中, CaSR的激活能够抑制碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)和表皮生长因子(epidermal growth factor, EGF)介导的致癌基因*cyclinD1*的表达[50]。但是, 当环境中没有bFGF或EGF的时候, CaSR对*cyclinD1*的表达没有相应的抑制效果, 说明甲状旁腺中CaSR与这些生长因子相互影响。

2.4 钙敏感受体在其他癌症中的抑癌作用

研究显示, 胃癌组织中CaSR的表达与正常组织相比明显偏低, CaSR表达的降低可以促进胃癌细胞的迁移[51]。在胰腺癌中, 激活的CaSR通过抑制Wnt通路抑制肿瘤的形成[52]。子宫内膜癌细胞中CaSR表达同样偏低, 而CaSR的激活可以降低细胞活力, 促进细胞凋亡, 并通过促进β-catenin/E-cadherin复合体的形成抑制肿瘤细胞的迁移[53]。对肺腺癌临床样品的分析显示, CaSR表达降低与病人的不良预后相关, 但其具体机制尚不清楚[54,71]。

3 钙敏感受体与乳腺癌

乳腺癌是女性中比较常见的一种恶性肿瘤, 多发于有乳腺癌发病史的家族中未生育或晚生育的女性。有研究显示, 乳腺癌细胞尤其是骨转移的癌细胞中CaSR的mRNA水平和蛋白水平与正常乳腺细胞相比明显增高[55]。然而, 也有研究团队对乳腺癌组织样本进行免疫组化分析, 结果发现癌变组织与癌旁组织相比, CaSR的表达量是降低的[56]。目前, 与正常乳腺组织相比CaSR在乳腺癌中表达量是增加还是减少、具体表达的调控机制、是否与个体特异性相关, 都还没有明确的解释, 因此需要更多的实验证实。

有关于乳腺癌的报道中, 除了表达水平方面存在的矛盾, 在CaSR的功能方面也不尽相同。在乳腺癌细胞系MCF-7细胞中, CaSR可以转激活表皮生长因子受体, 从而磷酸化ERK, 上调瞬时受体I型电位离子通道蛋白的表达, 促进癌细胞的增殖[12]。胞外钙离子可以激活CaSR进而通过经典磷脂酶C途径增加乳腺癌细胞的迁移能力[57]。雌激素受体在肿瘤介导的骨溶解中有重要作用, CaSR的激活还可以上调雌激素受体的转录活性, 促进乳腺癌细胞的骨转移[58]。胆碱激酶是一种可以通过致癌因子ras促进乳腺癌细胞生长和恶化的胞质酶, CaSR的激活通过Gα12/Rho信号通路增加胆碱激酶的表达, 促进肿瘤细胞的生长、迁移和耐药性[59]。正常乳腺细胞中, CaSR的激活会降低PTHrP的分泌, 但在乳腺癌细胞中CaSR的激活却会增加PTHrP的分泌。这是由于正常细胞内, CaSR偶联Gαo蛋白, 抑制细胞内的cAMP水平, 但乳腺癌细胞内CaSR偏向性偶联Gαs蛋白, 增加了细胞cAMP的积累, cAMP可以促进*PTHrP*基因的表达, 进而促进乳腺癌细胞的骨转移[60]。然而, 也有研究显示, 同样在乳腺癌细胞MCF-7中CaSR激活可以抑制肿瘤的恶性发展, 如增殖、迁移、非锚定生长, 并能够增加化疗药物如紫杉醇对肿瘤细胞的细胞毒性[13]。乳腺癌1号基因(breast cancer 1, *BRCA1*)是一个抑癌基因, *BRCA1*基因突变的女性更容易患乳腺癌。BRCA1可以上调*CaSR*基因的转录活性同时下调抗凋亡蛋白survivin的表达, 从而促进肿瘤细胞的凋亡[61]。目前还没有一个单一可靠的理论很好地解释CaSR在乳腺癌细胞中表达和功能上的矛盾, 可能是不同的研究小组使用不同药物浓度造成的, 也可能是受体下游复杂信号网络的一个综合结果, 对此还需要更深层次的研究[62]。

4 总结与展望

作为维持细胞钙稳态的重要受体, CaSR广泛表达于各种类型的细胞中。如上所述, CaSR与肿瘤的发生发展过程密切相关, 该受体扮演的角色因肿瘤类型的不同而不同(表1)。在前列腺癌、肾癌中, CaSR的表达量随肿瘤的恶性程度的增高而增高; 在结肠直肠癌和成神经细胞瘤中, 随肿瘤的恶性程度的增高而降低, 由此CaSR有望成为检测癌症恶性程度和临床等级的一个参考指标。

不同肿瘤类型中CaSR受体的作用为何不同, 乳腺癌中CaSR的作用为何会不一致, 各种类型的肿瘤中CaSR更详尽的作用机制目前都未了解, 这些都是未来针对CaSR功能研究的方向和热点。CaSR在肿瘤中与PTHrP和维生素D的具体关系以及是否还有其他重要的未知蛋白参与相关过程的调控都有待深入阐明。另外, 在成神经细胞瘤中, CaSR的激活

表1 钙敏感受体在不同肿瘤中的表达水平和作用机制

Table 1 CaSR expression and functional mechanismsin different types of cancer

肿瘤类型 Cancer type	表达 Expression	作用 Function	机制 Mechanism
Prostate cancer	Increased	Tumor promotor	CaSR activation→proliferation, micro-invasion, tissue inflammation[15] PKB→skeletal metastasis[20] ZnR→cell growth, invasion[21]
Renal cell carcinoma	Unknown	Tumor promotor	PKB, MAPK, PTEN→bone metastasis and proliferation[22,24]
Melanoma	Unknown	Tumor promotor	CaSR antagonist→ autophagy and apoptosis[27]
Colon cancer	Decreased	Tumor suppressor	CaSR activation→ Wnt 5a secretion→β-catenin degradation and good prognosis[36] VD→CaSR→ survivin ↓ → proliferation ↓ and invasion ↓ [39-40] CaSR overexpression→ EMT ↓ and invasion ↓ [40-41]
Neuroblastoma	Decreased	Tumor suppressor	CaSR overexpression→ proliferation ↓ and tumorigenicity ↓ [42,45]
Parathyroid carcinoma	Decreased	Tumor suppressor	CaSR inhibition→ prolifration[49] CaSR activation→oncogenic cyclinD1 ↓ [50]
Gastric cancer	Decreased	Tumor suppressor	Downregulated CaSR→invasion and malignancy[51]
Pancreatic cancer	Decreased	Tumor suppressor	CaSR activation→ NCX1/Ca^{2+}→β-catenin ↓ → tumorigenesis ↓ [52]
Endometrial cancer	Decreased	Tumor suppressor	CaSR overexpression→ VEGFR3 ↓ , invasion ↓ and apoptosis[53] EGFR→ERK1/2→proliferation[12]
Breast cancer	Increased	Tumor promotor	PLC→PKC→migration[57] ER transcriptional activity→bone metastasis[58] Gα12/Rho→cholinekinase→drug resistance[59,63] Gαs→cAMP→PTHrP→bone metastasis[60]
Breast cancer	Decreased	Tumor suppressor	BRCA1→CaSR→survivin ↓ →apoptosis[61]

↓: 下调。

↓: downregulation.

可以上调肿瘤相关抗原CTA的表达, 肿瘤免疫治疗作为当下的研究热点和肿瘤治疗的有效方式, CaSR有可能作为肿瘤免疫治疗的一个调控因素。在其他类型的肿瘤中是否存在同样的现象, 还需要更多的实验证明。作为第一个上市的G蛋白偶联受体(G protein-coupled receptor, GPCR)变构药物, CaSR的正向变构调节剂Cinacalcet被用于治疗甲状旁腺机能亢进, 由于CaSR在肿瘤进展中的重要作用, 使Cinacalcet具备了作为抗肿瘤药物的潜力。综上, 本文总结了CaSR在不同肿瘤中的作用及机制, 为以CaSR为靶点的抗肿瘤药物研发和针对肿瘤进行特异性治疗提供理论基础。

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