综 述

间隙连接蛋白43羧基端在恶性肿瘤中的作用及机制

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摘要 间隙连接蛋白43(connexin 43, CX43)是间隙连接蛋白家族的重要成员之一, 参与体内众多生理和病理过程的调控。结构上, 该蛋白由氨基端、跨膜结构及羧基端三部分组成, 其羧基端上存在大量蛋白结合位点。通过这些位点, CX43能够与不同的蛋白发生相互作用: 一方面, 影响CX43自身的磷酸化状态, 从而调控其降解、亚细胞定位以及装配等过程; 另一方面, CX43羧基端还能够通过某些特定的结合位点, 调控其他蛋白分子的功能状态, 从而影响信号转导, 调节细胞的生物学功能。近年来研究发现, 该蛋白的羧基端(carboxyl terminal)显著地影响肿瘤细胞/肿瘤干细胞的生物学特性。该文就CX43羧基端的结构特点、与蛋白质的相互作用位点、调控肿瘤细胞/肿瘤干细胞增殖、迁移、自我更新和成瘤能力的作用机制进行简要综述。

关键词 间隙连接蛋白43; 羧基端; 蛋白相互作用; 肿瘤干细胞; 增殖; 迁移

Function of Carboxyl Terminal of Connexin 43 in Malignant Tumor

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Abstract Connexin 43 (CX43), a member of connexin family, performs crucial roles in regulating diverse physiological and pathological processes in eukaryotic cells. This protein is composed of three parts, including the amino terminal, transmembrane structure and carboxyl terminal. In the carboxyl terminal of CX43, there are many protein binding sites. Through these sites, CX43 can interact with different proteins. On the one hand, those sites influence the phosphorylation state of CX43 and then regulate its degradation, subcellular localization, assembly process, etc. On the other hand, the carboxyl terminal of CX43 also influences the functional state of other protein molecules, thus affecting the signal transduction and regulating the biological function of cell. Recent studies revealed that the carboxyl terminal of CX43 played a critical role in regulating the biological features of cancer cells or cancer stem cells. Here, we make a brief review about the structural features, the protein-protein interaction sites and the molecular mechanisms for regulating tumor cell proliferation/migration of this domain in CX43.

Keywords connexin 43; carboxyl terminal; protein interaction; cancer stem cell; proliferation; migration

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间隙连接蛋白(connexins, Cxs)是构成细胞间间隙连接的分子基础, 其中间隙连接蛋白43(connexin 43, CX43)组织分布最为广泛, 并与恶性肿瘤关系密切。结构上, CX43由六个哑铃状的跨膜连接蛋白排列成一个亲水孔道, 细胞间两侧膜上的连接蛋白端–端相连, 形成间隙连接通道, 允许水分子及小分子, 如离子(Na^+、K^+、Ca^{2+}等)、代谢物(ADP/ATP、葡萄糖、谷氨酸、谷胱甘肽等)、第二信使(三磷酸肌醇、环腺苷酸等)、抗原, 甚至microRNA等通过[1-3], 从而形成细胞间隙连接通讯(gap junction intercellular communication, GJIC)。

长期以来, 人们认为CX43主要通过形成细胞膜间的通道样结构, 从而介导相邻细胞间的小分子转移, 调控电信号的传播及物质交换, 最终协调相邻细胞间的生长、兴奋、增殖、迁移和空间定位。然而深入观察表明, CX43生物学功能的发挥并不完全依赖于间隙连接通道的形成: (1)CX43的亚细胞定位多元化。该蛋白不仅定位于肿瘤细胞膜, 而且在细胞质、细胞核中也能检测到其存在[4]。(2)非完整的间隙连接也会影响细胞的生物学性质。在转染了CX43突变体以致无法形成完整间隙连接的Hela细胞, 其生长仍受到明显抑制[5]。(3)过表达CX43后, 细胞的生物学性质发生了明显的变化, 但是间隙连接通讯却没有改变, 而给予间隙连接通讯抑制剂并不能逆转过表达CX43所引起的效应[6-8]。这表明, CX43还能够通过非GJIC依赖性机制影响细胞的生物学性质。进一步研究发现, 仅仅干预CX43蛋白羧基末端包含132个氨基酸的序列, 就能对细胞特别是恶性肿瘤细胞的增殖、迁移/侵袭等恶性表型产生显著的影响。这提示, 羧基末端在CX43通过非GJIC依赖性机制调控肿瘤细胞生物学特性的过程中扮演了重要的角色。因此, 我们就CX43羧基端在调控恶性肿瘤细胞生物学性质方面的研究进展及分子机制进行简要的综述。

1 CX43羧基端的结构特点

CX43蛋白包含9个主要结构域: 4个跨膜结构、2个胞外环、氨基端、胞质环以及羧基端。采用核磁共振结合镜像共振成像技术对CX43羧基端结构进行观察后发现, CX43羧基端为132个氨基酸组成的无规则线圈样结构, 包括2个螺旋结构域以及从细胞质膜延生出来的膜连接通道(membrane-docked channel)[9](图1)。

深入研究发现, CX43羧基端上存在大量的蛋白结合位点(表1和图2)。通过这些位点, CX43能够与不同的蛋白发生相互作用。一方面, 影响CX43自身的磷酸化状态, 从而调控其降解、亚细胞定位以及装配等过程。比如, 在近膜的CX43羧基端E227-G242氨基酸区域可与CX43作用蛋白150 kDa(connexin 43 interacting protein 150 kDa, CIP150)相互作用, 诱导CX43磷酸化, 从而调控CX43在细胞膜上的亚定位; S325、S328、S330区域还可与酪蛋白激酶1(casein

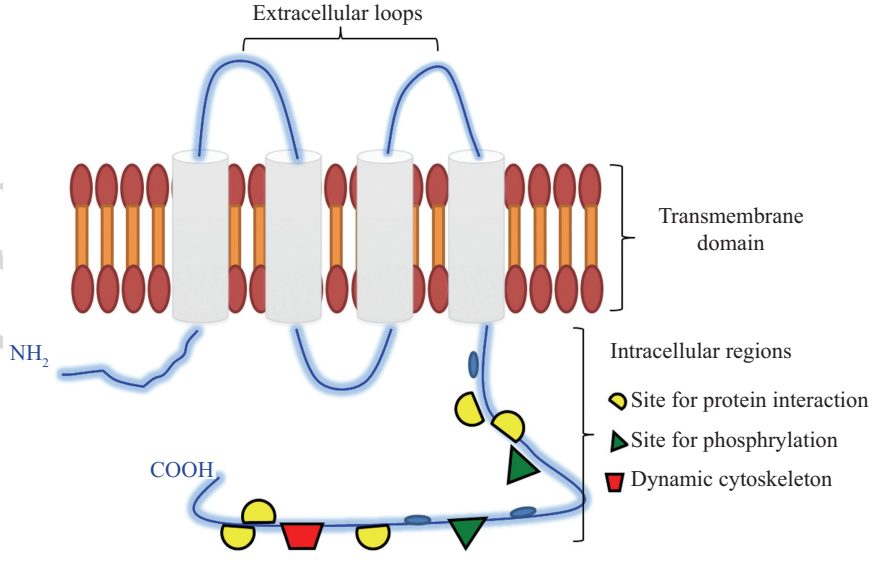

图1 CX43结构模式图

Fig.1 Diagram model of CX43 structure

表1　与CX43羧基端相互作用的蛋白

Table 1　Interaction proteins with CX43 carboxyl terminal

与CX43羧基端相互作用的蛋白 Interaction proteins with CX43 carboxyl terminal	氨基酸位点 Amino acid sites	作用 Function
Connexin 43 interacting protein 150 kDa (CIP150)	E227-G242	Phosphorylation[10]
Microtubule	K234-S262	Regulates the activation of TGF-β signaling pathway and influences cellular polarity or migration[14,16-17]
Calmodulin protein kinase II (CaMKII)	S244; S255; S257; S296; S297; S306; S314; S325; S328; S330; S364; S365; S369; S373	Phosphorylation[18]
v-Src	Y247; Y265; S262; S279/282; S368	Phosphorylation[19]
c-Src	Y247; Y265	Inhibits cell proliferation[12]
Connexin 43 interacting protein 85 kDa (CIP85)	A253-P256	Promotes the degradation of CX43[20]
PKC	S262; S365; S368	Phosphorylation[11,21-22]
PKC/mitogen-activated protein kinases (MAPK)	S255; S262; S368	Phosphorylation[23]
Hsc70	Q263-I382	Inhibits cell proliferation[13]
Connexin 43 interacting protein 75 kDa (CIP75)	K264-N302	Promotes the degradation of CX43[24-25]
Neuronal precursor cell expressed developmentally downregulated 4 (Nedd4)	S282-Y286	Regulates the degradation of CX43[26]
CK1	S325; S328; S330	Phosphorylation[27]
14-3-3	S373	Phosphorylation[28]
ZO-1	D379-I382	Regulates the interaction between ZO-1 and CX43[29]
Proteins with unclear sites for interaction		
Cyr61/connective tissue growth factor/nephroblastoma-overexpressed 3, NOV/CCN3		Inhibits cell proliferation[30]
Caveolin-1/-2/-3		Regulates the functional status of GJIC[31-32]
Developmentally regulated brain protein, drebrin		Regulates the subcellular distribution of CX43 and GJIC[33]

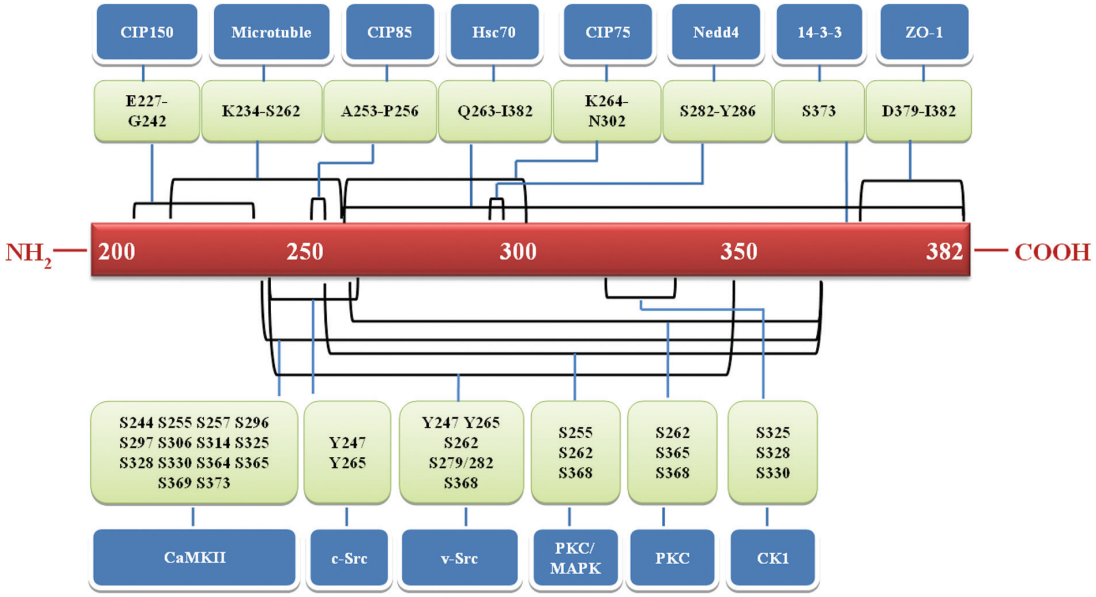

图2　与CX43羧基端相互作用的蛋白位点

Fig.2　Sites for protein-protein interaction located at the CX43 carboxyl terminal

kinase 1, CK1)相结合, 磷酸化CX43, 并调节CX43的装配[10]。更为重要的是, CX43羧基端能够通过某些特定的结合位点, 调控其他蛋白分子的功能状态, 从而影响信号转导, 调节细胞的生物学功能。比如, CX43羧基端的S262氨基酸残基可与参与多种有关细胞增殖、分化、存活相关信号通路的蛋白激酶C(protein kinase C, PKC)相作用, 磷酸化其位点进而抑制DNA的合成[11]; Y247和Y265氨基酸残基可与原癌基因表达蛋白(c-Src)相结合, 充当c-Src的底物, 从而调控细胞的生长、发育和分化[12]; Q263-I382区域可同热休克蛋白70(heat shock cognate protein 70, Hsc70)的385-543区域发生相互作用, 从而竞争性地抑制细

胞周期蛋白D1(cyclin D1)与Hsc70在胞质中的结合, 进而减少cyclin D1在细胞核中的集聚[13]; K234-S262区域还可与微管蛋白(microtubule)发生相互作用, 影响高尔基体和微管组织中心在迁移前沿的极化分布以及微管骨架的定向排列[14]; 此外, CX43羧基端的D379-I382区域还可与闭锁小带蛋白-1(zonula occludens-1, ZO-1)的PDZ2区域相结合, 调节紧密连接和间隙连接的相互作用[15]。

2 CX43羧基端对肿瘤细胞增殖的影响

CX43作为抑癌因子, 在多种恶性肿瘤中低表达, 同患者的预后呈正相关。临床研究发现, 在非小细胞肺癌患者的肿瘤组织中, CX43的表达显著低于癌旁组织, 并随着肿瘤的进展, CX43的阳性率逐渐降低(从I-II期的66.67%降至III-IV期的39.14%)[34]。同样, 在外源性超表达CX43的胶质瘤细胞系U6(U6-CX43)中, 细胞增殖显著降低: 流式细胞术检测发现, U6-CX43完成一个细胞周期的时间较对照U6细胞明显延长(34 h *vs* 16 h), 而在整个细胞周期中, 64%以上的U6-CX43都处于分裂间期(G_0/G_1期), 仅有5%的细胞在14 h内进入增殖分裂期[12]。深入研究发现, 仅对CX43羧基端进行修饰, 肿瘤细胞的DNA合成受到显著抑制, 显示CX43对肿瘤细胞增殖能力的抑制效应与其羧基端密切相关[35]。

2.1 通过蛋白–蛋白相互作用影响增殖

通过羧基端存在的多个蛋白作用位点影响其他蛋白质的稳定性、催化活性以及磷酸化状态, 是CX43调节肿瘤细胞增殖的重要方式之一。Herrero-González等[12]研究发现, CX43羧基端氨基酸247、265残基可与c-Src特异性结合, 降低活化c-Src/总c-Src比率, 上调p21/p27表达, 促进Retinoblastoma(Rb)蛋白磷酸化, 抑制转录因子E2F释放, 减少细胞周期蛋白E(cyclin E)表达, 进而减少细胞周期依赖性激酶2(cyclin-dependent kinase, CDK2)的激活, 诱导G_0/G_1期阻滞, 最终抑制细胞增殖。Hatakeyama等[13]发现, CX43羧基端氨基酸263-382区域能与cyclin D1相竞争结合Hsc70的氨基酸385-543区域, 阻止cyclin D1与Hsc70在胞质中的结合, 从而阻止Hsc70介导的cyclin D1胞质–胞核易位, 减少cyclin D1在细胞核中的集聚, 抑制细胞增殖。CX43羧基端结构域(244-382)可特异性结合CCN3, 减少CCN3的核易位, 从而抑制其刺激细胞生长的作用[30]。此外, 位于CX43羧基端的丝氨酸262残基还可被PKC特异性地磷酸化[21]。

2.2 作为核转录因子或转录辅助因子调控增殖

CX43羧基端除通过蛋白–蛋白相互作用来调节细胞增殖外, 还可能直接进入细胞核, 作为核转录因子或转录辅助因子调控增殖相关基因的表达。在转染了*src*、*neu*等癌基因的大鼠肝上皮转化细胞中, 研究者发现, 胞膜上的CX43明显减少, 并发生明显的细胞核转位, 激光共聚焦显微镜下可见CX43大部分定位于细胞核[36]。Dang等[4]进一步证实, 在过表达CX43羧基端(CT-CX43)的HeLa和心肌细胞中, CT-CX43除了广泛地定位于细胞质膜外, 也可在细胞核中检测到, 且稳定表达的CT-CX43还可抑制HeLa细胞的增殖。由于CX43是一个细胞膜蛋白, 除羧基端以外的其他部分结构难以到达细胞核, 因此推测CX43的羧基端可能具有核靶向功能, 以调节细胞生长。研究者对CX43蛋白的氨基酸序列分析发现, 其羧基端133-148处存在类似核定位序列(nuclear localization sequence, NLS)的序列, CX43可能借助该序列直接进入细胞核[37]。不过, 也有研究者认为, CX43羧基端并不存在经典的NLS, 但其相互作用蛋白如Src、PKC等均可转移到细胞核, CX43是通过蛋白–蛋白相互作用的间接机制进入细胞核的[38-40]。在转染CX43的狗肾上皮细胞系中, 发现细胞周期调节基因, 如cyclin A、cyclin D1、cyclin D2以及*CDK5*、*CDK6*的表达均下调, 细胞发生明显的G_0/G_1期阻滞, 提示CX43可能影响细胞周期相关基因的表达, 从而参与增殖的调控[36]。上述结果提示, 定位于细胞核的CX43羧基端具有非间隙连接通讯依赖性生长抑制能力, 可能作为转录因子或转录因子相关辅助因子, 直接或间接影响细胞增殖相关基因的表达。

3 CX43羧基端对肿瘤细胞迁移侵袭的影响

CX43对肿瘤细胞侵袭转移能力的影响较为复杂。部分研究结果表明, CX43的表达水平同肿瘤细胞的侵袭转移呈负相关。对肝癌患者组织标本的免疫组化检测发现, 未发生肿瘤转移的患者组织中CX43的表达显著高于转移患者, 且早期复发的患者*CX43* mRNA的水平显著低于未复发的患者, 显示CX43的表达可抑制肝癌的转移及早期复发[41]。在*CX43*突变的乳腺癌模型鼠中, 所有的*CX43*突变鼠均出现了肺转移, 给予外源性间隙连接抑制剂后, 转

移灶的数目进一步增加3倍, 显示CX43可抑制乳腺癌细胞的肺转移[42]。同样, 上调胶质瘤U251细胞的CX43表达水平, 其侵袭能力则显著降低[43]。

然而, 在某些肿瘤中, CX43的高表达却能促进肿瘤细胞迁移和运动。Tang等[44]发现, CX43在胃癌原发组织中低表达, 而在腹腔脱落癌细胞以及腹膜转移瘤组织中的表达水平则显著上升; 外源性超表达CX43后, 胃癌细胞向腹膜转移显著增多, 这表明CX43在人胃癌向腹膜转移中发挥着重要的促进作用; 在低剂量的γ-射线(10~20 cGy)诱导下, 脑胶质瘤细胞CX43的表达明显增强, 活化p38并促进细胞迁移, 而当下调CX43后, 细胞的迁移能力及p38的激活均受到抑制[45]。此外, 在前列腺癌细胞中过表达SNAI1后, CX43的表达及细胞的侵袭能力均显著上调; 反之下调CX43后, SNAI1表达下降, 细胞的侵袭能力减弱。这提示, CX43可与SNAI1相互作用形成CX43-SNAI1反馈环路, 促进肿瘤细胞的侵袭转移[46]。转移性的黑色素瘤细胞系与非转移性细胞系相比, CX43的表达显著升高[47]。

不过, 单就CX43羧基端而言, 绝大多数研究结果表明, 其对肿瘤细胞的迁移能力起显著的促进作用。过表达全长CX43的C6细胞迁移运动能力显著增强, 而当截断CX43羧基端后这种效应就消失; 单独转染CX43羧基端即可显著增强人胶质瘤LN18细胞和宫颈癌Hela细胞的迁移能力[48-49], 说明CX43的羧基端在胶质瘤细胞迁移中也发挥着重要的作用[50]。加入p38MAPK磷酸化特异性抑制剂后, 表达CX43羧基末端的Hela细胞迁移能力显著下降, 由于p38MAPK可磷酸化MAPK活化蛋白激酶2/3(MAPK-activated protein kinase 2/3, MAPKAPK2/3), 进而激活热休克蛋白27(heat shock protein 27, HSP27), 导致肌动蛋白(actin)重组而促进细胞迁移。因此, 活化p38MAPK可能是CX43羧基端调节细胞迁移的机制之一[48,51]。此外, CX43羧基端还可与细胞骨架蛋白(如F-actin、β-tubulin、N-cadherin)及actin结合蛋白(如drebrin、cortactin)发生相互作用进而促进细胞迁移[52]。采用时移成像技术可见, 过表达CX43羧基端的LN18细胞在迁移前沿形成伪足样的结构[49]。

4　CX43羧基端对肿瘤干细胞的影响

近年来的研究表明, 肿瘤是一个异质性的群体, 由肿瘤干细胞、祖细胞和分化肿瘤细胞共同构成金字塔形的分层分级结构。居于顶端的肿瘤干细胞, 尽管数量稀少, 却是恶性肿瘤发生、演进、治疗抵抗和复发的根源[53-55]。研究发现, CX43在肿瘤干细胞亚群中低表达甚至缺失, 而这种低表达对于维持肿瘤干细胞的恶性表型至关重要。在肿瘤干细胞中, 外源性超表达CX43能够抑制其自我更新、促进分化并降低其侵袭性和肿瘤起始能力[6,56-57]。进一步研究发现, 这种效应同CX43的羧基末端密切相关。Gangoso等[56]发现, 根据CX43羧基末端的245-283序列(CX43与c-Src的结合位点)所合成的穿膜肽, 可与c-Src结合, 从而抑制Src-DNA结合抑制子1(inhibitor of DNA binding 1, ID1)通路的活化, 降低性别决定区Y框蛋白2(sex determining region Y-box 2, SOX2)和神经型–钙黏蛋白(N-cadherin)的表达, 并上调上皮型–钙黏蛋白(E-cadherin)的水平, 进而抑制胶质瘤干细胞的自我更新和成瘤能力; 而小分子莱菔硫烷(sulforaphane)则能够提高CX43羧基末端S368、S279/282位点的磷酸化水平, 影响CX43的稳定性和亚细胞定位, 下调肝细胞生长因子受体(hepatocyte growth factor receptor, HGFR)和肿瘤干细胞标记物CD133的表达, 进而抑制胰腺癌干细胞的恶性表型[57]。

5　展望

综上, CX43的羧基端可独立地影响肿瘤细胞的生长和迁移侵袭, 其机制可能与蛋白–蛋白相互作用, 进而调控其自身装配、磷酸化状态、亚细胞定位以及重要信号通路的活性有关。我们知道, 蛋白质是生命活动的执行者, 蛋白与蛋白之间的相互作用是蛋白质功能发挥的关键环节。因此, 在蛋白质结构域水平上阐明CX43的羧基末端与其他蛋白之间的相互作用并建立网络, 将有利于我们全面了解CX43在恶性肿瘤发生、进展过程中所扮演的角色。然而, 下列问题尚待探讨: (1)CX43羧基端与调节肿瘤增殖、迁移相关功能蛋白(如NOV/CCN3等)的作用位点(域)尚不清楚; (2)CX43羧基端是否可以直接入核作为转录因子来调控肿瘤增殖相关基因的表达; (3)CX43羧基端与动态细胞骨架的相互作用在影响肿瘤细胞迁移侵袭中的地位如何; (4)除影响cyclin D1的入核, CX43羧基端与分子伴侣HSC70的相互作用还有哪些重要的意义; (5)还有哪些重要的蛋白分子与CX43羧基端存在相互作用。因此, 从蛋白–

蛋白相互作用组学的角度, 建立完整的CX43羧基末端–蛋白质相互作用组(interactome)图谱, 将有助于开发更具靶向性的短肽或者活性小分子, 直接或间接地作用于CX43羧基端, 保留其对肿瘤细胞的增殖抑制能力的同时抑制其促迁移作用, 为临床恶性肿瘤的治疗提供新的思路和选择。

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