肿瘤细胞外泌体对肿瘤血管新生的调控作用

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摘要 外泌体可将其内容物蛋白质、脂类、RNA、循环DNA等生物活性分子由供体细胞转运至受体细胞, 对细胞与细胞间的通讯发挥重要调控作用。肿瘤细胞可以主动释放包括外泌体在内的胞外囊泡进入周围微环境。血管为肿瘤的生长提供氧气和营养物质, 因此血管新生是肿瘤生长所必需的。研究发现, 蛋白或非编码RNA在不同肿瘤细胞衍生的外泌体中存在特异性表达的现象。肿瘤外泌体将其内含的非编码RNA以及蛋白转运至内皮细胞, 上调促血管新生因子的表达, 进而提高内皮细胞的活性, 促进其增殖、迁移和管腔形成。

关键词 肿瘤细胞; 外泌体; 血管新生; 非编码RNA; 蛋白

The Role of Tumor Cell Derived Exosomes in Angiogenesis

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Abstract Exosomes transport bioactive molecules such as proteins, lipids, RNA and circulating DNA from donor cells to recipient cells, which play an important role in regulating cell-cell communication. Tumor cells can actively release extracellular vesicles including exosomes into surrounding microenvironment. In addition, vessels provide oxygen and nutrients for tumor growth which is necessary for tumor growth. Some studies have demonstrated that the expression of proteins or non-coding RNAs are specifcally showed in different tumor cell-derived exosomes. To be exact, tumor exosomes can transport the non-coding RNA and protein contained in the tumor into endothelial cells, up-regulate the expression of pro-angiogenic factors, and then increase the activity of endothelial cells to promote their proliferation, migration and tube formation.

Keywords tumor cells; exosomes; angiogenesis; non-coding RNAs; protein

外泌体是细胞释放的脂质囊泡, 其内容物包括非编码RNA、DNA在内的核酸、蛋白质、脂类等生物活性分子[1]。外泌体可将其内容物由供体细胞转运至受体细胞, 进而在细胞与细胞间的通讯中发挥重要调节作用[2]。目前提取分离外泌体主要通过差速离心、密度梯度离心、超滤离心、磁珠免疫法或相关的试剂盒来实现。外泌体通过改变基因表达、信号通路以及增加肿瘤内的免疫抑制在肿瘤发展进程中发挥重要作用[3]。血管新生是在已有血管的基础上发展形成新毛细血管的过程, 在许多生理病理事件中都具有关键性作用[4]。血管新生涉及到内皮细胞激活、增殖、迁移和成熟在内的多个步骤[5]。

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血管新生对于维持机体血管平衡具有非常重要的生理作用, 并受到相关激活因子和抑制因子的严格调控, 正常血管发展失衡会导致相关疾病发生如: 癌症、骨关节炎等[6]。肿瘤血管新生与肿瘤的增殖和迁移密切相关[7-8], 血管新生增加会使肿瘤快速再生并增加肿瘤转移的可能性[9]。肿瘤细胞来源的外泌体可对肿瘤或周围组织的微环境产生相应的调控作用, 尤其是血管新生。因此, 本文通过通过综述相关的文献报道, 旨在阐述肿瘤细胞衍生的外泌体对血管新生的调控作用。

1 外泌体的来源

Exosome一词最早是在1987年由Johnstone和他的同事[10]提出的, 最开始外泌体被认为是排泄细胞成分的垃圾袋, 直到1998年Sebastian Amigorena的研究团队[11]提出外泌体在免疫系统和细胞间通讯中的作用, 才使得外泌体以细胞间通讯介质的身份亮相。胞外囊泡可根据尺寸划分为微囊泡和外泌体。微囊泡是细胞激活、损伤或凋亡后从细胞膜脱落产生的, 直径在100~1 000 nm[12], 外泌体是尺寸较小、直径在30~100 nm的胞外囊泡[13]。外泌体由内体系统形成, 首先在胞吞过程中形成早期内涵体, 之后各种功能分子进入早期内涵体, 并发展成富含腔内囊泡的晚期内涵体即胞外囊泡[14]。目前, 功能分子进入早期内涵体的机制和在它们在早期内涵体向晚期内涵体转化过程中的功能尚不清楚。CD9、CD63、CD81是内体系统衍生的外泌体标记物[15]。外泌体的生物合成和释放依赖于SNAREs、Rabs等能量活性物质[16]。与健康人群相比, 外泌体在肿瘤患者血液中浓度较高, 并且其内容物的个体差异较大[13]。

2 肿瘤血管新生

在肿瘤发展期间血管网络为肿瘤细胞输送氧气和营养物质, 并转运代谢废物。在没有血管生成的情况下, 癌细胞或肿瘤的直径不会超过2 mm[17]。随着肿瘤的生长, 对氧和营养物质的需求也不断增加, 这使得血管新生成为肿瘤生长存活所必需的条件[18]。一般血管新生包括内皮细胞激活、迁移、增殖和管状结构形成四个步骤。这个过程受到促血管生成因子和抗血管生成因子的严格调控, 以维持机体血管网络的动态平衡[19]。常见的促血管生成因子主要有VEGF(vascular endothelial growth factor)、FGF(fibroblast growth factor)、TGF-β(transforming growth factor-β)、MMPs(matrix metalloproteinases)等, 抗血管生成因子主要有血管抑制素、血小板反应蛋白等[20]。在促血管生成因子中VEGF有高度的促血管新生作用, 是被研究最多的因子之一[21], VEGF可诱导内皮细胞的增殖、迁移[22], 促进管状结构在细胞外基质中的形成[23]。越来越多的研究表明, 肿瘤外泌体来源的lncRNA(long non-coding RNA)和microRNA可以调控内皮细胞中VEFG的表达, 进而影响血管新生(图1)。

3 肿瘤外泌体与肿瘤血管新生

肿瘤细胞外泌体在肿瘤的自噬、化疗抵抗、转移等生物学进程中发挥重要的调控作用[24]。肿瘤外泌体对血管新生的调控主要是通过转运内容物来实现, 外泌体可直接转运促血管新生相关蛋白至内皮细胞, 也可通过转运microRNA和lncRNA, 对促血管新生因子的表达产生一定的影响。

3.1 肿瘤外泌体来源的蛋白对血管新生的调控

肿瘤外泌体直接转运相关蛋白至内皮细胞, 并通过Wnt/β-catenin信号通路、Notch信号通路、

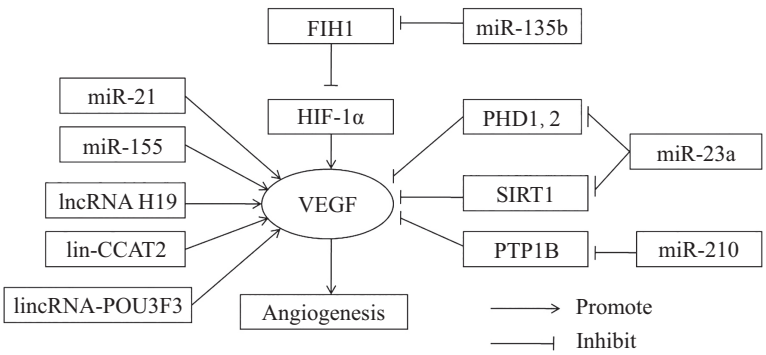

图1 肿瘤外泌体来源的microRNA和lncRNA对VEGF的调控

Fig.1 Regulation of VEGF by microRNA and lncRNA derived from tumor exosomes

EGFR(epidermal growth factor receptor)、MMP-9等影响肿瘤血管新生。

Wnt/β-catenin信号通路可诱导内皮细胞的增殖和迁移, Wnt4可增加β-catenin的核转运, 并激活Wnt/β-catenin信号通路[25]。直肠癌细胞在缺氧条件下释放的外泌体中, Wnt4表达显著增加, 并可通过外泌体转运至内皮细胞促进血管新生[26]。EGFR可以促进细胞的增殖, 激活EGFR可促进VEGF的表达, 而EDIL-3(EGF-like repeats and discoidin I-like domain-3)可激活EGFR[27]。EDIL-3在人膀胱癌细胞系外泌体中丰富表达, 外泌体将EDIL-3转运至内皮细胞后通过激活EGFR上调VEGF的表达, 并促进其血管新生[28]。PFKFB-3(enzymes 6-phosphofructo-2-kinase/fructose-2,6-bisphosphatase-3)可促进血管出芽和肿瘤生长[29]。在鼻咽癌患者外泌体中, PFKFB-3的表达较健康人显著升高, 外泌体将其转运至内皮细胞, 促进细胞的增殖、迁移和血管新生[30]。MMPs可通过细胞外基质降解和促血管有丝分裂原的释放促进血管新生, 在多发性骨肉瘤细胞外泌体中, MMP-9的表达显著高于细胞培养上清液, 且该外泌体可显著增加骨髓内皮细胞的活性, 促进血管新生[31]。Notch信号通路是在进化上高度保守的信号通路, 已有的研究证明, Notch信号通路涉及血管发育和血管新生, 其配体Dll-4(Delta-like 4)可通过调节尖端细胞的数量来控制血管发芽和分枝[32]。Sheldon等[33]的研究发现, 在过表达Dll-4的神经胶质瘤细胞系U87-GM中, Dll-4可被整合到外泌体中并被转运至内皮细胞, 导致内皮细胞中Notch信号传导的抑制和Notch受体的丧失, 进而使血管分支和血管密度增加。这表明, 肿瘤外泌体可通过转运Dll-4来抑制内皮细胞中Notch信号的传导, 使血管新生增加。

肿瘤外泌体通过Wnt/β-catenin信号通路、Notch信号通路、EGFR、MMP-9等, 使内皮细胞的增殖和迁移、细胞外基质降解情况、尖端细胞数量等产生变化, 进而对血管新生产生调控作用。除了直接转运促血管新生因子至内皮细胞外, 肿瘤外泌体还可通过转运膜蛋白对内皮细胞的PH和VEGF的转运产生影响。CA9(carbonic anhydrase 9)是定位在细胞表面的跨膜蛋白, 可通过促进碳酸氢盐的跨膜运输来调节细胞内的PH, 研究证明, CA9可以促进肿瘤细胞的迁移和侵袭[34]。CA9在肾癌细胞系OSRC-2外泌体中丰富表达, 并且可以被HUVEC摄取, 进而促进HUVEC迁移和管状结构的形成[35]。Myoferlin是一个具有多个C2结构域的跨膜蛋白, 已有的研究表明, 其可控制血管新生关键因子EGFR、VEGFR2的内吞作用和VEGF的胞吐作用[36-38]。Blomme等[39]的研究发现, 乳腺癌和胰腺癌细胞外泌体均可促进内皮细胞增殖和迁移, 且myoferlin在外泌体中丰富表达, 去除myoferlin后, 该外泌体对内皮细胞的促进作用减弱。这表明, 乳腺癌和胰腺癌细胞外泌体可通过其内容物myoferlin介导血管新生。跨膜蛋白在肿瘤外泌体促进血管新生中也发挥着重要的作用, 其主要通过对EGFR、VEGFR2、VEGF的转运, 促进内皮细胞的增殖、迁移, 使血管新生增加。

3.2 肿瘤外泌体来源的microRNA对血管新生的调控

MicroRNA是一类长度在18~22 bp的非编码RNA, 可通过与mRNA的3′-UTR特异性结合抑制蛋白翻译或使mRNA降解抑制基因表达[40]。不同肿瘤细胞外泌体中microRNA的表达各不相同, microRNA转运至内皮细胞后可直接调控促血管新生因子的表达, 也可通过其上游的SIT1、PHD、HIF-α等对VEGF、bFGF等的表达产生影响。

在氧气正常的情况下, 胶质母细胞瘤、伯基特淋巴瘤外泌体来源的microRNA, 可靶向地促进内皮细胞中VEGF的表达, 从而使血管新生增加。胶质母细胞瘤是血管新生最多的一种肿瘤, 它可以诱导肿瘤周围血管显著增长[41]。胶质母细胞瘤外泌体可以促进内皮细胞的增殖和管状结构形成, 用胶质母细胞瘤外泌体干预后肢缺血模型小鼠发现, 其可促进缺血后肢灌注的恢复以及血管的生成[42]。Sun等[43]研究发现, 胶质瘤干细胞外泌体中miR-21的表达量较胶质瘤干细胞高, miR-21可以促进内皮细胞VEGF的表达和管状结构的生成, 这表明胶质瘤干细胞外泌体可以通过miR-21/VEGF通路来促进血管新生。Paggetti等[44]的研究发现, 伯基特淋巴瘤细胞外泌体可被视网膜色素上皮细胞内化, 并通过其内容物miR-155促进VEGF-A在视网膜色素上皮细胞中的表达。这表明, 伯基特淋巴瘤细胞外泌体可能通过miR-155/VEGF-A通路来促进血管新生。此外, 也有研究发现, 横纹肌肉瘤细胞外泌体可能通过miR-1246-Smad1/5通路促进血管新生[45]。

肿瘤生长和发展过程中会在其核心部位形成

局部的缺氧, 这已成为肿瘤微环境的一个特征并可以推动肿瘤的发展进程[46]。肿瘤缺氧诱导的适应性机制主要依赖于肿瘤微环境中各种基质细胞表型的改变, 进而维持肿瘤细胞的存活并促进其发展和侵袭[47]。缺氧条件下肿瘤细胞外泌体中的microRNA的表达产生变化, 并通过VEGF通路上游的关键因子, 影响VEGF在内皮细胞中的表达。

乳腺癌细胞外泌体中miR-210的表达在缺氧情况下显著升高, 并且可以被肿瘤微环境中的肿瘤细胞、内皮细胞、干细胞等摄取, 通过抑制Ephrin-A3和PTP1B(protein tyrosine phosphatase-1B)的表达, 激活VEGF促进血管新生[48]。SIRT1(sirtuin 1)可抑制VEGF的表达[49], Sruthi等[50]的研究发现, 缺氧肝癌细胞集落释放的外泌体可以被HUVECs摄取, 同时miR-23a在外泌体中的表达较氧含量正常情况下增加, 在内皮细胞中miR-23a可作用于靶标SIRT1, 抑制其表达使VEGFR2表达显著升高。在氧气正常的情况下, HIF-1α(hypoxiainducible factor-1α)的异位表达可激活VEGF进而促进内皮细胞的增殖和管状结构形成[51], 而PHD(prolyl hydroxylase)家族蛋白, 可控制HIF-1α的水平[52]。肺癌细胞在缺氧情况下产生的外泌体含有丰富的miR-23a, miR-23a通过抑制其靶标PHD1、PHD2在内皮细胞中的表达进而促进HUVEC的迁移、渗透以及管状结构的形成[53]。此外, FIH-1(factor-inhibiting hypoxia-inducible factor-1)是一个可与HIF-1α结合的天冬酰胺酰羟化酶, 并能抑制HIF-1α的反式激活功能[54]。多发性骨髓瘤细胞在低氧情况下释放的外泌体可以促进HUVECs的管腔形成, miRNA表达谱分析发现, 低氧情况下miR-135b在外泌体中的表达较细胞内增高, 这表明, 多发性骨髓瘤细胞外泌体可能通过转运miR-135b促进HIF-1α的激活, 进而使血管新生增加[55]。

综合上述研究发现, 在氧气正常的情况下, 肿瘤外泌体中的microRNA主要通过靶向的作用于VEGF来调控血管新生, 而在缺氧的情况下肿瘤外泌体中的microRNA则主要通过作用于VEGF上游的因子影响血管新生。由于缺氧环境和血管新生对于肿瘤发展具有重要作用, 抑制肿瘤外泌体对VEGF的作用, 有可能会成为控制肿瘤发展的关键机制。

3.3 肿瘤外泌体来源的lncRNA对血管新生的调控

LncRNA是一类长度大于200 bp的非编码RNA, 其可参与表观遗传调控、转录调控、转录后调控过程[24]。虽然lncRNA不能编码功能性蛋白, 但可通过启动子结合、染色质重塑、组蛋白修饰和干扰RNA剪切来调节基因表达[56]。已有研究表明, 肿瘤细胞外泌体来源的lncRNA可诱导VEGF、bFGF、TGFβ等信号通路表达增加, 进而促进血管新生。

$CD90^+$肝癌细胞释放的外泌体可被内皮细胞摄取, 该外泌体可使内皮细胞中VEGF和VEGFR的表达显著上调, 管状结构的长度和数量增加; 使用siRNA抑制lncRNA H19在内皮细胞中的表达后, $CD90^+$肝癌细胞外泌体对内皮细胞的促血管新生作用减弱, 这表明, $CD90^+$肝癌细胞外泌体通过转运lncRNA H19促进血管新生[57]。Lang等[58]的研究发现, 胶质瘤细胞外泌体可以被人脑微血管内皮细胞内化并促进其增殖、迁移和管状结构形成, 其可能机制是胶质瘤细胞外泌体来源的lincRNA-POU3F3可促进人脑微血管内皮细胞中VEGFA表达。与其他神经胶质瘤细胞系相比, lin-CCAT2在神经胶质瘤细胞系U87-MG中表达最高, U87-MG细胞外泌体中富含lin-CCAT2且可以被人脐静脉内皮细胞吸收, 在体外环境下U87-MG细胞外泌体可以促进人脐静脉内皮细胞增殖、迁移和管状结构的形成, 在体情况下可以促进小动脉的形成, 其可能机制是U87-MG细胞外泌体中的lin-CCAT2可以促进VEGFA、TGFβ(transforming growth factor-β)、Bcl-2(B-cell lymphoma-2)的表达同时抑制Bax(Bcl-2 associated X protein)、蛋白酶3的表达[59]。

综合上述研究发现, 肿瘤细胞外泌体通过转运特异性表达的蛋白和非编码RNA对血管新生产生影响(表1)。其主要的作用机制可能是直接转运促血管新生因子或通过转运非编码RNA调控相关的信号通路, 促进内皮细胞的增殖、迁移、管腔形成以及血管分枝和萌芽。这为抑制肿瘤血管新生提供了有力的理论依据。

4 小结

众多研究表明, 肿瘤细胞外泌体可以促进血管新生, 进而对肿瘤的发展进程产生影响。外泌体通过传递蛋白、lncRNA、microRNA等分子调控血管新生的进程, 这一属性受到外泌体细胞起源、微环境以及靶细胞的影响, 因此可以通过筛选供体细胞或改变微环境来调控外泌体促血管新生的活性。基于外泌体可以有效地避开免疫系统, 其在临床疾病

表1 肿瘤细胞外泌体来源的非编码RNA对血管新生的调控作用

Table 1 The regulation of angiogenesis by non-coding RNA derived from tumor cell exosomes

外泌体来源的细胞 Exosome-derived cells	外泌体来源的非编码RNA Exosome-derived non-coding RNAs	功能 Functions	参考文献 References
Glioma stem cell	miR-21	Promoting VEGF expression	[43]
Burkitt lymphoma cell	miR-155	Promoting VEGF-A expression	[44]
Rhabdomyosarcoma cell	miR-1246	Activating Smad1/5/8 signaling pathway	[45]
Breast cancer cell	miR-210	Inhibiting Ephrin-A3 and PTP1B expression	[48]
Hypoxic liver cancer cells	miR-23a	Inhibiting SIT1 expression	[50]
Lung cancer cell	miR-23a	Inhibiting the binding of PHD1 and PHD2 to ZO-1	[53]
Multiple myeloma cells	miR-135b	Inhibiting FIH-1 expression	[55]
liver cancer cells	lncRNA H19	Promoting VEGF and ICAM-1expression	[56]
Glioma cells	lincRNA-POU3F3	Promoting bFGF, VEGFA and bFGFR expression	[57]
Glioma cell line (U87-MG)	lin-CCAT2	Promoting the expression of VEGFA, TGFβ, Bcl-2 and Inhibiting the expression of Bax and Protease 3	[58]

的治疗中具有较大的应用价值, 但有关外泌体的形成、释放、内化等机制以及临床应用问题仍需不断研究。此外, 在肿瘤血管新生中尚未见有外泌体对抗血管新生因子影响的研究, 这还需要广大学者不断探索。

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