综述

线粒体DNA变异与恶性肿瘤发生及进展关系

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摘要 线粒体是细胞进行氧化磷酸化产生ATP的主要场所, 在能量代谢、细胞凋亡、氧化应激等生命活动中扮演重要角色。近年来随着研究的不断深入, 线粒体DNA(mtDNA)变异在恶性肿瘤发生及进展中的作用机制逐渐引起人们的广泛关注, 成为肿瘤基础研究与临床研究领域的新兴热点。研究表明, 肿瘤细胞中mtDNA变异包括点突变、缺失、插入以及拷贝数变异, 并且这些变异可参与肿瘤细胞的增殖、生长、侵袭和转移。因此, 进一步深入理解mtDNA变异的发生规律及作用机制, 将为恶性肿瘤诊断、治疗及预后判断提供有意义的参考。

关键词 线粒体DNA; 恶性肿瘤; 变异; 活性氧

Somatic Variations of Mitochondrial DNA in Carcinogenesis and Tumor Progression

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Abstract Mitochondria is one of intracellular organelles responsible for generating ATP through respiration and oxidative phosphorylation, producing reactive oxygen species, and initiating and executing apoptosis. In recent years, there is increasing evidence that somatic mitochondrial DNA (mtDNA) variations are associated with various cancers, and these may be involved in carcinogenesis and tumor progression. Therefore, understanding mtDNA variations in cancer cells promises to provide major new insights into the etiology of solid tumors and be of great potential benefit for prognosis and possible treatment of cancer.

Key words mitochondrial DNA (mtDNA); cancer; variation; ROS

线粒体是细胞进行氧化磷酸化产生ATP的细胞器, 在能量代谢、细胞凋亡、氧化应激等生命活动中扮演着重要角色。线粒体拥有独立于核基因组之外的遗传物质——线粒体DNA(mtDNA), 并与核基因协同调控细胞生命活动。mtDNA为全长16 569 bp的双链环状分子, 与核DNA相比, mtDNA具有高拷贝数、高突变率、高度多态性、严格母系遗传等特点。mtDNA可分为编码区和非编码区, 编码区编码13个氧化磷酸化相关多肽、2个rRNA和22个tRNA; 而非编码区(即D-loop区)主要负责mtDNA重链复制起始和轻重链转录调控[1]。1988年, 人们首次发现mtDNA变异可导致人类疾病, 同年共报道了三种mtDNA变

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异相关疾病, 包括Leber遗传性视神经病[2]、线粒体脑肌病[3]以及KSS综合征[4]。近年来随着研究的不断深入, 人们在多种恶性肿瘤中发现大量mtDNA变异, 且mtDNA变异与恶性肿瘤的发生及进展密切相关, 但其具体分子机制尚不十分明确。因此, 为进一步理解mtDNA变异在肿瘤发生进展中的作用机制, 本文将从肿瘤细胞中mtDNA变异的类型、规律及其在临床和基础研究等方面的最新进展进行综述。

1 恶性肿瘤细胞中mtDNA的变异类型

1.1 突变

mtDNA高突变率主要由三个原因造成: (1) mtDNA是裸露DNA, 缺乏组蛋白保护; (2)缺乏有效的DNA损伤修复系统; (3)处于高浓度活性氧(ROS)中。研究表明, 恶性肿瘤中线粒体呼吸链功能异常可导致大量ROS蓄积, 使鸟嘌呤在C8位点羟基化生成高浓度8-氧鸟嘌呤核苷(8-oxo-G), 8-oxo-G可损伤DNA, 从而造成大量mtDNA突变[5], 而mtDNA突变又可进一步影响线粒体氧化呼吸链功能, 促进ROS产生。正常细胞中存在着一系列的抗氧化防御机制, 可以有效抵御ROS的损伤, 如ROS净化剂、抗氧化酶(SOD、过氧化氢酶以及谷胱甘肽)等, 然而这些抗氧化物质在肿瘤细胞中常常缺失, 导致细胞中的DNA持久氧化损伤[6]。目前的研究表明, 恶性肿瘤细胞中主要存在三种不同类型的mtDNA突变, 包括点突变、缺失及插入。

1.1.1 点突变 点突变是恶性肿瘤细胞中最常见的mtDNA变异。1998年, Polyak等[7]首次在人结直肠癌细胞系中报道mtDNA点突变, 发现十个结直肠癌细胞系中有七个存在mtDNA突变, 包括11个点突变和1个插入。2001年, Nishikawa等[8]在肝癌组织中发现, mtDNA突变频率显著高于正常肝组织。随着研究的深入, 到目前为止人们几乎在所有恶性肿瘤中均发现mtDNA点突变, 如Fliss等[9]报道43%的肺癌、46%的头颈癌及64%的膀胱癌中含mtDNA点突变; Liu等[10]报道卵巢癌mtDNA点突变率为61%; Parrella等[11]和Tan等[12]分别报道61%和55%的乳腺癌mtDNA含点突变以及Maximo等[13]报道甲状腺癌中mtDNA点突变率为49%等。

D-Loop区是mtDNA转录复制的调控区, 同时也是mtDNA突变的热点区域(表1)。D-loop区包含一个多聚胞嘧啶区(np303-np315), 被命名为D310。

表1 恶性肿瘤中线粒体DNA D-loop区突变

Table 1 D-loop mutation in different cancers

肿瘤类型 Cancer type	突变频率 *n/N*, % of patients with mutation	参考文献 Reference	肿瘤类型 Cancer type	突变频率 *n/N*, % of patients with mutation	参考文献 Reference
Bladder cancer	3/15, 2	[16]	Head & neck cancer	6/34, 18	[18]
	4/16, 25	[17]		23/109, 21	[19]
	5/14, 36	[9]		19/51, 37	[16]
				6/13, 46	[9]
Breast cancer	18/60, 3	[20]	Hepatocellular carcinoma	2/56, 3.6	[24]
	3/20, 15	[17]		17/50, 34	[25]
	5/17, 29	[16]		24/61, 39.3	[26]
	17/45, 38	[21]		37/62, 59	[27]
	7/18, 39	[11]		13/19, 68	[28]
	25/59, 42	[22]			
	13/30, 43	[23]			
	12/19, 63	[12]			
Colorectal cancer	3/13, 23	[29]	Lung cancer	16/100, 16	[16]
	7/25, 28	[16]		34/202, 17	[31]
	20/45, 44	[30]		17/55, 31	[32]
Gastric cancer	2/45, 4	[33]	Renal cancer	1/8, 12.5	[37]
	4/32, 12.5	[34]		4/15, 27	[38]
	15/31, 48	[35]			
	15/24, 62	[36]			
Thyroid cancer	32/66, 49	[39]			

D310序列被认为是恶性肿瘤mtDNA最常见的突变区。体外分析显示, 与其他mtDNA区域相比D310序列对氧化损伤更为敏感。Xu等[14]报道77.1%乳腺导管细胞癌和75.5%浸润性乳腺导管细胞癌含D310序列突变, 并且在35.3%的癌旁中也检测到该序列突变。Legras等[15]发现结直肠癌D310序列突变率远远高于正常组织, 且与肿瘤组织的恶性程度呈正相关, 提示D310突变可能是组织向恶性转化的一个重要标志。

1.1.2　缺失　迄今为止, 人们已在肿瘤细胞中发现超过一百种mtDNA片段缺失。其中, mtDNA 4 977 bp (np8 470-np13 447)大片段缺失是最为常见的一种mtDNA缺失。Pang等[40]最早报道, 经常受日光照射的皮肤组织中蓄积大量mtDNA 4 977 bp片段缺失, 同时在扁平鳞状细胞癌和具有癌前病变的皮肤组织中也检测到该片段缺失。随后他们的研究又发现4 977 bp片段缺失存在于口腔癌及良性口腔肿瘤中[41]。Shen等[42]在13个胃癌细胞系、52例胃癌样本及相应癌旁组织中检测到了mtDNA 4 977 bp片段缺失, 发现缺失率分别为92.3%、73.1%和52%, 提示mtDNA 4 977 bp大片段缺失可能在胃癌发生过程中发挥重要作用。尽管mtDNA 4 977 bp片段缺失常见于多种肿瘤组织, 然而Dani等[43]在胃癌、乳腺癌、结直肠癌和头颈癌及其相应癌旁中研究发现, 癌组织中mtDNA 4 977 bp片段缺失率远低于癌旁和正常组织, 推测mtDNA 4 977 bp片段缺失可能对细胞增殖起抑制作用。此外, Tan等[44]也在对食管癌的研究中得到类似结果。同时, 一些小片段mtDNA缺失在肿瘤中也相继被报道, 如Horton等[45]发现50%肾细胞癌中存在mtDNA 264 bp(np3323-3588)片段缺失, Jin等[46]研究发现一个mtDNA 9 bp片段缺失与肝癌发病风险显著相关。

1.1.3　插入　mtDNA 12418insA突变在肝癌、胃癌和结直肠癌中被检测到, 研究表明在ρ^0细胞中转入12418insA突变mtDNA可造成线粒体氧化呼吸链功能障碍和乳酸的大量产生, 进一步荷瘤小鼠体内实验表明, 在肿瘤细胞中导入该突变可促进肿瘤生长[47]。尽管如此, 大部分已发现的mtDNA插入突变呈随机性, 且未发现与恶性肿瘤的发生与进展具有明显相关性。如Wu等[35]在胃癌组织的mtDNA D-loop区中发现两种插入突变, 分别位于np303-309和np568-573区域并以两个或三个重复单核苷酸的方式插入。随后Hung等[24]在不同类型的癌组织中发现约5%存在以上两种插入, 并且在相应癌旁组织中也可检测到上述插入突变。虽然不具有肿瘤特异性, 但这种串联重复插入早在线粒体肌病中被发现, 并且可能参与该疾病的发生。

1.2　拷贝数变异

mtDNA功能的发挥既依赖于mtDNA分子结构的完整性, 同时也与细胞中mtDNA拷贝数密切相关。研究表明, mtDNA拷贝数的改变在不同类型肿瘤中有着明显差异。与癌旁组织相比, 头颈癌、食管癌、结直肠癌、子宫内膜癌、乳头状甲状腺癌及神经胶质瘤组织中mtDNA拷贝数明显增加, 而肝癌、肾癌、胃癌、肺癌、乳腺癌、前列腺癌及尤文氏肉瘤组织中mtDNA拷贝数显著降低(表2)。

在前期研究中我们首次发现, 正常个体mtDNA拷贝数主要由遗传因素决定, 遗传度可达65%[48]。而在恶性肿瘤中mtDNA拷贝数还受到其他非遗传因素的影响。如Lee等[49]在肝细胞肝癌、胃癌、肺癌组织中对D-loop区突变及mtDNA拷贝数分析中发现, D-loop区突变可导致mtDNA拷贝数降低。随后在肝细胞肝癌研究中进一步发现靠近重链复制起始位置的D-loop区突变点, 可能影响mtDNA复制相关蛋白结合, 从而降低了mtDNA复制速率。而Turner等[50]报道mtDNA D-loop区以外np3243位点的碱基突变也可导致mtDNA拷贝数显著降低。此外mtDNA复制关键基因的突变或表达水平的改变, 也可参与mtDNA拷贝数的异常改变。如Singh等[51]在63%的乳腺癌组织中发现了mtDNA复制相关*POLG*基因突变, 并证明该基因突变可导致mtDNA拷贝数显著降低。此外, 在*TFAM*基因敲除的小鼠模型中mtDNA拷贝数下降了35%~40%[52]。另有报道证明, p53可直接与mtDNA中特异的反应元件相结合, 进而调控mtDNA拷贝数[53]。

2　恶性肿瘤中线粒体DNA变异与临床研究

大量临床研究表明, mtDNA突变与多种恶性肿瘤的发生及进展显著相关。Bai等[58]利用156例乳腺癌样本及260例正常样本研究发现, G9055A、A10398G、T16519C三个位点的mtDNA突变可显著增加乳腺癌发病风险, 而T3197C、G13708A位点mtDNA突变则可降低乳腺癌发病风险; Choi等[59]利用线粒体测序芯片对70例肺癌样本及相应癌旁

表2 线粒体DNA拷贝数在恶性肿瘤中的改变

Table 2 Alterations in mtDNA content of human cancers

肿瘤类型 Cancer type	n/N n/N	增加或降低 Increase or decrease	突变频率(%) Frequency(%)	参考文献 Reference
Breast cancer	38/60	Decrease	63	[20]
	46/59	Decrease	78	[22]
	20/25	Decrease	80	[54]
Colorectal cancer	60/153	Increase	39	[55]
	10/25	Increase	40	[49]
	43/44	Increase	98	[56]
Gastric cancer	17/31	Decrease	55	[49]
Hepatocellular carcinoma	31/54	Decrease	57.4	[49]
	37/61	Decrease	60.5	[26]
Lung cancer	7/31	Decrease	22.6	[49]
Renal cancer	34/37	Decrease	91	[57]
Thyroid cancer	13/20	Increase	65	[54]

的线粒体全基因组研究发现, 肺癌组织中mtDNA突变频率约为正常组织的100倍。其中, np8 701和np10 398是两个突变热点(分别位于*ATPase6*和*ND3*基因内), 且与肺癌发病风险显著相关; 此外, 作者还发现吸烟者mtDNA突变率明显大于非吸烟者, 且np8701和np10398两个突变位点与患者吸烟史具有显著关系; Lièvre等[60]在对365例结直肠癌患者的mtDNA分析中发现, 肿瘤组织D-loop区发生突变的患者预后更差; 同时结直肠III期患者接受化疗后, 含D-loop区突变的患者三年存活率明显低于未突变者; Matsuyama等[31]对202例非小细胞肺癌患者研究发现, 处于IIIB期和IV期的患者肿瘤组织中mtDNA D-loop区突变率明显高于其他分期, 并且D-loop区发生突变的患者比未发生突变的患者预后差。

除此之外, 大量临床研究也证实mtDNA拷贝数变异对多种恶性肿瘤发生及进展具有重要意义。我们利用病例对照研究的实验设计, 在国际上首次证实患者外周血中低的mtDNA拷贝数与升高的肾细胞癌、肝细胞肝癌发病风险显著相关; 而患者外周血中mtDNA拷贝数升高则与高的大肠癌发病风险显著相关[61-62]。随后, Thyagarajan等[63]的研究发现外周血mtDNA拷贝数与结直肠癌发病风险呈U形关系, 即mtDNA拷贝数较高的和拷贝数相对较低的人群发病风险高。上述结果提示, 淋巴细胞中的mtDNA拷贝数可独立或与其他已知的风险评估因子共同构建风险评估模型, 用于对肿瘤发病高危人群进行筛选和鉴别。此外, Feng等[56]在结直肠癌组织中发现I期和II期患者mtDNA拷贝数明显高于III期和IV期患者, Xia等[64]发现乳腺癌I期患者外周血中mtDNA拷贝数明显比其他分期低。Mambo等[54]的研究发现mtDNA拷贝数变异具有肿瘤特异性, 如与正常组织相比, 80%乳腺癌组织中mtDNA拷贝数降低而65%甲状腺癌组织中mtDNA拷贝数升高, 但并未发现其与肿瘤病理分期和转移相关, 提示肿瘤mtDNA拷贝数变异可能对上述肿瘤形成早期敏感。而Yamada等报道肝癌组织中的mtDNA拷贝数明显降低, 并且与肿瘤的大小和肝硬化有关。进一步Yamada等[65]还发现mtDNA拷贝数越低的肝癌患者五年存活率越低。上述结果表明维持mtDNA拷贝数的稳态对于肿瘤的发生和发展可能具有重要意义, 但其具体机制尚有待进一步阐明。

综上所述, 无论mtDNA突变还是拷贝数变异均可直接或间接参与恶性肿瘤发生及进展, 同时随着我们对恶性肿瘤中mtDNA变异的进一步深入研究, 某些mtDNA特异性改变有望应用于临床, 作为新的生物标志用于诊断和监测恶性肿瘤。

3 恶性肿瘤中线粒体DNA变异与基础研究

Wheelhouse等[27]在研究HBV感染和肝细胞癌变之间的关系中发现, 携带HBV的肝癌细胞中D-loop区突变数量高于正常肝组织, 作者认为慢性病毒性肝炎所致肝组织反复损伤和再生, 造成了mtDNA突变累积, 并导致线粒体氧化呼吸链功能异常、氧自由基产生增加, 进一步造成mtDNA和核DNA损伤, 由此参与早期肝细胞癌变。为了探索mtDNA突变对恶性肿瘤的影响, Petros等[66]将致病的mtDNA T8993G(ATPase6)突变利用胞质杂合技术导入前列腺癌PC3细胞系并进行了裸鼠成瘤实验。结果表明, 肿瘤组织ROS含量显著增加, 并且携有该mtDNA突变(T8993G)的肿瘤体积是对照组(野生型T8993T)的7倍。Shidara等[67]发现mtDNA含T8993G(ATPase6)突变型细胞比野生型细胞的凋亡频率低, 推测致病的mtDNA点突变可能是通过抑制细胞凋亡从而促进肿瘤细胞的增殖, 而且这种突变也能够抑制由顺铂诱导的细胞凋亡, 说明mtDNA突变可能参与相关化疗药物的耐药性。Ishikawa等[68]用相同的方法将mtDNA G13997A(ND6)和13885insC(ND6)突 变

导入小鼠的肿瘤细胞系中, 也检测到了ROS大量积累, 同时ROS可介导核编码的某些基因表达上调, 如*MCL-1*、*HIF-1a*以及*VEGF*等, 进而增加肿瘤的转移潜能。

无mtDNA的ρ^0细胞系的成功诱导, 为mtDNA拷贝数变异的功能研究提供了良好的细胞模型。Li等[69]通过建立人肝癌mtDNA缺失ρ^0SK-Hepl细胞以及转线粒体细胞SK-HeplCyb, 发现mtDNA缺失对肿瘤细胞凋亡有显著拮抗作用。Lin等[70]发现mtDNA缺失的HeLa ρ^0细胞系对阿霉素和光动力性疗法诱导的细胞凋亡具有一定的耐受性, 而正常HeLa细胞却非常敏感, 据此作者认为非小细胞肺癌(NSCLC)中mtDNA拷贝数较低可能是其化疗敏感性差的原因之一。Delsite等[71]研究发现, 下调mtDNA拷贝数不但可诱发乳腺癌细胞的氧化应激, 造成脂类过氧化水平显著升高, 还可进一步引起众多核基因的表达异常, 而这些基因的功能主要涉及细胞生长、能量代谢、信号传递以及凋亡等重要生物学功能。

4　结语与展望

mtDNA变异发生于多种人类恶性肿瘤中, 并且被证实与恶性肿瘤发生及进展密切相关, 因此对其发生规律、作用机制的研究有助于将mtDNA变异作为新兴标志物或靶点应用于临床肿瘤诊断、治疗及预后判断。然而, mtDNA变异的研究尚存在很大的局限性及技术瓶颈, 如尚未开展大规模的肿瘤mtDNA基因组测序工作、尚缺乏有效的技术手段对细胞中mtDNA进行定点诱变、mtDNA变异的临床表型存在多样性等, 都成为mtDNA变异在肿瘤基础及临床研究中面临的挑战与机遇。

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