乳头状甲状腺癌中线粒体DNA突变的研究

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摘要 该文探究了线粒体DNA(mtDNA)突变与甲状腺癌的发生发展的相关性, 评估了mtDNA拷贝数对甲状腺癌的诊断价值。根据对结节性甲状腺肿、滤泡状甲状腺腺瘤和乳头状甲状腺癌3组病人的mtDNA全基因测序和单倍型分型结果, 统计3组病人mtDNA突变率及单倍型的差异, 分析乳头状甲状腺癌病人的mtDNA突变率与临床资料的联系, 最后通过荧光定量PCR检测3组病人的组织和血液样本中mtDNA的拷贝数。结果显示, 乳头状甲状腺癌患者mtDNA的复合体I亚基编码区和tRNA编码区的突变率明显高于结节性甲状腺肿, 在乳头状甲状腺癌患者中线粒体单体型M相对于单体型N有更低的淋巴结转移率, 荧光定量PCR结果显示, 甲状腺腺瘤和甲状腺癌组织中的mtDNA拷贝数明显高于结节性甲状腺肿, 而在血液标本中, 两者的mtDNA拷贝数均低于结节性甲状腺肿。这些结果表明, mtDNA拷贝数的变化和复合体I亚基编码区的突变可能作为甲状腺癌诊断的生物指标, 而线粒体单体型N可能可以作为乳头状甲状腺癌恶性变化的预警指标。

关键词 乳头状甲状腺癌; 线粒体DNA; 突变

The Study on the mtDNA Mutation in Papillary Thyroid Carcinoma

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Abstract This study explored the correlation between mtDNA (mitochondrial DNA) mutation and thyroid carcinoma, and evaluated the diagnostic value of mtDNA copy number for thyroid carcinoma. Analyzed the relationship between mutation rate and clinical data after mtDNA sequencing and haplotype typing in patients with nodular goiter, follicular thyroid adenoma and papilla thyroid carcinoma. Finally, the mtDNA copy number in tissue and blood samples of three groups was measured by fluorescence quantitative PCR. The results showed that the mtDNA mutation rates in complex I subunit coding region and tRNA coding region of patients with papillary thyroid carcinoma were significantly higher than those in nodular goiter, and mtDNA haplotype M had lower lymph node metastasis than haplotype N in patients with papilla thyroid carcinoma. The results of fluorescence quantitative PCR showed that the mtDNA copy number in the tissue of patients with thyroid adenoma and thyroid carcinoma was significantly higher than that in nodular goiter, but both of them had lower mtDNA copy number in the blood samples than that in nodular goiter. These results suggest that the mtDNA copy number changes and complex I subunit coding region mutations may be used as biological indexes for the diagnosis of thyroid carcinoma, and the mtDNA haplotype N may be used as an early warning index for malignant changes of papillary thyroid carcinoma.

Keywords papillary thyroid carcinoma; mitochondrial DNA; mutation

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甲状腺癌是内分泌系统中常见的恶性肿瘤, 约占全身恶性肿瘤的1%, 其发病机制至今尚未完全阐述, 是一种由基因和环境等多种因素共同调节的疾病, 找到甲状腺癌发生发展的机制对于其治疗有着重要意义。

线粒体是哺乳动物细胞进行有氧呼吸的主要场所, 通过氧化磷酸化为细胞的代谢提供大量的能量, 线粒体功能异常与肿瘤的发生密切相关, 异常的线粒体功能已经成为多种肿瘤的显著性特征[1-3]。线粒体DNA(mitochondrial DNA, mtDNA)编码线粒体内的功能蛋白及RNA分子, 它包含37个编码基因和非编码的D-loop区, 其中编码区的13个基因(*ND1*、*ND2*、*ND3*、*ND4*、*ND4L*、*ND5*、*ND6*、*CytB*、*COI*、*COII*、*COIII*、*ATP6ase*、*ATP8ase*)用来参与线粒体复合体亚基的生物合成, 此外, mtDNA非编码的D-loop区可调控mtDNA的复制。mtDNA的突变导致细胞的线粒体功能障碍和较高的氧化应激状态[4], 从而促进肿瘤的发生和发展。目前研究发现, mtDNA突变与肝癌[5]、乳腺癌[6]、结直肠癌[7]等多种肿瘤的发生密切相关, 其中, 与甲状腺癌密切相关的mtDNA突变区域包括13个复合体亚基基因以及D-Loop区。TRISKA等[8]在儿童恶性肿瘤中发现了mtDNA的复合体I亚基和复合体IV亚基的突变, 并在肿瘤和正常组织中有显著差异。由此可见, mtDNA突变与甲状腺癌发生发展的联系值得进一步的研究。本研究对结节性甲状腺肿、滤泡状甲状腺腺瘤和乳头状甲状腺癌病人的冰冻组织切片和外周血进行线粒体全基因测序, 分析mtDNA基因突变和线粒体单倍型在3组病人中的差异, 以及乳头状甲状腺癌患者中mtDNA的突变和单倍型与临床资料的联系, 最后通过荧光定量PCR来检测结节性甲状腺肿、滤泡状甲状腺腺瘤和乳头状甲状腺癌中mtDNA拷贝数的变化。

1 材料和方法

1.1 材料

1.1.1 病人标本 本研究获得温州医科大学第一附属医院伦理委员会批准, 血液和冰冻组织标本来源于温州医科大学第一附属医院的甲状腺癌和良性甲状腺疾病患者,并签署知情同意书。在温州医学院第一附属医院随机采集92例甲状腺癌和69例良性甲状腺疾病患者术后冰冻病理组织切片和外周静脉血。经石蜡切片确诊, 92例甲状腺癌组织标本均为乳头状甲状腺癌, 69例良性甲状腺疾病组织标本包括28例滤泡型甲状腺腺瘤和41例结节性甲状腺肿。所有冰冻病理组织切片均为苏木精–伊红染色(hematoxylin-eosin staining), 每张切片厚度为5 μm, 各采集3张。冰冻病理组织切片和血液标本采集后储存于实验室–40 °C冰箱, 备用。161例甲状腺疾病患者均来自温州地区, 乳头状甲状腺癌组男女比例为2:7, 年龄分布范围为14~81岁, 平均年龄为47.96±11.20岁; 滤泡型甲状腺腺瘤组男女比例为3:11, 年龄分布范围为17~73岁, 平均年龄为46.79±17.00岁; 结节性甲状腺肿组男女比例为8:33, 年龄分布范围为30~72岁, 平均年龄为49.41±10.60岁。同时收集患者临床病历资料, 包括年龄、性别、身高、体质量、其他病史和家族史、冰冻和石蜡病理诊断结果、肿块大小、癌转移与否和甲状腺相关激素水平(包括甲状腺素、三碘甲腺原氨酸、TSH、游离FT4、游离FT3等)。甲状腺癌以Tc为前缀, 甲状腺腺瘤以Tb为前缀, 结节性甲状腺肿以Th为前缀; 癌组织、腺瘤组织、结节组织均以t为后缀, 血液标本以b为后缀。

1.1.2 试剂 细胞裂解缓冲液: 10 mmol/L Tris-HCl(pH=8.0)、2 mmol/L EDTA(pH=8.0)、0.5% SDS(m/V)。红细胞裂解液: 20 mmol/L Tris-HCl(pH=7.6)。蛋白k、Ex Taq DNA Polymerase、DL2000 DNA Marker和Premix Ex Taq™ (Perfect Real Time)均购自宝生物工程(大连)有限公司。PCR反应引物由宝生物工程(大连)有限公司合成, 见表1, 琼脂糖购自BBI公司。

1.2 方法

1.2.1 癌组织、腺瘤组织及结节组织的鉴定 在病理医师的指导下, 用普通倒置光学显微镜观察甲状腺癌、甲状腺腺瘤及结节性甲状腺肿冰冻病理切片, 根据相应组织的细胞形态, 用记号笔在载玻片的反面对病变细胞聚集区做标记。

1.2.2 冰冻病理组织切片全基因组DNA提取法 (1)通过普通倒置光学显微镜下观察, 玻片的背面标出病变细胞丰富区; (2)加二甲苯滴在盖玻片周围, 小心揭掉盖玻片, 再用二甲苯冲洗几次, 用无水乙醇、75%乙醇及蒸馏水依次冲洗玻片, 通风处自然干燥; (3)移液枪吸取适量细胞裂解缓冲液滴到已标记好的区域, 用枪头小心刮取相应标本转入加400 μL细胞裂解缓冲液的离心管中, 然后加入3 μL蛋白酶k, 液体

混匀后放于56 °C恒温水浴箱中消化5~6 h; (4)将消化液冷却至室温, 加入预冷的醋酸钾240 μL, 用手充分振荡混匀后冰浴15 min, 然后4 °C, 18 000 r/min, 离心15 min, 取上清液, 加入预冷的无水异丙醇800 μL, 充分混匀后, 4 °C, 18 000 r/min, 离心10 min; (5)弃上清, 向管中加入75%乙醇800 μL, 颠倒混匀后, 4 °C, 18 000 r/min, 离心10 min; (6)弃上清, 室温下干燥40 min或65 °C干燥仪上干燥10 min, 使管中液体完全挥发; (7)加入高压灭菌蒸馏水50 μL, 65 °C干燥仪上溶解1 h或4 °C冰箱溶解过夜。

1.2.3 血液全基因组DNA提取法 (1)在1.5 mL离心管中加入200 μL全血标本, 每管中加入900 μL红细胞裂解液, 颠倒混匀后室温下放置10 min, 中间颠倒混匀几次; (2)18 000 r/min, 室温, 离心3 min, 弃上清, 向细胞沉淀中加入600 μL细胞裂解缓冲液, 然后加入3 μL蛋白酶k, 用枪头将液体混匀后放于56 °C恒温水浴箱中消化3~5 h; (3)将消化液冷却至室温, 经短暂离心后加入预冷的醋酸钾200 μL, 用手充分振荡混匀后冰浴10 min, 然后4 °C, 18 000 r/min, 离心3 min; (4)吸上清液加入600 μL异丙醇, 充分混匀后, 4 °C, 18 000 r/min, 离心1 min; (5)弃上清, 向管中加入75%乙醇800 μL, 颠倒混匀后, 室温, 18 000 r/min, 离心1min; (6)弃上清, 室温下干燥40 min或65 °C干燥仪上干燥10 min, 使管中液体完全挥发, 加入高压灭菌蒸馏水50 μL, 65 °C干燥仪上溶解1 h或4 °C冰箱溶解过夜。

1.2.4 mtDNA全基因测序和突变筛查 参照MITOMAP数据库中的mtDNA修正剑桥参考序列(revised Cambridge Reference Sequence, rCRS), 设计24对连续且首尾重叠的特异性引物PCR扩增mtDNA全序, 各引物序列详情见表1, 将提取的基因组进行PCR反应, 反应完后的产物再经过1%琼脂凝胶鉴定, 鉴定成功后, 电泳合格的PCR扩增产物送上海华

表1 PCR扩增mtDNA全序的24对引物

Table 1 24 pairs of primers which used for the amplification of the whole sequence mtDNA

引物 Primer	引物序列(5′→3′) Primer sequence (5′→3′)	引物 Primer	引物序列(5′→3′) Primer sequence (5′→3′)	扩增范围 Amplification range
1F	CTC CTC AAA GCA ATA CAC TG	1R	TGC TAA ATC CAC CTT CGA CC	611-1 411
2F	CGA TCA ACC TCA CCA CCT CT	2R	TGG ACA ACC AGC TAT CAC CA	1 245-2 007
3F	GGA CTA ACC CCT ATA CCT TCT GC	3R	GGC AGG TCA ATT TCA CTG GT	1 854-2 669
4F	AAA TCT TAC CCC GCC TGT TT	4R	AGG AAT GCC ATT GCG ATT AG	1 499-3 346
5F	TAC TTC ACA AAG CGC CTT CC	5R	ATG AAG AAT AGG GCG AAG GG	3 169-3 961
6F	TGG CTC CTT TAA CCT CTC CA	6R	AAG GAT TAT GGA TGC GGT TG	3 796-4 654
7F	ACT AAT TAA TCC CCT GGC CC	7R	CCT GGG GTG GGT TTT GTA TG	4 485-5 420
8F	CTA ACC GGC TTT TTG CCC	8R	ACC TAG AAG GTT GCC TGG CT	5 255-6 031
9F	GAG GCC TAA CCC CTG TCT TT	9R	ATT CCG AAG CCT GGT AGG AT	5 855-6 642
10F	CTC TTC GTC TGA TCC GTC CT	10R	AGC GAA GGC TTC TCA AAT CA	6 469-7 315
11F	ACG CCA AAA TCC ATT TCA CT	11R	CGG GAA TTG CAT CTG TTT TT	7 148-8 095
12F	ACG AGT ACA CCG ACT ACG GC	12R	TGG GTG GTT GGT GTA AAT GA	9 737-8 797
13F	TTT CCC CCT CTA TTG ATC CC	13R	GTG GCC TTG GTA TGT GCT TT	8 621-9 397
14F	CCC ACC AAT CAC ATG CCT AT	14R	TGT AGC CGT TGA GTT GTG GT	9 230-10 130
15F	TCT CCA TCT ATT GAT GAG GGT CT	15R	AAT TAG GCT GTG GGT GGT TG	9 989-10 837
16F	GCC ATA CTA GTC TTT GCC GC	16R	TTG AGA ATG AGT GTG AGG CG	10 672-11 472
17F	TCA CTC TCA CTG CCC AAG AA	17R	GGA GAA TGG GGG ATA GGT GT	11 314-12 076
18F	TAT CAC TCT CCT ACT TAC AG	18R	AGA AGG TTA TAA TTC CTA CG	11 948-12 772
19F	AAA CAA CCC AGC TCT CCC TAA	19R	TCG ATG ATG TGG TCT TTG GA	12 571-13 507
20F	ACA TCT GTA CCC ACG CCT TC	20R	AGA GGG GTC AGG GTT CAT TC	13 338-14 268
21F	GCA TAA TTA AAC TTT ACT TC	21R	AGA ATA TTG AGG CGC CAT TG	14 000-14 998
22F	TGA AAC TTC GGC TCA CTC CT	22R	AGC TTT GGG TGC TAA TGG TG	14 856-15 978
23F	TCA TTG GAC AAG TAG CAT CC	23R	GAG TGG TTA ATA GGG TGA TAG	15 811-5
24F	CAC CAT TCT CCG TGA AAT CA	24R	AGG CTA AGC GTT TTG AGC TG	16 420-775

引物以数字命名, F表示正向引物, R表示反向引物。

The primers are named with numbers, F for forward primers and R for reverse primers.

大生物有限公司测序。应用软件Codoncode Aligner 4.0.4, 并以*rCRS*作为参考序列对测序序列进行比对分析, 筛查体细胞mtDNA突变位点和mtDNA单核苷酸多态性位点。我们将序列变化仅发生于病变组织而不发生于外周血淋巴细胞中的位点定义为体细胞mtDNA突变位点(包括替换突变和插入缺失突变), 而将序列变化同时发生于病变组织和外周血淋巴细胞中的位点定义为mtDNA单核苷酸多态性位点。细胞内mtDNA分子全为突变型或野生型的状态称为同质性, 同时含有突变性和野生型的状态为异质性, 根据测序峰图即可判断mtDNA的同异质性突变。

1.2.5 线粒体单倍型鉴定 参考YAO等[9-10]给出的东亚人群系统发育树对所有患者进行线粒体单倍型鉴定, 并通过MITOMAP数据库进行验证。

1.2.6 氨基酸改变及物种间保守性分析 通过MITOMAP数据库(http://www.mitomap.org/bin/view.pl/MITOMAP/HumanMitoCode)中提供的人类线粒体遗传密码, 分析mtDNA突变造成的氨基酸改变。运用Cluxtal软件1.83版本比对人类mtDNA编码的13个呼吸链复合体亚基氨基酸序列和9个物种对应的mtDNA编码的氨基酸序列(NCBI数据库), 分析各变异氨基酸的种间进化保守性。9个物种分别为: 黑猩猩、大鼠、小鼠、家犬、牛、鸡、爪蟾、斑马鱼和果蝇。

1.2.7 蛋白编码区突变的分类及错义突变对其编码蛋白结构和功能影响的预测根据突变是否造成相应编码氨基酸的改变, 我们将蛋白编码区的mtDNA突变分为同义突变(突变未造成相应编码氨基酸的替换)和非同义突变(突变造成了相应编码氨基酸的替换), 后者又分为无义突变(突变导致编码相应氨基酸的密码子变成终止密码子, 从而造成蛋白多肽合成终止)、移码突变(插入或缺失突变导致其后串联氨基酸的替换)和错义突变(突变仅造成相应编码氨基酸的替换)。由于无义突变和移码突变均可对线粒体蛋白亚基的合成造成严重的影响, 所以将这两种突变定义为破坏性突变[11]。通过运用PyloPhen-2数据库对mtDNA错义突变造成相应蛋白结构和功能的影响进行预测, 我们得到三种结果: Benign、Possibly Damaging、Probably Damaging(http://genetics.bwh.harvard.edu/pph2/)。参照IVAN等[12]将Possibly damaging mutations和Probably damaging mutations统称为Potentially damaging mutations, 我们称之为潜在破坏性突变, Benign为良性突变。由于破坏性突变和潜在破坏性突变都将可能影响线粒体复合体亚基的功能, 因此, 我们将这两类突变统称为恶性突变, 而将同义突变和预测结果为Benign的mtDNA突变称为良性突变, 突变位点是否报道参考标准为MITOMAP数据库、mtDB数据库和Google搜索。

1.2.8 实时荧光定量PCR 荧光定量的反应体系总体积为20 μL: Premix Ex Taq™ 10 μL(2×); ROX Peference Dye 0.4 μL(50×); F/R引物各0.4 μL(10 μmol/L); TaqMan Probe 0.8 μL(5 μmol/L); ddH_2O_2 6 μL; 样品2 μL。40个循环, 反应条件: 95 ℃预变性10 s, 95 ℃变性5 s, 58 ℃延伸30 s。做7个标准品梯度(标准品由本课题组成员提供, 标准品浓度梯度为10^1、10^2、10^3、10^4、10^5、10^6、10^7拷贝数/μL和一个阴性对照, 标准曲线R^2的范围: 0.99~1.00, 扩增效率百分数EFF%的范围: 90%~110%。采用两步法, 使用ABI StepOne荧光定量PCR仪, 每个标本做2次重复, ND1拷贝数代表mtDNA的拷贝数, *β-actin*基因拷贝数用于校正细胞数。探针的5′端采用FAM染料标记, 3′端采用ECLIPSE染料标记, 荧光定量引物和探针见表2。

表2 荧光定量引物及探针

Table 2 The primers and probes for fluorescent quantitative

基因 Gene	引物序列位置 Sequence position	引物序列(5′→3′) Sequence (5′→3′)	基因长度/bp Gene length /bp	退火温度/℃ *T*m /℃
β-actin	F(1997—2017)	ACC CAC ACT GTG CCC ATC TAC	107	58
	R(2081—2103)	TCG GTG AGG ATC TTC ATG AGG TA		
	P(2025—2045)	ATG CCC TCC CCC ATG CCA TC		
ND1	F(3789—3811)	CCT CTC CAC CCT TAT CAC AAC AC	73	56
	R(3840—3861)	TCA TAT TAT GGC CAA GGG TCA T		
	P(3813—3831)	AGA ACA CCT CTG ATT ACT C		

F代表正向引物, R代表反向引物, P代表荧光定量探针, Tm为引物最佳退火温度。

F stands for forward primers, R for reverse primers and P for fluorescent quantitative probes, Tm is the best annealing temperature for primers.

1.2.9 统计学分析 应用SPSS 16.0统计软件, 用卡方检验和Fisher's确切概率检验统计甲状腺癌和良性甲状腺疾病组间mtDNA突变、线粒体单倍型分布及临床资料之间的差异; 用独立样本*t*检验统计mtDNA拷贝数的组间差异, $P<0.05$为有统计学意义。

2 结果

2.1 乳头状甲状腺癌、滤泡状甲状腺腺瘤和结节性甲状腺肿病人中mtDNA突变结果

测序和分析结果如表3~表5所示, 在92例乳头状甲状腺癌(papillary thyroid cancer, PTC)病人中, 发现有52例病人在复合体亚基编码区发生mtDNA突变(56.5%), 一共包含65个mtDNA突变位点, 有10例病人包含2个或以上mtDNA突变, 有12例发生了tRNA编码基因突变(13.0%),有5例发生了rRNA编码基因突变(5.4%),有3例病人在mtDNA的D-loop区发生突变。在28例滤泡状甲状腺腺瘤(follicular thyroid adenoma, FTA)病人中, 有11例病人在复合体亚基编码区发生mtDNA突变(39.2%), 共有13个mtDNA突变位点, 有2例病人带有2个mtDNA突变, 有3例发生

表3 甲状腺癌组mtDNA突变情况

Table 3 The mtDNA mutation in thyroid carcinoma

样本编号 Sample ID	基因突变 Gene mutation	保守性 IC	mtDNA突变 mtDNA change	氨基酸突变 Amino acid change	突变类型 Mutation type	破坏性 Destructiveness	报道 Reported
Tc253	ND1	/	3571insC	L89frameshift	Homogeneity	D	Yes
Tc381	ND1	/	3571insC	L89frameshift	Homogeneity	D	Yes
Tc386	ND1	/	3571insC	L89frameshift	Homogeneity	D	Yes
Tc400	ND1	/	3571insC	L89frameshift	Homogeneity	D	Yes
Tc254	ND1	/	G3842A	W179Ter	Homogeneity	D	Yes
Tc303	ND2	/	G4720A	W84Ter	Heterogeneity	D	Yes
Tc259	ND4	/	11038delA	K93frameshift	Homogeneity	D	Yes
Tc283	ND4	/	11038delA	K93frameshift	Homogeneity	D	Yes
Tc335	ND4	*	10952insC	L65frameshift	Homogeneity	D	Yes
Tc373	ND4	*	11872insC	P371frameshift	Homogeneity	D	Yes
Tc322	ND5	/	12384insT	I16frameshift	Homogeneity	D	Yes
Tc346	ND5	/	12382delA	I16frameshift	Homogeneity	D	No
Tc364	ND5	/	12425insA	N30frameshift	Homogeneity	D	Yes
Tc365	ND5	/	12425insA	N30frameshift	Homogeneity	D	Yes
Tc269	CO1	/	G6727A	W275Ter	Heterogeneity	D	No
Tc276	CO1	*	G6321A	G140Ter	Homogeneity	D	No
Tc323	CO1	*	G6573A	G224Ter	Heterogeneity	D	No
Tc330	CO1	*	G6708A	G269Ter	Homogeneity	D	Yes
Tc342	CO1	/	G6789A	G296Ter	Heterogeneity	D	No
TC397	CO1	*	6582insGAG	D227frameshift	Homogeneity	D	No
Tc277	CO2	*	G8153A	G190Ter	Heterogeneity	D	Yes
Tc341	CytB	*	G14888A	G48Ter	Heterogeneity	D	No
Tc286	ND1	*	C3416A	P37H	Homogeneity	Probably D	No
Tc296	ND1	*	G3380A	R25Q	Heterogeneity	Probably D	Yes
Tc319	CO2	/	G7604A	V7M	Homogeneity	Benign	Yes
Tc386	CO2	*	A7828G	L81L	Homogeneity	—	Yes
Tc363	ND1	*	G3481A	E59K	Homogeneity	Probably D	Yes
Tc378	ND1	*	C3680T	S125L	Homogeneity	Probably D	No
Tc325	ND2	/	G4818A	E117K	Heterogeneity	Probably D	No
Tc289	ND3	*	T10252C	F65S	Homogeneity	Probably D	No
Tc391	ND3	/	T10060C	M1T	Homogeneity	Possibly D	No
Tc348	ND4L	/	T10563C	C32R	Heterogeneity	Probably D	Yes
Tc251	ND4	*	G11423A	E222K	Homogeneity	Probably D	No

续表3

样本编号 Sample ID	基因突变 Gene mutation	保守性 IC	mtDNA突变 mtDNA change	氨基酸突变 Amino acid change	突变类型 Mutation type	破坏性 Destructiveness	报道 Reported
Tc261	ND4	/	T11046C	L96P	Homogeneity	Probably D	Yes
Tc266	ND4	/	T11613C	L285P	Homogeneity	Probably D	Yes
Tc319	ND4	/	G11711A	A318T	Heterogeneity	Probably D	No
Tc351	ND4	*	G11169A	G137D	Heterogeneity	Probably D	No
Tc296	ND4	*	T11441C	S228P	Heterogeneity	Probably D	No
Tc256	ND5	*	T13706C	L457P	Homogeneity	Probably D	No
Tc296	ND5	/	T12467G	F44C	Heterogeneity	Probably D	No
Tc300	ND5	/	G13804A	A490T	Homogeneity	Probably D	No
Tc281	ND5	*	C13250T	S305F	Homogeneity	Probably D	No
Tc384	ND5	*	G13471A	A379T	Homogeneity	Probably D	No
Tc342	ND5	/	T13685C	L450P	Heterogeneity	Probably D	No
Tc252	CO1	/	T6229C	L109P	Heterogeneity	Possibly D	No
Tc304	CO1	*	G6729A	A276T	Heterogeneity	Probably D	No
Tc370	CO1	*	G7108A	G402D	Heterogeneity	Probably D	No
Tc342	CO1	/	G7075A	G391E	Heterogeneity	Probably D	No
Tc356	CO1	*	G6958A	G352D	Homogeneity	Probably D	No
Tc262	CytB	*	T15144C	L133P	Homogeneity	Probably D	No
Tc315	CytB	*	G15135A	G130D	Homogeneity	Probably D	No
Tc331	CytB	*	G14973A	G76D	Homogeneity	Probably D	No
Tc373	CytB	/	T14979C	I78T	Heterogeneity	Possibly D	No
Tc311	ATP6	*	A9135T	E203D	Homogeneity	Probably D	No
Tc316	ATP6	/	T8966C	I147T	Homogeneity	Possibly D	Yes
Tc323	ND2	/	C4940T	L157L	Heterogeneity	—	Yes
Tc261	ND4L	/	G10653A	A62T	Homogeneity	Benign	Yes
Tc362	ND4	/	G11914A	T385T	Homogeneity	—	Yes
Tc281	ND6	/	T14326C	V116V	Homogeneity	—	No
Tc363	CO3	/	G9305A	M33M	Homogeneity	—	Yes
Tc300	CytB	*	G15093A	G116D	Homogeneity	Benign	No
Tc311	ATP6	*	A8784G	G86G	Homogeneity	—	Yes
Tc311	ATP6	/	C8829T	N101N	Homogeneity	—	Yes
Tc311	ATP6	/	G9053A	S176N	Homogeneity	Benign	Yes
Tc316	ATP6	/	A9120G	L198L	Homogeneity	—	Yes
Tc297	D-loop		G260A		Heterogeneity		Yes
Tc336	D-loop		T16093C		Heterogeneity		Yes
Tc348	D-loop		T152C		Heterogeneity		Yes
Tc302	16S rRNA		G1748A		Heterogeneity		No
Tc325	16S rRNA		2 463delA		Homogeneity		No
Tc335	16S rRNA		T2285C		Homogeneity		No
Tc363	12S rRNA		G1285T		Homogeneity		No
Tc400	16S rRNA		G3091A		Homogeneity		No
Tc251	tRNA-Asp		T7579C		Homogeneity		No
Tc297	tRNA-Gln		G4358A		Heterogeneity		No
Tc316	tRNA- Lys		T8306C		Heterogeneity		Yes
Tc329	tRNA-Val		G1606A		Heterogeneity		Yes
Tc338	tRNA-Asn		G5667A		Homogeneity		No
Tc339	tRNA-Leu(UUR)		A3243T		Heterogeneity		Yes
Tc349	tRNA-Leu(UUR)		G3 44A		Homogeneity		Yes
Tc367	tRNA-Ala		A5649G		Homogeneity		No

续表3

样本编号 Sample ID	基因突变 Gene mutation	保守性 IC	mtDNA突变 mtDNA change	氨基酸突变 Amino acid change	突变类型 Mutation type	破坏性 Destructiveness	报道 Reported
Tc370	tRNA-Lys		T8355C		Homogeneity		Yes
Tc384	tRNA-Leu(UUR)		G3244A		Heterogeneity		Yes
Tc397	tRNA-Met		G4412A		Homogeneity		No
Tc334	tRNA-Trp		T5574C		Heterogeneity		No

mtDNA变化栏中数字代表突变位点, 数字左右的字母分别代表突变前后的碱基。氨基酸改变栏中数字代表在相应亚基中的氨基酸位点, 数字左右边的字母分别为替换前后的氨基酸缩写。IC表示种间进化保守性, *为高度保守性位点, /为非高度保守的位点。D代表破坏性突变, Probably D和Possibly D代表潜在破坏性突变, Benign为良性突变, “—”为同义突变。

In the mtDNA change column, the number represents the mutation site, and the letters around the number represent the base before and after the mutation. The number in the amino acid change column represents the amino acid sites in the corresponding subunits, and the letters on the left and right sides of the numbers are amino acid abbreviations before and after replacement, respectively. IC indicates the conservatism of interspecific evolution, * is a highly conserved site, and / is a non-highly conserved site. D represents destructive mutations, probably D and possibly D represent potential destructive mutations, Benign is benign mutations, and “—” is synonymous with synonymous mutations.

表4 滤泡状甲状腺腺瘤中mtDNA突变

Table 4 The mtDNA mutation in follicular thyroid adenoma

样本编号 Sample ID	基因突变 Gene mutation	保守性 IC	MtDNA突变 MtDNA change	氨基酸突变 Amino acid change	突变类型 Mutation type	破坏性 Destructiveness	报道 Reported
Tb131	ND1	/	3 571insC	L89frameshift	Homogeneity	D	Yes
Tb103	ND2	*	G4 974A	G169Ter	Homogeneity	D	No
Tb105	ND3	/	G10 288A	W77Ter	Homogeneity	D	No
Tb142	ND4	*	11 872insC	P371frameshift	Homogeneity	D	Yes
Tb116	ND5	/	12 425insA	N30frameshift	Homogeneity	D	Yes
Tb135	ND1	*	G3 910A	E202K	Heterogeneity	Probably D	Yes
Tb141	ND1	/	G3 955A	A217T	Homogeneity	Probably D	No
Tb124	ND5	/	T12 638C	M101T	Heterogeneity	Probably D	No
Tb111	CytB	/	T14 748C	M1T	Heterogeneity	Possibly D	No
Tb142	ATP6	/	T8 789C	L88P	Homogeneity	Probably D	No
Tb141	ND4	/	T11 827C	A356A	Homogeneity	—	Yes
Tb136	ND6	/	G14 384A	A97V	Homogeneity	Benign	Yes
Tb139	CO1	*	C7 196A	L251L	Homogeneity	—	Yes
Tb108	D-loop		C16 111A		Heterogeneity		Yes
Tb105	16S rRNA		T2 352C		Homogeneity		
Tb111	12S rRNA		T1 371C		Heterogeneity		
Tb142	12S rRNA		G1 393A		Homogeneity		

mtDNA变化栏中数字代表突变位点, 数字左右的字母分别代表突变前后的碱基。氨基酸改变栏中数字代表在相应亚基中的氨基酸位点, 数字左右边的字母分别为替换前后的氨基酸缩写。IC表示种间进化保守性, *为高度保守性位点, /为非高度保守的位点。D代表破坏性突变, Probably D和Possibly D代表潜在破坏性突变, Benign为良性突变, “—”为同义突变。

In the mtDNA change column, the number represents the mutation site, and the letters around the number represent the base before and after the mutation. The number in the amino acid change column represents the amino acid sites in the corresponding subunits, and the letters on the left and right sides of the numbers are amino acid abbreviations before and after replacement, respectively. IC indicates the conservatism of interspecific evolution, * is a highly conserved site, and / is a non-highly conserved site. D represents destructive mutations, probably D and possibly D represent potential destructive mutations, Benign is benign mutations, and “—” is synonymous with synonymous mutations.

了rRNA编码基因突变(10.7%),有1例病人在mtDNA的D-loop区发生突变。在41例结节性甲状腺肿病人的对照组(nodular goiter, NG)中, 有11例病人的mtDNA在复合体亚基编码区发生突变(26.8%), 共有13个mtDNA突变位点, 有2例病人带有2个mtDNA突变位点。此外, 有1例病人在D-loop区发生突变。

在PTC组的65个mtDNA突变中, 发现22个破坏

表5 结节性甲状腺肿中mtDNA突变情况

Table 5 The mtDNA mutation in nodular goiter

样本编号 Sample ID	基因突变 Gene mutation	保守性 IC	MtDNA突变 MtDNA change	氨基酸突变 Amino acid change	突变类型 Mutation type	破坏性 Destructiveness	报道 Reported
Th158	ND1	/	3 571 insC	L89frameshift	Homogeneity	D	Yes
Th211	ND2	/	5 063delT	P198frameshift	Homogeneity	D	No
Th150	ND3	/	10 299insT	T81frameshift	Homogeneity	D	No
Th203	CO1	*	G6 280A	W126Ter	Homogeneity	D	No
Th218	ND3	/	G10 197A	A47T	Heterogeneity	Probably D	Yes
Th228	ND4	*	G11 562A	G268D	Homogeneity	Probably D	No
Th203	ND5	/	A12 536T	H67L	Homogeneity	Possibly D	No
Th159	ND6	/	T14 378C	E99G	Heterogeneity	Probably D	No
Th122	CO3	/	T9 840C	S212P	Homogeneity	Probably D	No
Th228	CytB	/	T15 132C	M129T	Homogeneity	Probably D	No
Th213	ND2	/	G5 417A	Q313Q	Homogeneity	—	Yes
Th225	CO1	/	G5 970A	G23S	Heterogeneity	Benign	Yes
Th231	CO2	*	T8 119C	R178R	Homogeneity	—	Yes
Th113	D-loop		G203A		Homogeneity		Yes

mtDNA变化栏中数字代表突变位点, 数字左右的字母分别代表突变前后的碱基。氨基酸改变栏中数字代表在相应亚基中的氨基酸位点, 数字左右边的字母分别为替换前后的氨基酸缩写。IC表示种间进化保守性, *为高度保守性位点, /为非高度保守的位点。D代表破坏性突变, Probably D和Possibly D代表潜在破坏性突变, Benign为良性突变, “—”为同义突变。

In the mtDNA change column, the number represents the mutation site, and the letters around the number represent the base before and after the mutation. The number in the amino acid change column represents the amino acid sites in the corresponding subunits, and the letters on the left and right sides of the numbers are amino acid abbreviations before and after replacement, respectively. IC indicates the conservatism of interspecific evolution, * is a highly conserved site, and / is a non-highly conserved site. D represents destructive mutations, probably D and possibly D represent potential destructive mutations, Benign is benign mutations, and “—”is synonymous with synonymous mutations.

性突变和31个潜在性破坏突变, 共53个恶性突变, 其中22个破坏性突变为16个同质性和6个异质性, 有8个在高度保守位点, 31个潜在破坏性突变有13个异质性和18个同质性, 有18个在高保守位点。在FTA组的13个mtDNA突变中, 发现5个破坏性突变和5个潜在性破坏突变, 共10个恶性突变,有1人同时患有破坏性突变和潜在破坏性突变, 其中5个破坏性突变均为同质性, 并且有2个在高度保守位点, 5个潜在性破坏突变3个异质性和2个同质性, 有1个在高度保守位点。在NG组的13个mtDNA突变中, 有4个破坏性突变和6个潜在性破坏突变, 共10个恶性突变, 有1人同时患有破坏性突变和潜在性破坏突变。4个破坏性突变均为同质性, 并有1个在高度保守位点, 而6个潜在性破坏突变中有2个异质性突变和4个同质性突变, 有1个在高度保守位点。

2.2 mtDNA突变在乳头状甲状腺癌、滤泡状甲状腺腺瘤及结节性甲状腺肿3组间的比较分析

我们通过卡方检验对3组病人的mtDNA突变进行统计学分析, 结果(表6和表7)显示, 乳头状甲状腺癌病人的mtDNA在13个复合体亚基编码区和tRNA编码区的突变率明显高于结节性甲状腺肿病人的突变率。乳头状甲状腺癌的mtDNA的突变率与滤泡状甲状腺腺瘤的mtDNA相比也存在上升的趋势。而在D-loop区, 3组病人的mtDNA突变并没有明显统计学差异。因此, 我们进一步分析3组病人的mtDNA在不同的复合体亚基编码区发生突变的情况, 我们发现, 乳头状甲状腺癌组和结节性甲状腺肿组的mtDNA在编码复合体I亚基区的突变率存在统计学差异, 乳头状甲状腺癌的mtDNA突变率要高于结节性甲状腺肿病人, 而在其他复合体亚基编码区, 3组病人之间并没有发现差异。

2.3 甲状腺结节、甲状腺腺瘤和甲状腺癌病人线粒体单倍型及组间比较

参照东亚人群系统发育树, 根据筛查到的mtDNA单核苷酸多态性位点, 我们对所有研究对象进行了线粒体单体型的划分, 然后对乳头状甲状腺癌、滤泡状甲状腺腺瘤及结节性甲状腺肿3组间线粒体单体型分布进行两两比较, 结果见表8~表10。M单体型下的亚单体型CZ在乳头状甲状腺癌和结节性甲状腺肿中的分布存在统计学差异(P=0.031), 其余线粒体单

表6 PTC、FTA和NG之间mtDNA突变比较

Table 6 The mtDNA mutations between PTC, FTA and NG

突变区域 Mutation region	PTC (*n*=92)	FTA (*n*=28)	NG (*n*=41)	*P*
mtDNA	58 (63.0%)	12 (42.9%)	12 (29.3%)	0.244 5[a]
				0.000 3***[b]
				0.057 8[c]
Complex coding region	52 (56.5%)	11 (39.2%)	11 (26.8)	0.275 6[a]
				0.001 5**[b]
				0.109 8[c]
tRNA coding region	12 (13.0%)	0 (0%)	0 (0%)	-
				0.015 3*[b]
				0.044 0*[c]
rRNA coding region	5 (5.4%)	3 (10.7%)	0 (0%)	0.032 1*[a]
				0.128 1[b]
				0.326 8[c]
D-loop region	3 (3.2%)	1 (3.6%)	1 (2.4%)	0.802 8[a]
				0.812 6[b]
				0.945 7[c]

PTC为甲状腺癌, FTA为甲状腺腺瘤, NG为正常对照, *n*代表样本数, ()内的数字为占总人数的百分比, -表示没有意义, 应用卡方检验和 Fisher's确切概率检验进行统计分析, *P*<0.05为有统计学意义, *代表*P*<0.05, **代表*P*<0.01, ***代表*P*<0.001, a为NG *vs* FTA, b为NG *vs* PTC, c为PTC *vs* FTA。

PTC is papillary thyroid cancer, FTA is follicular thyroid adenomas, NG is nodular goiter, and *n* represents the number of samples. "the number of ()" stand for percentage of total number. "-" indicates that there is no significance. Chi-square test and fisher's exact probability test are used for statistical analysis. *P*<0.05 is statistically significant, * *P*<0.05, ** *P*<0.01, ****P*<0.001, a stand for NG *vs* FTA, b for NG *vs* PTC, c for PTC *vs* FTA.

表7 PTC、FTA和NG之间4类呼吸链复合体亚基编码区mtDNA突变

Table 7 The mtDNA mutation in the coding region of four kinds of respiratory chain complex subunits between PTC, FTA and NG

突变区域 Mutation region	PTC (*n*=92)	FTA (*n*=28)	NG (*n*=41)	*P*
Complex I	34 (37.2%)	9 (34.5%)	8 (19.5%)	0.231 8[a]
				0.045 7*[b]
				0.641 8[c]
Complex III	6 (7.4%)	1 (3.4%)	1 (2.4%)	0.783 1[a]
				0.330 2[b]
				0.559 7[c]
Complex IV	14 (16.0%)	1 (3.4%)	4 (9.8%)	0.330 5[a]
				0.395 2[b]
				0.102 8[c]
Complex V	2 (2.1%)	1 (3.4%)	0 (0.0%)	0.222 9[a]
				0.341 5[b]
				0.678 3[c]

PTC为甲状腺癌, FTA为甲状腺腺瘤, NG为正常对照, *n*代表样本数, ()内的数字为占总人数的百分比, 应用卡方检验和Fisher's确切概率检验进行统计分析, *P*<0.05为有统计学意义, *代表*P*<0.05, a为NG *vs* FTA, b为NG *vs* PTC, c为PTC *vs* FTA。

PTC is papillary thyroid cancer, FTA is follicular thyroid adenomas, NG is nodular goiter, and *n* represents the number of samples. "the number of ()" stand for percentage of total number. Chi-square test and fisher's exact probability test are used for statistical analysis. *P*<0.05 is statistically significant, **P*<0.05, a stand for NG *vs* FTA, b for NG *vs* PTC, c for PTC *vs* FTA.

体型分布在2组间均无统计学差异; 乳头状甲状腺癌和滤泡状甲状腺腺瘤间及滤泡状甲状腺腺瘤和结节性甲状腺肿间线粒体单体型分布均无统计学差异。

2.4 mtDNA突变与乳头状甲状腺癌患者临床病历资料间的相关性分析

为了探讨mtDNA突变是否与甲状腺癌患者临

表8 PTC和FTA两组间线粒体单体型分布的比较分析

Table 8 The mtDNA haplotype between PTC and FTA

mtDNA单倍型 MtDNA haplotype	PTC (*n*=92)	FTA (*n*=28)	*P*
M	36 (39.1%)	14(50.0%)	0.307
M7	7 (7.6%)	2 (7.1%)	1.000
M7b	5 (5.4%)	1 (3.6%)	1.000
M7c	2 (2.2%)	1 (3.6%)	0.553
D	19 (20.7%)	7 (25.0%)	0.625
D5	4 (4.3%)	2 (7.1%)	0.623
D5a	3 (3.3%)	1 (3.6%)	1.000
D4	15 (16.3%)	5 (17.9%)	0.781
D4a	6 (6.5%)	1 (3.6%)	1.000
D4b	7 (7.6%)	0 (0.0%)	0.198
M8	3 (3.3%)	2 (7.1%)	0.331
CZ	1 (1.1%)	0 (0.0%)	1.000
M8a	2 (2.2%)	2 (7.1%)	0.232
M9	4 (4.3%)	0 (0.0%)	0.572
M10	0 (0%)	1 (3.6%)	0.233
G	1 (1.1%)	2 (7.1%)	0.135
N	56 (60.9%)	14 (50.0%)	0.307
R	40 (43.5%)	11 (39.3%)	0.694
B	21 (22.8%)	8 (28.6%)	0.534
B4	12 (13.0%)	6 (21.4%)	0.363
B5	9 (9.8%)	2 (7.1%)	1.000
R9	18 (19.6%)	3 (10.7%)	0.397
F	17 (18.5%)	3 (10.7%)	0.401
F1	4 (4.3%)	1 (3.6%)	1.000
F2	8 (8.7%)	2 (7.1%)	1.000
N9	10 (10.9%)	2 (7.1%)	0.730
N9a	10 (10.9%)	1 (3.6%)	0.454
Y	0 (0.0%)	1 (3.6%)	0.233
A	6 (6.5%)	1 (3.6%)	1.000

PTC为甲状腺癌, FTA为甲状腺腺瘤, *n*代表样本数, ()内的数字为占总人数的百分比, 应用卡方检验和Fisher's确切概率检验进行统计分析, $P<0.05$为有统计学意义。

PTC is papillary thyroid cancer, FTA is follicular thyroid adenomas and *n* represents the number of samples. "the number of ()" stand for percentage of total number. Chi-square test and fisher's exact probability test are used for statistical analysis. $P<0.05$ is statistically significant.

床表现存在一定的相关性, 我们收集了患者的临床病历料, 包括年龄、性别、肿块大小、是否发生淋巴节转移及促甲状腺激素水平(thyroid stimulating hormone, TSH)等, 并将它们与mtDNA突变进行了相关性分析, 表11和表12为甲状腺癌mtDNA突变与临床资料的统计分析结果, 结果显示, 甲状腺癌mtDNA突变与患者临床资料间并不存在显著的相关性。

2.5 线粒体单体型与乳头状甲状腺癌患者临床病历资料间的相关性分析

为了探讨线粒体单体型是否与乳头状甲状腺癌患者临床表现存在一定的相关性, 我们也对乳头状甲状腺癌患者线粒体单体型与其临床资料进行了相关性统计分析, 结果如表13所示, 线粒体单体型与淋巴结转移存在显著的相关性, 线粒体单体型M的甲状腺癌患者相对于线粒体单倍型N的甲状腺癌患者有更低的淋巴结转移率。

2.6 乳头状甲状腺癌、滤泡状甲状腺腺瘤及结节性甲状腺肿患者组织标本和血液标本中mtDNA拷贝数的检测

为了比较分析乳头状甲状腺癌、滤泡状甲状

表9 PT和NG两组间线粒体单体型分布的比较分析

Table 9 The mtDNA haplotype between PTC and NG

mtDNA单倍型 Mt DNA haplotype	PTC (*n*=92)	NG (*n*=41)	*P*
M	36 (39.1%)	17(41.5%)	0.800
M7	7 (7.6%)	1 (2.4%)	0.434
M7b	5 (5.4%)	0 (0.0%)	0.323
M7c	2 (2.2%)	1 (2.4%)	1.000
D	19 (20.7%)	10 (24.4%)	0.630
D5	4 (4.3%)	3 (7.3%)	0.676
D5a	3 (3.3%)	1 (2.4%)	1.000
D4	15 (16.3%)	7 (17.1%)	0.912
D4a	6 (6.5%)	1 (2.4%)	0.436
D4b	7 (7.6%)	1 (2.4%)	0.434
M8	3 (3.3%)	5 (12.2%)	0.106
CZ	1 (1.1%)	4 (9.8%)	0.031*
M8a	2 (2.2%)	1 (2.4%)	1.000
M9	4 (4.3%)	0 (0.0%)	0.311
G	1 (1.1%)	1 (2.4%)	0.523
N	56 (60.9%)	24 (58.5%)	0.800
R	40 (43.5%)	20 (48.8%)	0.570
B	21 (22.8%)	11 (26.8%)	0.618
B4	12 (13.0%)	4 (9.8%)	0.775
B5	9 (9.8%)	7 (17.1%)	0.256
R9	18 (19.6%)	9 (22.0%)	0.752
F	17 (18.5%)	9 (22.0%)	0.641
F1	4 (4.3%)	3 (7.3%)	0.676
F2	8 (8.7%)	4 (9.8%)	1.000
N9	10 (10.9%)	3 (7.3%)	0.754
N9a	10 (10.9%)	2 (4.9%)	0.342
Y	0 (0.0%)	1 (2.4%)	0.308
A	6 (6.5%)	1 (2.4%)	0.436

PTC为甲状腺癌, NG为正常对照, *n*代表样本数, ()内的数字为占总人数的百分比, 应用卡方检验和Fisher's确切概率检验进行统计分析, *P*<0.05为有统计学意义, **P*<0.05。

PTC is papillary thyroid cancer, NG is nodular goiter and *n* represents the number of samples. "the number of ()" stand for percentage of total number. Chi-square test and fisher's exact probability test are used for statistical analysis. *P*<0.05 is statistically significant, **P*<0.05.

腺腺瘤和结节性甲状腺肿3组间组织和血液标本中mtDNA拷贝数的差异, 我们通过SPSS 16.0统计软件, 应用独立样本*t*检验, 分别对乳头状甲状腺癌、滤泡状甲状腺腺瘤和结节性甲状腺肿3组间组织标本及血液标本中mtDNA拷贝数进行了统计分析, 结果如图1所示, 乳头状甲状腺癌患者癌组织中mtDNA拷贝数及滤泡状甲状腺腺瘤患者腺瘤组织中mtDNA拷贝数均显著高于结节性甲状腺肿患者结节组织中mtDNA拷贝数, *P*值分别为0.003和0.042(*P*<0.05具有统计学意义); 乳头状甲状腺癌患者癌组织中mtDNA拷贝数与滤泡状甲状腺腺瘤患者腺瘤组织中mtDNA拷贝数无统计学差异。乳头状甲状腺癌患者血液中mtDNA拷贝数及滤泡状甲状腺腺瘤患者血液中mtDNA拷贝数均显著低于结节性甲状腺肿患者血液中mtDNA拷贝数, P值分别为0.034和0.004(*P*<0.05有统计学意义); 乳头状甲状腺癌患者血液中mtDNA拷贝数与滤泡状甲状腺腺瘤患者血液中mtDNA拷贝数无统计学差异。

3 讨论

甲状腺癌是内分泌系统中常见的恶性肿瘤, 约占全球癌症新发病例的1%, 在过去的几十年中, 甲

表10 FTA和NG线粒体单体型比较

Table 10 The mtDNA haplotype between FTA and NG

mtDNA单倍型 Mt DNA haplotype	FTA (*n*=28)	NG (*n*=41)	*P*
M	14 (50.0%)	17 (41.5%)	0.484
M7	2 (7.1%)	1 (2.4%)	0.562
M7b	1 (3.6%)	0 (0.0%)	0.406
M7c	1 (3.6%)	1 (2.4%)	1.000
D	7 (25.0%)	10 (24.4%)	0.954
D5	2 (7.1%)	3 (7.3%)	1.000
D5a	1 (3.6%)	1 (2.4%)	1.000
D4	5 (17.9%)	7 (17.1%)	1.000
D4a	1 (3.6%)	1 (2.4%)	1.000
D4b	0 (0.0%)	1 (2.4%)	1.000
M8	2 (7.1%)	5 (12.2%)	0.693
CZ	0 (0.0%)	4 (9.8%)	0.141
M8a	2 (7.1%)	1 (2.4%)	0.562
M10	1 (3.6%)	0 (0.0%)	0.406
G	2 (7.1%)	1 (2.4%)	0.562
N	14 (50.0%)	24 (58.5%)	0.484
R	11 (39.3%)	20 (48.8%)	0.436
B	8 (28.6%)	11 (26.8%)	0.874
B4	6 (21.4%)	4 (9.8%)	0.296
B5	2 (7.1%)	7 (17.1%)	0.294
R9	3 (10.7%)	9 (22.0%)	0.335
F	3(10.7%)	9 (22.0%)	0.335
F1	1 (3.6%)	3 (7.3%)	0.641
F2	2 (7.1%)	4 (9.8%)	1.000
N9	2 (7.1%)	3 (7.3%)	1.000
N9a	1 (3.6%)	2 (4.9%)	1.000
Y	1 (3.6%)	1 (2.4%)	1.000
A	1 (3.6%)	1 (2.4%)	1.000

FTA为甲状腺腺瘤, NG为正常对照, *n*代表样本数, ()内的数字为占总人数的百分比, 应用卡方检验和Fisher's确切概率检验进行统计分析, *P*<0.05为有统计学意义。

FTA is follicular thyroid adenomas, NG is nodular goiter, and *n* represents the number of samples. "the number of ()" stand for percentage of total number. Chi-square test and fisher's exact probability test are used for statistical analysis. *P*<0.05 is statistically significant.

状腺癌的发病率在全球都有明显上升趋势, 其发病率在女性中明显增加[13], 严重威胁着人民的生命健康, 甲状腺癌是一种多因素疾病, 其病因仍不明确, 发病因素包括异常碘饮食、接触放射物质、促甲状腺激素及雌激素等激素刺激、遗传因素, 也可由结节性甲状腺肿及甲状腺腺瘤等良性甲状腺疾病发展演变而来。

线粒体是哺乳动物细胞中唯一携带核外遗传物质的细胞器, mtDNA为全长16 569 bp的闭合环状双链DNA分子, 它编码了37个线粒体基因, 包括13条呼吸复合体亚基编码基因、2个rRNA和22个tRNA编码基因。除了37个编码基因外, mtDNA还含有调控mtDNA复制和转录的非编码区(D-loop区)。由于长期浸润于线粒体内高浓度的ROS中, 同时缺乏组蛋白的保护和有效的mtDNA修复系统, mtDNA极易受到氧化损伤而发生突变, 其突变发生率约为细胞核DNA突变发生率的10~20倍, 近年来已有大量的研究表明, mtDNA突变普遍存在于包括甲状腺肿瘤在内的多种人类肿瘤中, MAXIMO等[14]早期发现, 甲状腺肿瘤中体细胞mtDNA突变发生率为51.5%(34/66), 并且mtDNA编码的线粒体复合体I基因突变与甲状腺恶性肿瘤存在显著的相关

表11 乳头状甲状腺癌患者的mtDNA突变与临床资料间的相关性

Table 11 The correlation between mtDNA mutation and clinical data in patients with papilla thyroid carcinoma

参数 Parameter	全部突变 Total mutation		*P*	复合体突变 Complexs mutation		*P*	tRNAs突变 tRNAs mutation		*P*
	+	-		+	-		+	-	
Gender									
Male	13	7	0.838	13	7	0.280	2	18	1.000
Female	45	27		37	35		10	62	
Age									
≥50	22	16	0.391	19	19	0.482	4	34	0.755
<50	36	18		31	23		8	46	
Lymphatic metastasis									
Yes	22	13	0.977	23	14	0.282	6	29	0.525
No	36	21		29	28		6	51	
Tumor size									
≥1.0cm	42	25	0.908	38	29	0.455	9	58	1.000
<1.0cm	16	9		12	13		3	22	
TSH									
Normal	46	27	1.000	39	34	1.000	9	64	1.000
Abnormal	6	4		5	5		1	9	
Unknow	6	3		6	3		2	7	

应用卡方检验和Fisher's确切概率检验进行统计分析, "+"为存在突变, "-"为不存在突变。*P*<0.05有统计学意义。

Chi-square test and fisher's exact probability test were used for statistical analysis, "+"for the existence of mutation, "-"for no mutation, *P*<0.05 has statistical significance.

表12 乳头状甲状腺癌患者mtDNA突变类型与临床资料间的相关性

Table 12 The correlation between mtDNA mutation type and clinical data in patients with papilla thyroid carcinoma

参数 Parameter	恶性突变 Malignant		*P*	破坏性突变 Destructive		*P*	潜在破坏性突变 Potential destructive		*P*
	+	-		+	-		+	-	
Gender									
Male	12	8	0.839	4	16	0.772	8	12	0.293
Female	45	27		18	54		20	52	
Age									
≥50	21	17	0.267	9	29	0.966	9	29	0.238
<50	36	18		13	41		19	35	
L ymphatic metastasis									
Yes	22	13	0.889	8	27	0.852	13	22	0.273
No	35	22		14	43		15	42	
Tumor size									
≥1.0cm	42	26	0.877	17	50	0.591	25	44	0.184
＜1.0cm	15	10		5	20		5	20	
TSH									
Normal	45	28	1.000	19	54	0.438	22	51	0.717
Abnormal	6	4		1	9		4	6	
Unknow	6	3		2	7		2	7	

应用卡方检验和Fisher's确切概率检验进行统计分析, "+"为存在突变, "-"为不存在突变。*P*<0.05有统计学意义。

Chi-square test and fisher's exact probability test were used for statistical analysis, "+" for the existence of mutation, "-" for no mutation, *P*<0.05 has statistical significance.

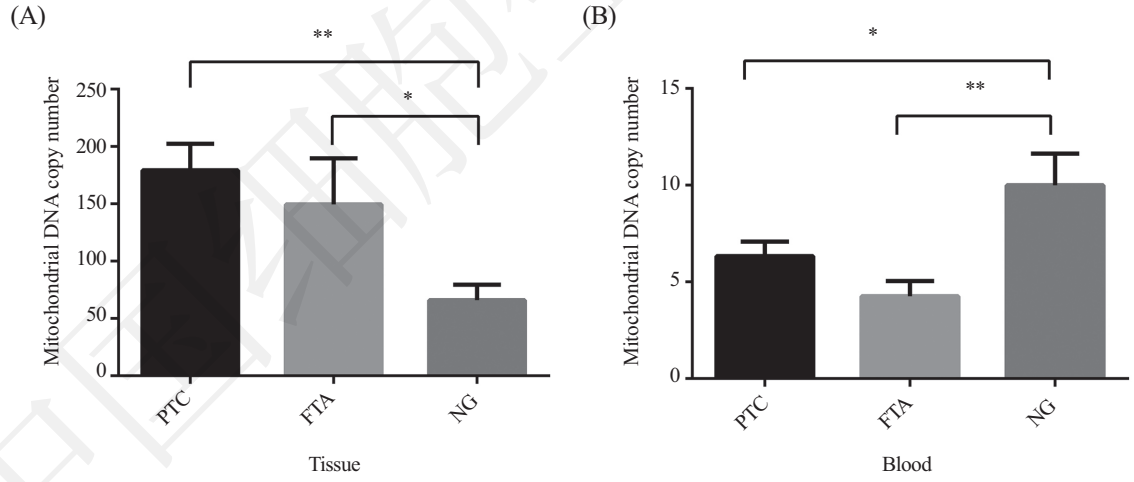
表13 甲状腺癌患者的线粒体单倍型与临床资料间的相关性

Table 13 The correlation between mtDNA haplotype and clinical data in patients with thyroid carcinoma

参数 Parameter	mtDNA单倍型 mtDNA haplotype		*P*
	M (*n*=36)	N (*n*=56)	
Gender			
Male	9	11	0.543
Female	27	45	
Age			
≥50	15	23	0.955
<50	21	33	
lymphatic metastasis			
Yes	9	26	0.039*
No	27	30	
Tumor size			
≥1.0cm	26	41	0.917
<1.0cm	10	15	
TSH			
Normal	25	48	1.000
Abnormal	3	7	
Unknow	8	1	

应用卡方检验和Fisher's确切概率检验进行统计分析, "+"为存在突变, "-"为不存在突变。*P*<0.05有统计学意义, *代表有统计学差异。

Chi-square test and fisher's exact probability test were used for statistical analysis, "+"for the existence of mutation, "-"for no mutation, *P*<0.05 has statistical significance, * represents statistical difference.



A: PTC、FTA和NG病人组织中的mtDNA拷贝数; B: PTC、FTA和NG病人血液中的mtDNA拷贝数。*P<0.05, **P<0.01。

A: the mtDNA copy number in the tissue of patients with PTC, FTA and NG; B: the mtDNA copy number in the blood of patients with PTC, FTA and NG. *P<0.05, **P<0.01.

图1 PTC、FTA和NG病人的组织和血液中mtDNA拷贝数

Fig.1 The mtDNA copy number in the tissue and blood of patients with PTC, FTA and NG

性, ABUAMERO等[15]对19例乳头状甲状腺癌标本进行mtDNA突变筛查发现, 有7例携带mtDNA突变(36.8%), 其中大部分突变都发生在复合体I编码基因中。CONTI等[16]在甲状腺肿瘤中发现, mtDNA的4977 bp片段的缺失, mtDNA拷贝数的改变能够增加患甲状腺癌的风险[17], 而在中国胃癌患者中发现了大量的mtDNA的D-loop区的突变, 这些突变能够促进胃癌的进展[18], 使得mtDNA改变有望成为癌症早

期诊断的一个重要标记物, 这些研究让人们对于甲状腺癌和mtDNA突变之间的关系产生浓厚的兴趣。

虽然线粒体与甲状腺肿瘤的研究取得了很大进展, 但这些研究均局限于小样本量及mtDNA中的部分编码区或非编码区, 此外, 线粒体遗传存在很大的地域差异。为了全面的探讨温州地区乳头状甲状腺癌与mtDNA突变的关系, 本课题在温州地区收集了92例乳头状甲状腺癌、28例滤泡状甲状腺腺瘤和41例结节性甲状腺肿患者的术后冰冻病理组织切片和外周静脉血标本, 同时收集相应的临床病历资料。对所有标本进行mtDNA全序扩增并测序, 然后进行线粒体基因组突变和多态性筛查, 分析mtDNA突变在整个线粒体基因组中的分布情况, 应用东亚人群系统发育树并根据筛查的多态性位点对所有甲状腺疾病患者进行线粒体单体型划分, 然后统计分析乳头状甲状腺癌、滤泡状甲状腺腺瘤和结节性甲状腺肿3组间mtDNA突变、mtDNA单体型分布及临床病历资料间的联系。

我们的研究发现, 在乳头状甲状腺癌、滤泡状甲状腺腺瘤和结节性甲状腺肿三组中, 甲状腺癌患者呼吸复合体亚基编码区mtDNA的突变率(56.5%)和恶性突变率(52.2%)分别高于结节性甲状腺肿的突变率(26.8%)和恶性突变率(19.5%), 并且, 线粒体复合体I基因编码区是3组mtDNA突变的好发部位, 甲状腺癌的突变率(37.2%)和恶性突变率(34.8%)分别高于结节性甲状腺肿的突变率(19.5%)和恶性突变率(17.1%), 统计学分析有显著差异。从以上结果可推测, 呼吸链复合体编码区mtDNA突变, 尤其是恶性突变, 可能跟乳头状甲状腺癌的发生发展相关, 这种相关性更进一步体现在线粒体复合体I编码区mtDNA恶性突变中, 这与 ABU-AMERO等[15]的研究发现是一致的。13个呼吸复合体亚基编码基因组成了整个mtDNA分子的70%左右, 这是成为mtDNA突变高发区的重要因数。复合体I是电子进入呼吸链的限速酶, 也是最大的线粒体呼吸复合体, 同时也是线粒体内产生ROS的主要部位, ROS可造成mtDNA的氧化损伤[19], 使得碱基发生突变和缺失[20-21], 这些可能是mtDNA突变好发于复合体I的原因, 而肿瘤细胞中ROS含量往往增加, 这就提高了肿瘤细胞的mtDNA突变的机率。

值得注意的是, 在92例乳头状甲状腺癌患者中检出12例患者发生了tRNA基因编码区mtDNA突变(13%), 但28例滤泡状甲状腺癌和41例结节性甲状腺肿患者tRNA基因编码区mtDNA突变的检出率均为0, 乳头状甲状腺癌和结节性甲状腺肿两组间tRNA编码区mtDNA突变率存在统计学差异, *P*值为0.015 3, 由此推测tRNA基因编码区mtDNA突变与乳头状甲状腺癌存在一定的相关性, tRNA编码基因的突变可能在乳头状甲状腺癌的发生发展中起着一定的修饰作用。线粒体编码的22个tRNA均在线粒体蛋白合成中起着不可或缺的作用, 发生在这些tRNA编码区的基因突变可能通过改变线粒体的生物合成来介导肿瘤的发生发展, 但仍需要进一步的功能研究加以证实。

近年来, 线粒体DNA非编码区D-loop区被视为mtDNA突变发生的热点区域, 已有大量研究报道, 口腔癌[22]、乳腺癌[23]、胃癌[24]、结直肠癌[25]等癌症类型中存在高频率的mtDNA的D-loop区点突变、插入缺失突变及线粒体微卫星不稳定性。然而, 我们的研究发现, 92例乳头状甲状腺癌中仅有3例发生了mtDNA的D-loop区点突变(3.3%), 28例滤泡状甲状腺腺瘤中仅有1例发生了mtDNA的D-loop区点突变(3.6%), 41例结节性甲状腺肿中仅有1例发生了mtDNA的 D-loop区点突变(2.4%), 可见乳头状甲状腺癌、滤泡状甲状腺腺瘤和结节性甲状腺肿中mtDNA的D-loop区突变发生率都比较低。可能是不同的癌症类型mtDNA突变分布存在一定的差异, 甲状腺癌mtDNA突变高发区域为呼吸复合体编码区, 尤其是复合体I编码区。

在对mtDNA突变与乳头状甲状腺癌患者临床资料进行相关性分析中, 我们并没有发现mtDNA突变与患者性别、年龄、淋巴结转移、肿块大小、TSH水平存在显著的相关性。线粒体单体型M下的子单体型CZ的分布在乳头状甲状腺癌患和结节性甲状腺肿间存在统计学差异, 单体型CZ可能是乳头状甲状腺癌的保护因素。值得注意的是, 线粒体单体型M的乳头状甲状腺癌患者比线粒体单体型N的乳头状甲状腺癌患者淋巴结转移发生率更低, 由此推测, 线粒体单倍型N可能增加甲状腺癌发生淋巴结转移的风险, 线粒体单倍型N可能在甲状腺癌恶性表现中起着一定的作用。

近年来已有很多研究报道, 肿瘤细胞中存在mtDNA拷贝数的改变, 但mtDNA拷贝数的变化有肿瘤特异性, 通过检测91例乳头状甲状腺癌患者、28

例滤泡状甲状腺腺瘤患者和31例结节性甲状腺肿患者组织和血液标本中mtDNA 拷贝数, 发现乳头状甲状腺癌和滤泡状甲状腺腺瘤组织中mtDNA拷贝数均显著高于结节性甲状腺肿组织中mtDNA拷贝数,而甲状腺癌和甲状腺腺瘤之间无统计学差异, 但是甲状腺癌组织的mtDNA拷贝数有上升的趋势。而乳头状甲状腺癌患者和滤泡状甲状腺腺瘤患者血液中mtDNA拷贝数均低于结节性甲状腺肿患者。mtDNA拷贝数在甲状腺癌和甲状腺腺瘤之间无统计学差异, 但在甲状腺癌中有下降趋势。肿瘤细胞内mtDNA的增多可能与肿瘤发展过程中由于mtDNA氧化损伤, 为满足肿瘤细胞的快速增殖, 细胞内代偿性的增加mtDNA的拷贝数有关。而血液中游离DNA主要有两种形式存在, 一种是完全游离于血液中[26], 另一种是吸附于血细胞表面随着血液循环[27-28], 两种形式的循环DNA保持一种动态平衡的状态[29]。甲状腺癌血液中游离mtDNA的减少可能是因为mtDNA更多的吸附于血细胞表面。

本文的研究揭示了mtDNA突变与甲状腺癌的发生密切相关, mtDNA的复合体亚基编码区基因突变, 尤其是复合体I亚基编码区基因突变与甲状腺癌的发生密切相关, 有望作为甲状腺癌筛查的辅助指标, 另外, 甲状腺癌组织和血液中mtDNA拷贝数可作为临床甲状腺癌辅助诊断的一个生物学指标, 但这尚且需要进行大样本量的流行病学研究以确定正常mtDNA拷贝数的参考范围。

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