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• 研究快报 •

食管鳞癌组织III型胶原和纤维粘连蛋白表达的临床意义

李小飞, 刘 锟, 程庆书, 王小平, 汪 健, 卢 强, 刘 勇

李小飞, 刘锟, 程庆书, 王小平, 汪健, 卢强, 刘勇, 中国人民第四军医大学唐都医院胸外科 陕西省西安市 710038
项目负责人: 李小飞, 710038, 陕西省西安市, 中国人民第四军医大学唐都医院胸外科.
电话: 029-83377437 传真: 029-83377436
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摘要

目的: 食管癌的发生、发展和预后虽与多种因素有关，但未定论. 癌间质是影响癌临床行为的重要因素, III型胶原和纤维粘连蛋白(FN)是肿瘤基质的主要成分. 我们研究了III型胶原和FN在食管癌组织的表达及其临床意义.

方法: 食管鳞癌手术切除存档蜡块标本102例，用免疫组化方法观察FN和III型胶原在食管鳞癌组织中的分布特征，并和食管鳞癌临床特征诸如淋巴结转移、肿瘤浸润深度、癌分化程度、临床TNM分期作对比分析.

结果: 食管鳞癌组织中III型胶原主要分布于癌细胞质，阳性率71.6%，其次分布于间质，呈纤维状. FN分布于癌间质呈纤维状包绕癌巢，阳性率73%; 淋巴结转移组FN阳性率63%，而无淋巴结转移组阳性率83%($P<0.05$)，表明FN阳性的食管鳞癌不易发生淋巴结转移.

结论: 食管鳞癌细胞具有产生III型胶原的能力. FN对癌的扩散可能有限制作用.

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0 引言

食管癌是我国最常见的恶性肿瘤之一[1-7]，其发生、发展和预后虽与多种因素有关，但迄今未定论[8-9]. 癌间

质是影响癌临床行为的重要因素，III 型胶原和纤维粘连蛋白(FN)是肿瘤基质的主要成分. 目前，有关 III 型胶原和FN在食管癌中的分布及其临床行为的关系报道甚少，为此我们用免疫组化方法对此进行了研究.

1 材料和方法

1.1 材料 我院 1988/1991 年胸段食管鳞癌手术切除标本 102 例，男 63 例，女 39 例，年龄 36–71(平均 53)岁. 按 1987 年 UICC 的 TNM 分期标准，结合临床和病理，对本组病例进行组织学分级(G 级)，肿瘤侵润深度分级(PT 级)及 TNM 分期，并根据区域淋巴结光镜下是否转移分为食管鳞癌伴淋巴结转移组 51 例(至少有 1 个以上区域淋巴结阳性)和无淋巴结转移组 51 例(术中摘除的全部区域淋巴结均无转移). 随访 3.5 a 以上者 73 例，随访率 72%. 兔抗人 FN(Dako 公司); 鼠抗人 III 型胶原(第四军医大学病理教研室制备); 鼠 ABC 复合物、羊抗兔 IgG 和羊抗鼠 IgG(Vector Lab 公司).

1.2 方法 石蜡包埋标本制成 4–5 μm 切片，脱蜡切至水; 3 mol/L尿素消化30 min; 30 mL/L H_2O_2甲醇阻断内源性过氧化物酶; 加 100 mL/L 正常小牛血清湿盒 37 ℃，封闭 20 min; 滴加稀释后的一抗，4 ℃过夜; 复温后滴加生物素化二抗孵育; 加鼠 ABC 复合物. 以上各步中间均用 PBS 缓冲液振洗 3 次. DAB–H_2O_2 显色液中显色，苏木精复染，脱水封片. 染色后光镜下观察阳性呈深棕色，癌细胞或间质有阳性产物深积者定为阳性，不考虑阳性产物的多少. 实对照验: 正常食管组织作对照 PBS 分别取代一抗和二抗作阴性对照; 自身对照.

统计学处理 采用 χ^2 检验，$P<0.05$ 有意义.

2 结果

2.1 食管鳞癌 III 型胶原的表达 在 102 例食管鳞癌中 III 型胶原主要位于癌细胞胞质中，间质较少，高倍视野下可见 III 型胶原呈棕黄色细颗粒状均匀布满癌细胞胞质，癌巢内角化株边缘 III 型胶原呈纤维样层状分布. 在 102 例食管鳞癌中胞质 III 型胶原阳性着色 73 例，占 71.6%. 少数病例可见 III 型胶原位于间质，呈细纤维样分布，间质分布者 27 例，占 26.5%，胞质和间质 III 型胶原阳性着色可共存于同一组织. III型胶原阳性的癌细胞有时散在分布于癌巢内. 对照为阴性. 正常食管组织中可见 III 型胶原分布于平滑肌间，少量位于间质呈纤维样，偶见鳞状上皮阳性. III 胶原阳性与食管鳞癌淋巴结转移、浸润深度、分期的关系不大($P>0.05$)，如果除外 G4 级，则胞质 III 型胶原阳性率随高分化至低分化渐次减低. 间质 III 型胶原阳性 27 例中，淋巴结转移组阳性率为14/51(27.5%)，而在非淋巴结转移组中阳性率为 13/51(25. 5%)，间质 III 型胶原与癌的淋巴结转移的关系不明显(表 1).

2.2 食管鳞癌 FN 的表达 在 93 例食管鳞癌中经免疫组化染色表明 FN 阳性 68 例，阳性率 73.0%，染成棕黄色的 FN 主要分布于间质，或小血管基底膜. 鳞癌间质内 FN 呈包绕癌巢的条索状阳性着色，有的 FN 突入癌巢内完整或不完整地包绕癌巢，FN 也可位于癌巢内形成长索状分隔，在染色中还发现 FN 可分布于癌巢基底膜部位，构成完整或不完整线状阳性着色. 在癌组织与

表 1 食管鳞癌临床特征与癌细胞 III 型胶原阳性的关系

病理特征		n	III型胶原(+)	
			n	%
淋巴结	PN_0	51	37	72.5
	PN_1	51	36	70.8
癌侵润	PT_1	2	2	100.0
	PT_2	51	37	72.5
	PT_3	48	33	68.8
	PT_4	1	1	100.0
组织学分级	G_1	39	30	76.9
	G_2	36	25	69.4
	G_3	26	17	65.3
	G_4	1	1	100.0
TNM 分期	I	2	2	100.0
	II^a	48	33	68.8
	II_b	15	9	60.0
	III	36	28	77.8
	IV	1	1	100.0
总计		102	73	71. 6

$P>0.05$.

表 2 食管鳞癌临床特征与细胞外纤维粘连蛋白阳性反应的关系

病理特征		n	FN(+)	
			n	%
淋巴结	PN_0	47	39	83.0
	PN_1	46	29	63.0
癌侵润	PT_1	2	1	50.0
	PT_2	49	36	73.5
	PT_3	41	31	75.6
	PT_4	1	0	0.0
组织学分级	G_1	32	23	71.9
	G_2	35	26	72.3
	G_3	25	18	72.0
	G_4	1	1	100.0
TNM 分期	I	2	1	50.0
	II^a	43	34	79.1
	II^b	15	9	60.0
	III	32	23	71.9
	IV	1	1	100.0
总计		93	68	73.1

$^aP<0.05$ *vs* PN_0.

肌层间有FN不连续的阳性着色，有时可在癌细胞间形成网格状阳性着色. 我们把以上间质或基底膜FN统称为细胞外FN，未发现癌细胞胞质FN，正常食管组织可见间质FN分布于小血管基底膜，FN阳性的小血管周围间质内可见细纤维样FN分布. 对照实验为阴性. 食管鳞癌患者伴淋巴结转移组细胞外FN(以下简称FN)阳性率63%，明显低于无淋巴结转移组83%($P<0.05$，表2).

3 讨论

Ⅲ型胶原蛋白与肿瘤的关系是目前基质生物学研究的主要内容之一[10]. 但是食管鳞癌中有关Ⅲ型胶原的研究属薄弱环节，Ⅲ型胶原在食管鳞癌中的分布以及Ⅲ型胶原与食管鳞癌临床特征关系的研究还不明确[11-12]. 本研究显示，在食管鳞癌中Ⅲ型胶原主要位于癌细胞胞质，其次为间质，胞质Ⅲ型胶原阳性的癌细胞弥漫或散在分布于癌巢内，间质Ⅲ型胶原分布呈细纤维状. 由于受多种因素的影响，目前对Ⅲ型胶原蛋白在恶性肿瘤中的分布各家报道不一，许多人认为Ⅲ型胶原是间质成分[13-14]，但有人用免疫组化在肝细胞癌胞质中、甚至肝硬化肝细胞或乳腺癌癌细胞质内发现Ⅲ型胶原[15-16]，根据胶原结构和形成特点，目前认为间质中的胶原应该是前胶原和胶原，而胞质内只有前胶原，但是免疫组化中胶原单抗只对抗某些抗原决定族，所以用胶原抗体做免疫组化染色时，间质和胞质均可呈阳性，但胞质中发现的应该为前胶原，而胶原来源于前胶原，这就意味着胞质胶原阳性的癌细胞具有产生胶原的能力[17-18]. 由此可见，本结果表明食管鳞癌细胞具有产生Ⅲ型胶原的能力，或者至少可以说明食管鳞癌细胞本身参与了间质Ⅲ型胶原的形成. 这些反映了癌细胞和间质具有密切的关系，这种密切的关系可能影响癌的一系列生物学行为.

为了探讨食管鳞癌癌细胞质内Ⅲ型胶原是否影响癌的临床行为，我们把胞质Ⅲ型胶原阳性率与食管鳞癌临床特征加以比较，发现胞质Ⅲ型胶原阳性与食管鳞癌淋巴结转移、浸润深度及分期间无相关性. 但鳞癌组织由高分化到低分化胞质Ⅲ型胶原阳性率渐减少，虽然未达到统计上的差异但反映了一个趋势，在高分化组织中胞质Ⅲ型胶原阳性率高，而低分化组织中胞质Ⅲ型胶原较少[19]. 有证据表明在胰腺癌中高分化和中分化组织内Ⅲ型胶原RNA表达明显高于低分化和未分化组织[20-21]. 那么是高分化组织中Ⅲ型胶原生成多于低分化组织、还是低分化组织中胶原降解比高分化组织快尚不清楚，仍需要进一步详细和深入的研究.

我们发现FN与食管鳞癌淋巴结转移密切相关，实体恶性肿瘤中FN的分布仍未定论，有人认为FN可存在于癌细胞质、基底膜或间质[22-25]. 我们未发现癌细胞内FN阳性着色，而FN分布于细胞外，由于在实际观察中很难区别细胞表面FN，基膜FN和间质FN[26]，应把这些细胞外FN作为一个整体而分析[27-29]. 我们正是把细胞外FN看作一个整体来分析，发现细胞外FN与食管鳞癌淋巴结转移呈明显的负相关，即FN阴性的食管鳞癌易发生淋巴结转移，反之亦然. 这个结果表明，间质内FN可能对肿瘤细胞的淋巴结转移起限制作用. 但是有关间质FN与肿瘤淋巴结转移关系的研究结果大多是否定的[30-31]，有人认为间质FN与肿瘤的局部生长、浸润等有相关性[32-36]，甚至有人认为癌细胞表面FN减少可使细胞与间质的粘着力下降、使癌细胞更易脱落、从而有利于癌细胞转移[37-38]. 如果把FN的免疫组化应用于临床，对提示临床医师注意术中清除淋巴结或者合理选择治疗方案等无疑是有益的.

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