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错配修复蛋白与上皮间质转化标志物在大肠癌中表达及意义

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[摘　要]　**目的：**探讨错配修复(mismatch repair，MMR)蛋白与上皮间质转化(epithelial mesenchymal transition，EMT)标志物在大肠癌中的表达及意义，并分析二者关系。**方法：**采用免疫组织化学方法标记大肠癌组织中MMR蛋白(MSH1，MSH2，MSH6及PMS2)及EMT标志物(E-cadherin和Vimentin)的表达。MSH1，MSH2，MSH6及PMS2四种蛋白中的1种及以上表达缺失判定为错配修复基因缺陷(deficient mismatch repair，dMMR)，全部表达判定为错配基因完整(proficient mismatch repair，pMMR)。**结果：**242例大肠癌中186例pMMR，MMR蛋白表达率为76.86%；56例dMMR，MMR蛋白缺失率为23.14%。且大肠癌中dMMR与发病部位差异有统计学意义，68例右半结肠癌中31例dMMR(45.59%)，118例左半结肠癌中18例dMMR(15.25%)，56例直肠癌中7例dMMR(12.50%)。并且有淋巴结转移组和侵透肌层组dMMR要明显低于无淋巴结转移组及侵犯肌层组(P<0.05)。242例大肠癌中E-cadherin表达189例(78.10%)，低于癌旁组织(98.76%)，差异有统计学意义(P<0.05)；Vimentin表达38例(15.70%)，明显高于癌旁组织(0%)，差异有统计学意义(P<0.05)。低分化大肠癌、有淋巴结转移组及侵透肌层组E-cadherin的表达明显低于高-中分化大肠癌、无淋巴结组及侵犯肌层组(P<0.05)。而Vimentin的表达则相反(P<0.05)。发病部位上右半结肠癌E-cadherin的表达要高于左半结肠及直肠癌，Vimentin右半结肠癌的表达低于左半结肠癌及直肠癌(P<0.05)。**结论：**错配修复蛋白状态和EMT过程与大肠癌的发生及进展有关，影响其预后，提示存在MMR蛋白缺失状态的大肠癌其EMT过程也不明显。

[关键词]　大肠癌；错配修复蛋白；上皮间质转化

Expression and significance of mismatch repair protein and epithelial mesenchymal transition marker in colorectal cancer

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Abstract　**Objective:** To investigate the expression and significance of mismatch repair protein (MMR) and epithelial mesenchymal transition (EMT) markers in colorectal cancer, and to analyze their relationships. **Methods:** The

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expressions of mismatch repair proteins (MSH1, MSH2, MSH6, and PMS2) and E-cadherin and Vimentin in colorectal cancer tissues were marked by immunohistochemistry. The deletion of one or more of MSH1, MSH2, MSH6, and PMS2 proteins was determined as deficient mismatch repair (dMMR), and all expressions were judged as proficient mismatch repair (pMMR). **Results:** In 242 cases of colorectal cancer, there were 186 cases of pMMR, the expression rate of MMR protein was 76.86%, and in 56 cases of dMMR, the rate of deletion of MMR protein was 23.14%. And there were differences in dMMR and pathogenesis in colorectal cancer. There were 31 cases of dMMR (45.59%) in 68 cases of right colon cancer, 18 cases of dMMR (15.25%) in 118 cases of left colon cancer, and 7 cases of dMMR (12.50%) in 56 cases of rectal cancer. The dMMR in the lymph node metastasis group and the penetrating muscle group was significantly lower than that in the group without lymph node metastasis and in the muscle invading group. 189 cases (78.10%) of colorectal carcinoma had E-cadherin expression, which was lower than that in paraneoplastic tissue (98.76%), the difference was statistically significant (P<0.05); Vimentin expression in 38 cases (15.70%) was significantly higher than paraneoplastic tissue (0%), the difference was statistically significant (P<0.05). The expression of E-cadherin in poorly differentiated colorectal cancer, lymph node metastasis group, and penetrate muscle layer was significantly lower than that in high-grade differentiated colorectal cancer, no lymph node group, and muscle infiltration group (P<0.05). The expression was opposite (P<0.05). The expression of E-cadherin in the right colon was higher than that in the left colon and rectal cancer. The expression of Vimentin in colon cancer was lower than that in the left colon and rectal carcinoma (P<0.05). **Conclusion:** The status of mismatch repair protein and the process of epithelial-mesenchymal transition are related to the occurrence and progression of colorectal cancer, and affect its prognosis. It also suggests that the presence of MMR protein-deficient colorectal cancer has no obvious epithelial-mesenchymal transition.

Keywords colorectal cancer; mismatch repair protein; epithelial-mesenchymal transition

大肠癌是消化道常见恶性肿瘤，2014年世界癌症大会WHO[1]指出：所有恶性肿瘤中大肠癌发病率居第3位，2012年死亡69.4万，占所有恶性肿瘤死亡的8.5%。国家癌症中心[2]调查统计2015年中国大肠癌的发病率及病死率均居第5位。大肠癌发病原因多样，其发生发展是一个多因素、多基因、多步骤参与的复杂过程。大肠癌的发生、发展与微卫星不稳定性有重要关系，其中微卫星不稳定性是由错配修复(mismatch repair，MMR)基因表达缺失所造成的[3]。恶性肿瘤发生、发展过程中与上皮间质转化(epithelial mesenchymal transition，EMT)有重要关系，并且影响恶性肿瘤细胞的浸润和转移[4]。本实验通过免疫组织化学方法检测大肠癌中MMR蛋白及EMT标志物(E-cadherin，Vimentin)的表达并分析二者关系。

1 材料与方法

1.1 材料

收集承德市中心医院2015年6月至2017年12月经病理检查且明确诊断的242例大肠癌标本，作为实验组。实验组年龄32~71(56.00±7.32)岁，其中右半结肠癌68例，左半结肠癌118例，直肠癌56例，均符合2015年中国结直肠癌诊疗规范中的诊断标准。所有病例术前未接受放化疗及生物治疗，并排除远处转移，且所有临床病理资料完整。同时选取距癌组织5 cm处肠黏膜作为对照组。本研究获得承德市中心医院伦理委员会批准[批准文号：(2018)伦审批第014号]。

1.2 方法

采用免疫组织化学染色检测4种MMR蛋白(MLH1，MSH2，MSH6和PMS2)及EMT标志物E-cadherin和Vimentin的表达。4种蛋白均表达，即判定为错配基因完整(proficient mismatch repair，pMMR)；4种蛋白中一种及以上不表达，即判定为错配修复基因缺陷(deficient mismatch repair，dMMR)。试剂盒均购自福州迈新生物有限公司。

1.3 结果判定

MLH1，MSH2，MSH6及PMS2均定位于细胞核。肿瘤周围的正常上皮细胞、淋巴细胞、间质细

胞均出现阳性可作为内对照。E-cadherin，Vimentin阳性定位于细胞质，均以PBS作为阴性对照。

1.4 统计学处理

采用SPSS 19.0统计软件进行分析，计数资料比较进行χ^2检验。

2 结果

2.1 MMR 蛋白表达情况

242例大肠癌组织中186例(76.86%)MMR蛋白阳性(pMMR)，56例(23.14%)表达缺失(dMMR)。右半结肠癌68例中dMMR31例(45.59%)，左半结肠癌118例中dMMR18例(15.25%)，直肠癌56例中dMMR7例(12.5%；P<0.05)。并且有淋巴结转移组和侵透肌层组dMMR要明显低于无淋巴结转移组及侵犯肌层组(P<0.05；图1，表1)。

2.2 E-cadherin，Vimentin 在大肠癌中的表达

242例大肠癌中E-cadherin表达189例(78.10%)，低于癌旁组织(98.76%)，差异有统计学意义(P<0.05)；Vimentin表达38例(15.70%)，明显高于癌旁组织(0%)，差异有统计学意义(P<0.05)。发病部位上右半结肠癌E-cadherin的表达要高于左半结肠及直肠癌，Vimentin右半结肠癌的表达低于左半结肠癌及直肠癌。低分化大肠癌、有淋巴结组及侵透肌层组E-cadherin的表达明显低于高中分化大肠癌、无淋巴结组及侵犯肌层组(P<0.05)；而Vimentin的表达则相反(P<0.05；图2，表2)。

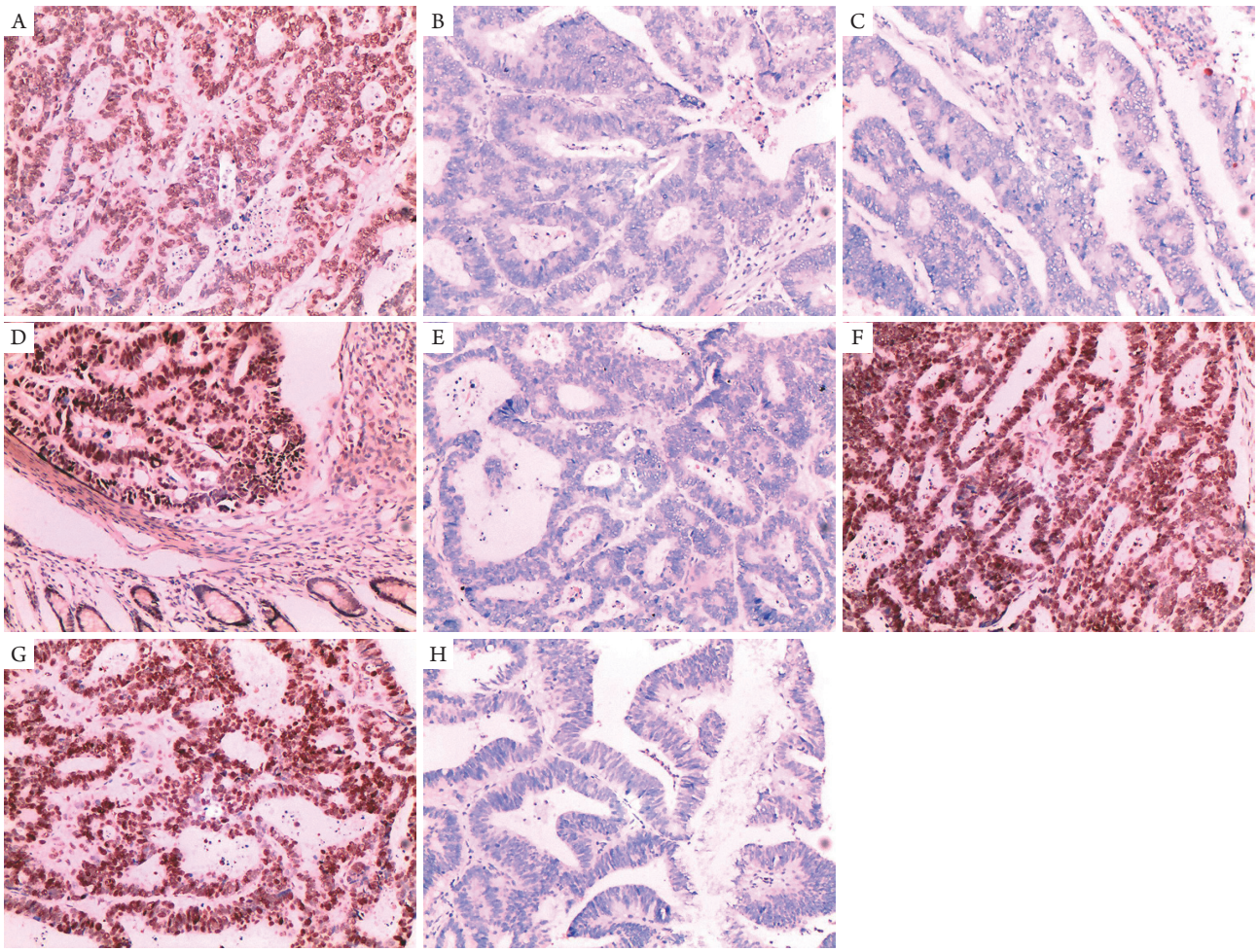

图1 MLH1，MSH2，MSH6，PMS2在大肠癌中的表达(SP，×100)

Figure 1 Expression of MLH1, MSH2, MSH6, PMS2 in colorectal cancer (SP, × 100)

(A) MLH1(+); (B) MSH2(–); (C) MSH6(–); (D) PMS2(+); (E) MLH1 (–); (F) MLH2(+); (G) MSH6(+); (H) PMS2(–).

表1 MMR蛋白表达与大肠癌病理参数的关系

Table 1 Relationship between MMR protein expression and pathological parameters of colorectal cancer

病理参数	*n*	MMR		*P*
		dMMR	pMMR	
发病部位				<0.05
右半结肠	68	31	37	
左半结肠	118	18	100	
直肠	56	7	49	
淋巴结转移				<0.05
有	103	14	89	
无	139	43	76	
浸润深度				<0.05
侵犯肌层	168	48	120	
侵透肌层	74	8	66	

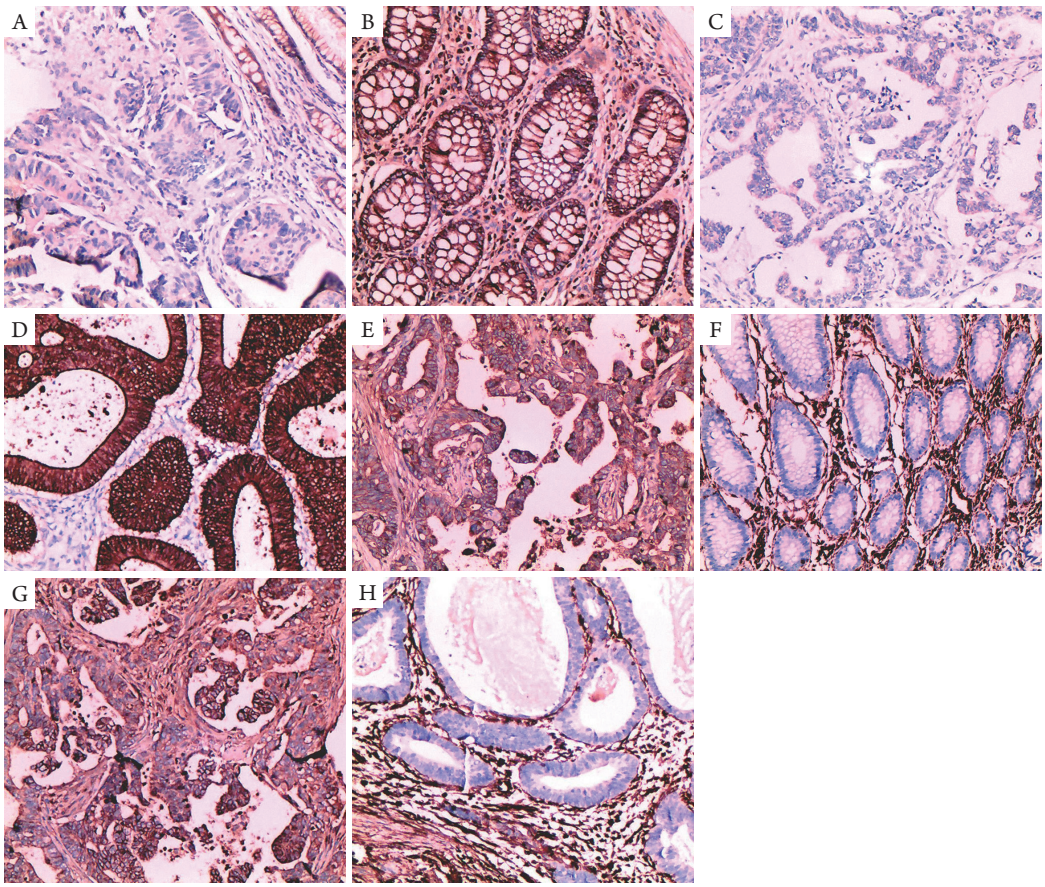

图2 E-cadherin，Vimentin在大肠癌及癌旁组织中的表达(SP，×100)

Figure 2 Expression of E-cadherin and Vimentin in colorectal cancer and paraneoplastic tissues (SP, × 100)

E-cadherin在大肠癌(A)中的表达低于及癌旁组织(B)；E-cadherin在低分化癌(C)中的表达低于及高-中分化癌(D)；Vimentin在大肠癌(E)中阳性表达，在癌旁组织(F)中阴性表达；Vimentin在低分化癌(G)中的表达高于高-中分化癌(H)。

Expression of E-cadherin in colorectal cancer (A) is lower than that in the paraneoplastic tissues (B); Expression of E-cadherin in poorly differentiated (C) is lower than that in high-to-middle differentiated carcinomas (D); Vimentin expression is positive in colorectal cancer (E), while negative in paraneoplastic tissues (F); Vimentin expression in poorly differentiated is higher (G) than that in high-to-middle-differentiated carcinomas.

表2 E-cadherin，Viemntin在大肠癌、癌旁组织的表达与病理参数的关系

Table 2 Relationship between E-cadherin and Viemntin in colorectal cancer, paracancerous tissues, and pathological parameters of cancer

参数	*n*	E-cadherin阳性表达/[例(%)]	Vimentin阳性表达/[例(%)]	*P*
组织部位				<0.05
癌	242	189 (78.10)	38 (15.70)	
癌旁	242	239 (98.76)	0	
肿瘤部位				<0.05
右半结肠	68	63 (92.65)	4 (5.88)	
左半结肠	118	91 (77.12)	24 (20.33)	
直肠	56	35 (62.50)	10 (17.86)	
分化程度				<0.05
低分化	117	79 (67.52)	31 (26.49)	
高-中分化	125	110 (88.00)	7 (5.60)	
淋巴结转移				<0.05
有	103	63 (61.16)	27 (26.21)	
无	139	126 (90.65)	11 (9.09)	
浸润深度				<0.05
侵犯肌层	168	145 (86.31)	16 (9.52)	
侵透肌层	74	44 (59.46)	22 (29.73)	

3 讨论

大肠癌是消化道常见肿瘤之一，依据不同的分子变异基础可划分为2类：一是由于染色体不稳定性(chromosomal instability，CIN)造成；另一类与dMMR有关[5-6]，约15%的大肠癌发生与错配功能缺失相关。目前免疫组织化学方法已被广泛应用，且报道[7]证实检测MMR基因的可靠性，因此应用免疫组织化学检测MMR基因被医院及科研机构所采用。dMMR结直肠癌有其特有的临床病理特征，包括发病年龄早、好发于右半结肠、组织学类型以黏液腺癌多见、存在Crohn's样淋巴细胞反应、预后好等特点[8]。本研究采用免疫组织化学方法检测MMR主导蛋白(MLH1，MSH2，MSH6及PMS2)，结果显示242例大肠癌中186例pMMR，MMR蛋白表达率为76.86%；56例dMMR，MMR蛋白缺失率为23.14%。同时也显示MMR蛋白与肿瘤发生部位相关，在dMMR组发生于右半结肠(31/68，45.59%)明显高于左半结肠(18/7118，15.25%)和直肠(7/56，12.5%)。这提示右半结肠在发生途径上可能与左半结肠和直肠有区别，右半结肠癌预后可能会更好。

EMT是指通过特定程序将上皮细胞转化为间充质细胞的生物过程。EMT导致上皮细胞失去细胞极性和上皮细胞表型特征，如与基底膜连接，因此获得高迁移性、侵袭性、抗凋亡性和细胞外基质降解的特征，浸润、侵袭、抗凋亡、免疫抑制、耐药性和肿瘤干细胞样细胞(cancer stem cell，CSC)等特性将会由经历上皮间质转化的细胞所获得[9]。EMT发生的标志之一是E-cadherin的低表达，其低表达可以降低细胞间的黏附性并增强细胞的运动及迁移能力[10]。E-cadherin是一种钙依赖性细胞黏附因子，可与β-catenin结合成复合物以维持上皮细胞极性和完整性，防止肿瘤细胞的脱落及转移。文献[11]报道：当E-cadherin表达降低或缺失可使肿瘤细胞发生间质转化，并与大肠癌的恶性程度及转移相关。本研究结果也显示：E-cadherin在癌旁组织中的表达高于癌组织，且高-中分化癌、无淋巴结转移及侵犯肌层组的E-cadherin表达要高于低分化癌、有淋巴结转移及侵透肌层组，提示E-cadherin的表达高低与大肠癌发生发展相关。癌细胞表达间叶分化标志是

EMT更为直接的表型，Vimentin是细胞骨架蛋白之一，主要表达于中胚层来源的细胞，是一种EMT相关肿瘤间叶标志物，具有高度选择性。Kidd等[12]研究发现在上皮肿瘤中也可以出现Vimentin的表达，认为其可能与肿瘤生物学行为有关，包括肿瘤增殖、侵袭、转移及预后。本研究证实大肠癌中Vimentin的表达要明显高于癌旁组织，并且高-中分化癌、无淋巴结转移及侵犯肌层组的Vimentin表达要低于低分化癌、有淋巴结转移及侵透肌层组。

本研究还发现E-cadherin和Vimentin在大肠癌不同部位表达不同，E-cadherin在右半结肠癌表达明显高于左半结肠癌及直肠癌，而Vimentin表达则相反。同时右半结肠癌中dMMR发生率要高于左半结肠癌及直肠癌。两组实验表明：存在dMMR状态的大肠癌预后可能会更好。MMR蛋白缺失是否与EMT相关，尚需进一步证实。

本研究结果显示MMR蛋白缺失越高，E-cadherin高表达及Vimentin低表达的大肠癌预后可能相对更好，反之预后差，同时也提示存在dMMR状态的大肠癌中EMT发生率亦低于其他状态大肠癌。

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