·临床研究·

血清 Periostin 表达与鼻咽癌患者临床病理因素的相关性研究

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摘 要:目的 探讨 Periostin 蛋白在鼻咽癌患者中的表达以及与临床病理因素的相关性。**方法** 选择 87 例鼻咽癌患者,分析患者的临床病理信息,包括年龄、性别、组织分化程度、肿瘤分级、淋巴结转移和远处转移等。每例患者采集手术前、后血清,用酶联免疫吸附测定(ELISA)检测患者血清 Periostin 蛋白水平,并分析其与临床病理因素的关系。65 例健康志愿者的血清作为对照。**结果** 鼻咽癌患者血清 Periostin 蛋白水平显著高于健康志愿者;患者血清 Periostin 蛋白水平与患者肿瘤组织分化程度、淋巴结转移有关;术后患者血清 Periostin 蛋白水平显著下降,但仍高于健康志愿者。化疗不影响血清 Periostin 蛋白水平。**结论** 鼻咽癌患者血清 periostin 蛋白水平显著高于健康者,与患者的临床病理因素、淋巴结转移相关。

关键词:鼻咽肿瘤;细胞分化;淋巴转移;细胞因子类;骨膜蛋白
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A correlational study on the serum Periostin expression and clinicopathologic factors of patients with nasopharyngeal carcinoma

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Abstract:Objective To explore Periostein protein expression in serum of patients with nasopharyngeal carcinoma and its correlation with clinicopathologic factors. **Methods** 87 patients with nasopharyngeal carcinoma were selected. Clinicopathological information including age,gender,degree of histological differentiation,tumor grade,lymphatic metastasis and distant metastasis were analyzed. Serum samples from each patient were collected preoperatively and postoperatively,and enzyme-linked immunosorbent assay (ELISA) was employed to detect the serum level of Periostin protein,and its relation to clinicopathological factors was assayed. Serum samples of 65 healthy volunteers served as control. **Results** Serum levels of Periostin protein of patients with nasopharyngeal carcinoma were significantly higher than those of healthy volunteers. The expression levels of serum Periostin protein were correlated with the histological differentiation degree of tumor and lymphatic metastasis. Serum Periostin levels of patients which weren't affected by chemotherapy decreased markedly after operation,but were still higher than those of healthy volunteers. **Conclusion** Serum level of Periostin protein of patients with nasopharyngeal carcinoma which correlated with clinicopathological factors and lymphatic metastasis is higher than that of healthy people.

Key words:nasopharyngeal neoplasms;cell differentiation;lymphatic metastasis;cytokines;periostin

Periostin 蛋白又称成骨细胞特异性因子-2,是一个相对分子质量为 93×10^3 的 N 型糖蛋白,Periostin 蛋白高表达于富含胶原的组织,如细胞外基质(extracellular matrix,ECM)和机械应激导致的组织修复和再生[1-3]。

Periostin 基因的蛋白或 mRNA 在许多实体瘤中高表达,包括乳腺癌[4]、结肠癌[5]、头颈癌[6]、胰腺癌[7]及甲状腺癌[8]等。Periostin 也是一个分泌蛋白,因此在乳腺癌、结直肠癌和非小细胞肺癌患者血清中存在着 Periostin 蛋白的高表达[9-11],其对肿瘤细胞的作用为促进增殖和增强对低氧和化学药物的耐受[12-13],从而成为一种潜在的预测肿瘤侵袭和转移的标志。

1 资料与方法

1.1 一般资料 选择本院收治的行外科手术切除的鼻咽癌患者 87 例(病例组),其中男 51 例,女 36 例;年龄 28~63 岁,平均(47.4±9.1)岁。按照病理分化程度分为Ⅰ级 21 例,Ⅱ级 25 例,Ⅲ级 22 例,泡状核细胞癌 19 例。其中 52 例有淋巴结转移(淋巴结转移组),35 例无淋巴结转移(无淋巴结转移组)。所有患者均行放疗后,再行手术治疗,并分别于放疗的前、后,以及手术治疗前、后取血清,采用室温自然凝固,再置 37 ℃温箱 1 h,然后放 4 ℃冰箱过夜,待血块收缩后分离血清,4 ℃保存备用。同时采集 65 例健康者(来自本院健康体检者)血清样本作为对照。

1.2 酶联免疫吸附测定(enzyme-linked immunosorbent assay,ELISA) 用 10 mmol/L PBS(pH7.4,1%BSA)将单抗稀释成 3 mL 溶液,包被 96 孔板,4 ℃过夜;用含 1%BSA 的 10 mmol/L PBS(pH7.4)、4 ℃封闭过夜;0.02%吐温 20 的 PBS 洗板 3 次,然后分别加 0.5、1.0、5.0、10.0、30.0、50.0 ng/mL 6 种不同浓度的 Periostin 标准品,以及对照组和患者血清 100 μL 至相应孔(每份标准品及待测样本均做复孔),置 37 ℃孵育 2 h 后,洗板 4 次。每孔分别加 5 μL 兔抗人 Periostin 单克隆抗体 (0.25 mL)和羊抗兔 IgG(HRP 标记),置 37 ℃孵育 1 h 后,加 DAB/H_2O_2 于 37 ℃避光显色 20 min,最后加 2 mol/L H_2SO_4 终止反应。空白孔调零,酶标仪 450 nm 处读取吸光度值。绘制标准曲线,计算待测样本中血清 Periostin 浓度。分别于放疗的前、后,以及手术治疗前、后取血清,应用 ELISA 方法检测患者血清 Periostin 蛋白的表达水平。

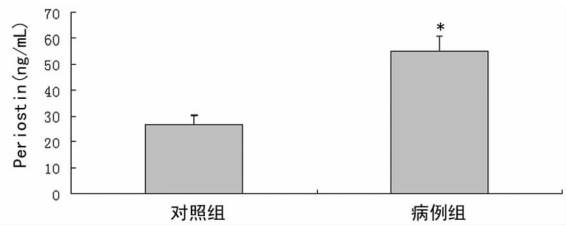
1.3 统计学分析 采用 SPSS14.0 统计软件分析实验数据。计量资料采用 $\bar{x}\pm s$ 表示,组间比较采用 t 检验分析。以 $P<0.05$ 为差异有统计学意义。

2 结 果

2.1 鼻咽癌患者血清 Periostin 蛋白的表达 鼻咽癌患者血

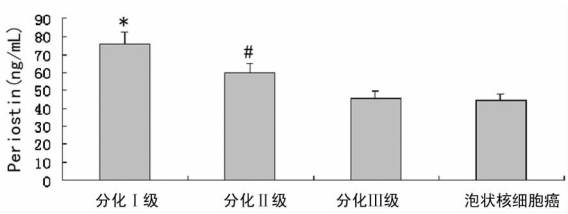
清 Periostin 蛋白水平比对照组显著增高，分别为(55.2±5.5)、(26.6±3.7)ng/mL($P<0.01$)，见图 1。

2.2 不同病理分级鼻咽癌患者血清 Periostin 蛋白的表达 按照鼻咽癌病理分化程度分为Ⅰ级 21 例，Ⅱ级 25 例，Ⅲ级 22 例，泡状核细胞癌 19 例。分别统计 4 组患者血清 Periostin 蛋白表达水平，发现病理分化Ⅰ、Ⅱ、Ⅲ级各组之间血清 Periostin 蛋白表达水平差异有统计学意义($P<0.05$)，即随病理恶性度的增高，血清 Periostin 亦增高；但泡状核细胞癌与病理分化Ⅲ级之间无差别，见图 2，其病理恶性度与分化Ⅲ级也很相近。



*：$P<0.01$，与对照组比较。

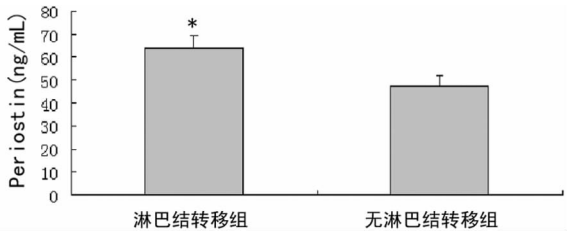
图 1 鼻咽癌患者血清 Periostin mRNA 的表达



*：$P<0.05$，与分化Ⅱ级比较；#：$P<0.05$，与分化Ⅲ级及泡状核细胞癌比较。

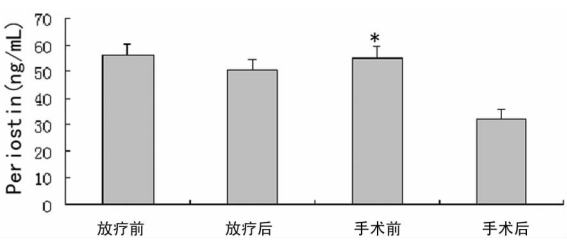
图 2 不同病理分级鼻咽癌的血清 Periostin 的表达水平

2.3 淋巴结转移对血清 Periostin 蛋白表达的影响 淋巴结转移组血清 Periostin 蛋白表达显著高于无淋巴结转移组，分别为(64.0±5.4)、(47.5±4.3)ng/mL($P<0.05$)，见图 3。



*：$P<0.05$，与无淋巴结转移组比较。

图 3 淋巴结转移对血清 Periostin 蛋白表达的影响



*：$P<0.05$，与手术后比较。

图 4 不同治疗前、后血清 Periostin 蛋白的表达

2.4 不同治疗前、后血清 Periostin 蛋白的表达 放疗前患者血清 Periostin 蛋白水平为(56.4±4.1)ng/mL，放疗后稍有降低[(50.3±4.4)ng/mL]，但差异无统计学意义($P>0.05$)；手术治疗能显著降低血清 Periostin 蛋白，从手术前的(55.0±4.5)ng/mL 下降到手术后的(32.2±3.4)ng/mL，差异有统计学意义($P<0.05$)，见图 4。

3 讨 论

Periostin 是间皮细胞特异性表达产物，其过表达能促使转化细胞由上皮向间皮转变(epithelial-mesenchymal transition，EMT)。鼻咽癌是一种由 EB 病毒感染并转化上皮细胞形成的恶性肿瘤，EB 病毒潜伏膜蛋白 1(latentmembraneprotein1，LMP1)能诱导鼻咽癌细胞产生 EMT 现象[14]。EMT 导致上皮来源的鼻咽癌细胞具有间皮细胞样性质，更容易发生转移。对于上皮来源的肿瘤，EMT 能使肿瘤细胞获得更强的转移潜能[15]。

本研究结果显示，与健康者相比，鼻咽癌患者血清 Periostin 蛋白水平显著增高。在不同病理分级的鼻咽癌患者之间，分化差的患者，其血清 Periostin 蛋白水平显著高于分化良好的患者。血清 Periostin 蛋白水平还与是否存在肿瘤转移有关，淋巴结转移患者的血清 Periostin 蛋白水平显著高于无转移者。不同的治疗方式对于血清 Periostin 蛋白水平有不同影响，放疗不影响血清 Periostin 蛋白水平，但手术治疗能显著降低鼻咽癌患者的血清 Periostin 蛋白水平。

本研究首次发现鼻咽癌患者血清中存在 Periostin 蛋白的高表达，证实了 Periostin 蛋白是一种在肿瘤中广泛表达的分泌蛋白。实际上，Periostin 蛋白的来源有两个：肿瘤细胞和周围的基质细胞。在肿瘤侵袭、浸润过程中，肿瘤细胞不断破坏周围基质，导致 Periostin 蛋白释放至血液循环中[16]。因此，肿瘤患者血清 Periostin 蛋白水平越高，肿瘤细胞的浸润和转移潜能也越强。有文献报道 Periostin 能诱导上皮来源的细胞发生 EMT 现象，使之具有类似间皮细胞的多向分化功能[17]。也有研究显示，EMT 与肿瘤干细胞有关，能使较成熟的肿瘤细胞获得干细胞的表型[18]。因此，在低分化组的鼻咽癌，高 Periostin 可能通过诱使 EMT 现象的产生，进而增加鼻咽癌干细胞的数量[19]。

本研究还观察了不同治疗方法对血清 Periostin 蛋白水平的影响。放疗对鼻咽癌患者血清 Periostin 蛋白水平无显著影响。虽然放疗能极大杀死肿瘤细胞，但表达并分泌 Periostin 蛋白的主要是癌旁基质细胞，因此不影响血清 Periostin 蛋白水平。手术切除肿瘤的鼻咽癌患者，由于一并切除了癌旁基质，其术后血清 Periostin 蛋白水平显著低于手术前。同时，鼻咽癌患者术后血清 Periostin 蛋白水平仍高于健康者，这可能是由于转移灶以及残留的鼻咽癌细胞合成并分泌的。

总之，本研究结果显示，鼻咽癌患者血清 Periostin 蛋白水平显著高于健康者，与患者的病理分级、淋巴结转移相关。

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