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Eotaxin-1 在浆液性卵巢癌组织中的表达及临床意义研究

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摘要 目的：研究嗜酸性粒细胞趋化因子 1(Eotaxin-1)在浆液性卵巢癌组织中的表达及临床病理意义。**方法：**收集 2013 年 4 月至 2014 年 5 月于我院妇产科手术切除的 60 例浆液性卵巢癌及对应癌旁组织，采用免疫组织化学染色检测 Eotaxin-1 表达，分析 Eotaxin-1 蛋白与肿瘤临床病理资料之间的相关性。**结果：**浆液性卵巢癌组织中 Eotaxin-1 蛋白表达水平较对应癌旁组织显著升高(P<0.05)，浆液性卵巢癌组织中高表达 Eotaxin-1 蛋白与恶性组织病理分级、淋巴结转移及高 TNM 分期呈显著正相关(P<0.05)。**结论：**Eotaxin-1 蛋白在浆液性卵巢癌组织中表达上调，并与肿瘤恶性临床病理特征有关；Eotaxin-1 可能成为浆液性卵巢癌早期诊断的重要标志物和生物靶向治疗的有效靶点之一，具有广阔的临床应用前景。

关键词：Eotaxin-1；浆液性卵巢癌；临床意义

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The Expression and Clinical Significance of Eotaxin-1 in Human Serous Ovarian Cancer

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ABSTRACT Objective: To investigate the expression and clinical significance of Eotaxin-1 in human serous ovarian cancer. **Methods:** Immunohistochemistry (IHC) was applied to detect the protein expression of Eotaxin-1 in erous ovarian cancer (n=60) in comparison to the corresponding tumor-adjacent normal tissues. Pearson chi-square test was used to analyze the relationship between the Eotaxin-1 expression and clinical features. **Results:** The protein expression level of Eotaxin-1 in serous ovarian cancer tissues was significantly higher than in adjacent normal tissues (P<0.05). The positive expression of Eotaxin-1 protein was also associated with malignant tissue pathologic grades, lymphatic metastasis and advanced TNM stage (P<0.05). **Conclusions:** The expression of Eotaxin-1 in serous ovarian cancer tissues was significantly higher than in tumor-adjacent normal tissues, and associated with tumor malignant clinicopathological features Eotaxin-1 may become a new diagnosed marker and effective therapeutic target.

Key words: Eotaxin-1; Serous ovarian cancer; Clinical significance

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前言

卵巢癌是女性最常见的恶性肿瘤之一，在女性生殖器肿瘤发病率中位居第 3 为[1]。全世界每年约有 191 000 名女性确诊为卵巢癌。目前，根治性手术及术后规范放、化疗是治疗浆液性卵巢癌的主要方法[2]，尽管新的化疗药物出现提高的卵巢癌患者的初治反应率[3]，但浆液性卵巢癌早期诊断困难、易发生淋巴转移及腹腔转移常使患者就诊时已失去根治性手术机会，因而患者的总体死亡率在过去的几十年间几乎没有明显下降[4]。近年来，针对表皮生长因子受体(Epidermal Growth Factor Receptor，EGFR)的西妥昔单抗[5]及针对血管内皮生长因子(vascular endothelial growth factor，VEGF)的贝伐单抗[6]在浆液性卵巢癌的治疗中显现出一定优势，因而对浆液性卵巢癌分子生物学诊断及生物靶向治疗的研究可以为浆液性卵巢癌早期诊治提供有力的理论依据。

嗜酸性粒细胞趋化因子 1(Eotaxin-1)属于 CC 类趋化因子家族，与巨噬细胞趋化蛋白(macrophage chemoattractant protein, MCP) 亚族具有高度的同源性[7]。其编码基因定位于人类染色体 17q11 上，Eotaxin-1 mRNA 在小肠、结肠、心肌组织中表达量较高而在肺脏、肝脏等组织中表达量较低[8]，但 Eotaxin-1 在人类恶性肿瘤包括浆液性卵巢癌中的表达及其临床病理意义尚不完全清楚。

本研究通过免疫组织化学染色等方法检测 Eotaxin-1 在浆液性卵巢癌组织中的表达情况，并分析其与肿瘤临床病理特征之间的相关性，旨在为 Eotaxin-1 成为浆液性卵巢癌诊断和治疗的分子标志物提供一定的研究基础。

1 材料与方法

1.1 材料

本科题经我院医学伦理委员会批准后开展。收集 2013 年 4 月至 2014 年 5 月月期间我院妇科手术切除并经石蜡病理证实为浆液性卵巢癌及对应癌旁组织（距切缘 >2 cm）标本 60

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例,年龄 29~65 岁,中位年龄 47 岁。所有入组患者术前均签署知情同意书且未行放化疗治疗。组织标本在切除后 30 min 内取材,置入 4℃、浓度为 40 g/L 的多聚甲醛溶液中固定保存。兔抗人 Eotaxin-1 多克隆抗体(sc-373767)购自美国 Santa Cruz 公司。组织自动脱水机及生物组织切片机均购自孝感市普诺电子科技有限公司。ZD-Ⅱ医用微波仪购自上海市中达医学应用研究所,电子恒温水浴锅购自汕头市医用设备厂,BX-51 光学显微镜购自日本奥林巴斯公司。

1.2 方法

组织标本经脱水、石蜡包埋后,使用自动切片机制作 4 μm 厚组织切片。切片经二甲苯及梯度酒精脱蜡、水化后,于 pH 6.0 用枸橼酸缓冲液进行抗原热修复;3% H_2O_2 水溶液阻断内源性过氧化物酶活性;10%山羊血清封闭后滴加兔抗人 Eotaxin-1 抗体(1:100),4℃孵育过夜;洗去多余一抗后,加生物素标记的山羊抗兔二抗,37℃孵育 30 min;洗去残余未结合二抗,滴加辣根过氧化物酶 - 链酶卵白素复合物进行反应,DAB 显色、苏木素复染,常规脱水、透明、中性树胶封片。每张切片经由 2 位高年资病理医师,在高倍镜(× 400)下随机选取 10 个视野,按以下标准[9,10]单独阅片、评分:染色强度评分:0 分:阴性;1 分:弱阳性;2 分:中度阳性;3 分:强阳性。阳性肿瘤细胞百分比评分:阳性细胞≤ 5% 为 0 分;6%~25% 为 1 分;26%~50% 为 2 分;51% ~75% 为 3 分;≥ 76% 为 4 分。每视野评分 = 染色强度评分× 阳性肿瘤细胞百分比评分,取 10 个视野的平均得分作为切片的最终评分。

1.3 统计学分析

采用 SPSS13.0 统计软件进行分析,采用 Pearson 卡方检验分析 Eotaxin-1 表达与肿瘤临床病理资料间的相关性。以 P<0.05 表示差异具有统计学意义。

2 结果

2.1 Eotaxin-1 蛋白在浆液性卵巢癌及对应癌旁组织中的表达情况

通过免疫组化方法检测 60 例浆液性卵巢癌及对应癌旁组织中 Eotaxin-1 的表达情况。结果如图 1 所示,肿瘤组织中 Eotaxin-1 阳性表达率为 71.67%(43/60),而癌旁组织中 Eotaxin-1 阳性表达率仅为 11.67%(7/60)。经 Pearson 卡方检验分析证明,Eotaxin-1 在癌与癌旁组织中表达水平具有显著性差异(X^2=44.434,P<0.001,表 1)。

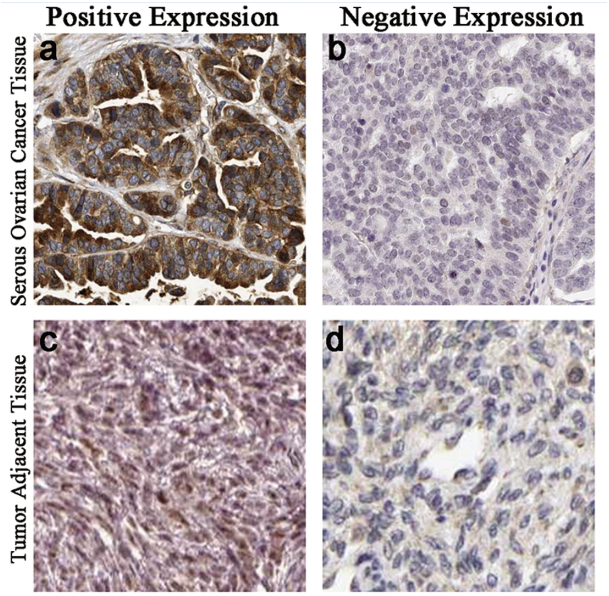

图 1 Eotaxin-1 蛋白在浆液性卵巢癌及对应癌旁组织中的表达

Fig.1 The expression of Eotaxin-1 in serous ovarian cancer and tumor adjacnet tissues

Note: a:The positive expression of Eotaxin-1 in serous ovarian cancer tissues;b:The negative expression of Eotaxin-1 in serous ovarian cancer tissues;c:The positive expression of Eotaxin-1 in tumor adjacnet tissues; d:The negative expression of Eotaxin-1 in tumor adjacnet tissues.

表 1 Eotaxin-1 蛋白在浆液性卵巢癌及对应癌旁组织中的表达情况 (n=60)

Table 1 The expression of Eotaxin-1 in serous ovarian cancer and tumor adjacnet tissues (n=60)

Specimen	Eotaxin-1 Positve	Eotaxin-1Negative	X^2	P
Serous ovarian cancer	43	17	44.434	<0.001*
Tumor adjacnet tissues	7	53		

Note:*P<0.05.

2.2 Eotaxin-1 表达与浆液性卵巢癌临床病理特征间的相关性分析

将浆液性卵巢癌中 Eotaxin-1 蛋白的表达情况与患者年龄、绝经与否、肿瘤体积、组织学分级、TNM 分期、有无淋巴结转移等临床病理资料间的的相关性进行卡方分析。结果如表 2 所示,在 43 例 Eotaxin-1 蛋白阳性表达的浆液性卵巢癌组织中,26 例(60.47%)病理分级为高度恶心的 G3 级;20 例(46.%)存在淋巴结转移;35 例(81.40%)TNM 分期为 III~IV 期。统计学分析证实,肿瘤组织中 Eotaxin-1 蛋白阳性表达与恶性组织病理分级(X^2=4.705,P=0.030)、淋巴结转移(X^2=4.294,P=0.038)及高 TNM 分期(X^2=5.044,P=0.025)均成正相关关系,提示肿瘤中 Eotaxin-1 蛋白阳性表达的患者可能预后不良。

3 讨论

在本研究中,我们通过免疫组化染色发现,Eotaxin-1 蛋白主要定位于肿瘤细胞或正常卵巢细胞的胞质内,并且在癌与癌旁组织中的表达存在显著差异。对 60 对样本中 Eotaxin-1 蛋白的表达强度分析证实,Eotaxin-1 蛋白在浆液性卵巢癌中的表达水平较癌旁组织显著升高。Agarwal 等 [11] 在前列腺癌以及 Georgiou 等[12]在肾癌中对 Eotaxin-1 蛋白表达的研究也得到了相似的结果,这提示 Eotaxin-1 蛋白可能在多种癌组织中存在表达上调现象,进而推断 Eotaxin-1 蛋白有可能作为一种生物

表 2 Eotaxin-1 表达与浆液性卵巢癌患者临床病理特征的关系 (n=60)

Table 2 The relationship between the expression of Eotaxin-1 and clinical features(n=60)

Clinical Features	Classification	Eotaxin-1 Negative (n=17)	Eotaxin-1 Positive (n=43)	X^2	P
Age	<50 year	7	8	2.216	0.137
	≥ 50 year	10	35		
Pausimenia	Yes	12	27	0.326	0.568
	No	5	16		
Tumor Valoum	＜2 cm	11	17	3.101	0.078
	≥ 2 cm	6	26		
Pathological Grade	G1~G2	12	17	4.705	0.030*
	G3	5	26		
Lymphatic Metastasis	No	14	23	4.294	0.038*
	Yes	3	20		
TNM Stage	I~II	8	8	5.044	0.025*
	III~IV	9	35		

Note:*P<0.05.

标志物,对浆液性卵巢癌患者发挥早期诊断作用。

分子生物学研究 [13,14] 证实,Eotaxin-1 可通过活化下游 MEK-1,ERK1/2 和 STAT3 信号通路来促进上皮性肿瘤细胞发生间质性转化(Epithelial-mesenchymal transition,EMT),从而增加肿瘤细胞的侵袭转移能力。我们通过统计学分析也发现,浆液性卵巢癌组织中高 Eotaxin-1 蛋白表达与患者恶性组织病理分级、淋巴结转移及高 TNM 分期显著相关，提示存在 Eotaxin-1 高表达的浆液性卵巢癌细胞恶性程度较高,易发生远处转移。临床实践证明,肿瘤转移是制约浆液性卵巢癌根治性手术的关键因素之一[15,16],而淋巴结转移及高 TNM 分期均预示着较差的放化疗效果及较短的术后生存期 [17,18]。因而,Eotaxin-1 很可能成为预测浆液性卵巢癌患者预后的有效指标之一。

目前,卵巢组织活检作为一种有创性及技术难度较高的检查[19],在临床实践中的应用并不广泛。目前,Umit Koc 等[20]的研究已经发现，胃癌患者血清中 Eotaxin-1 的表达量较正常对照组显著上升,但统计分析未发现其预后价值,提示 Eotaxin-1 表达量的改变可作为肿瘤早期诊断的分子标志物,但其与肿瘤临床病理特征及患者预后间关系尚待进一步证实。因而,在卵巢癌患者中进行大样本的血清学 Eotaxin-1 含量测定，并分析其临床价值，对进一步研究 Eotaxin-1 的肿瘤评价作用具有重要作用。

综上所述,Eotaxin-1 在浆液性卵巢癌中存在异常高表达,其表达水平与肿瘤恶性临床病理学特征显著相关。Eotaxin-1 可能成为浆液性卵巢癌早期诊断的重要标志物及分子靶向治疗的有效靶点之一,具有广阔的临床应用前景。

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