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跨膜型 TNF- α 与 NF- κ B 在三阴性乳腺癌中的表达及其意义*

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摘要 目的:探讨跨膜型 TNF- α 与磷酸化 NF- κ B 在三阴性乳腺癌(TNBC)中的表达及其临床意义。**方法:**采用免疫组化法检测 52 例三阴性乳腺癌与 30 例非三阴性乳腺癌组织中跨膜型 TNF- α 与磷酸化 NF- κ B 的表达,并分析其与三阴性乳腺癌各临床指标的相关性。**结果:**跨膜型 TNF- α 与磷酸化 NF- κ B 在三阴性乳腺癌中的阳性表达率分别为 82.7%(43/52)和 67.3%(35/52),均显著高于非三阴性乳腺癌,差异具有统计学意义($P<0.05$)。在三阴性乳腺癌中,跨膜型 TNF- α 及磷酸化 NF- κ B 的表达与肿瘤大小和淋巴结转移与否显著相关($P<0.05$),且跨膜型 TNF- α 与磷酸化 NF- κ B 的表达呈显著正相关($P<0.05$)。**结论:**跨膜型 TNF- α 及磷酸化 NF- κ B 高表达可能预示着 TNBC 更差的化疗敏感性和预后,有望成为 TNBC 预后的重要预测因子。跨膜型 TNF- α 通过激活磷酸化 NF- κ B 激活其下游的抗凋亡和促增殖因子,与三阴性乳腺癌的发生发展有关。

关键词:三阴性乳腺癌;跨膜型 TNF- α ;NF- κ B;耐药

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The Expressions and Clinical Significances of Transmembrane TNF-alpha and Nf-kappa B in Triple Negative Breast Cancer*

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ABSTRACT Objective: To discuss the expressions and clinical significances of transmembrane TNF-alpha and nf-kappa B in triple negative breast cancer. **Methods:** The expressions of transmembrane TNF-alpha and phosphorylated nf-kappa B in 52 cases of triple negative breast cancer and 30 cases of non-triple negative breast cancer were detected by immunohistochemical method and their correlation with the clinical indicators were analyzed. **Results:** The positive rates of transmembrane TNF- α and phosphorylated nf-lappa B in triple negative breast cancer were 82.7%(43/52) and 67.3% (35/52), which were significantly higher than those in the non-triple negative breast cancer. In TNBC, the expressions of transmembrane TNF- α and phosphorylated nf-kappa B were significantly positively correlated with the tumour sizes and lymph node metastasis ($P<0.05$), the expressions of transmembrane TNF- α and phosphorylated nf-kappa B were positively correlated ($P<0.05$). **Conclusions:** High expressions of transmembrane TNF alpha and phosphorylated nf-kappa B may indicate chemotherapy resistance and worse prognosis of triple negative breast cancer. Transmembrane TNF alpha activate the antiapoptotic factors and multiplication factors of downstream by activating phosphorylated nf-kappa B, which is associated with the occurrence and development of triple negative breast cancer.

Key words: Triple negative breast cancer; Transmembrane TNF-alpha; Nf-kappa B; Drug resistant

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前言

乳腺癌是目前全球女性发病率最高的恶性肿瘤之一,每年大约有 140 万新发病例^[1]。2000 年,Perou 等^[2,3]用 c-DNA 微阵列技术和免疫组化标记物将乳腺癌分为五种亚型:luminal A 型、luminal B 型、正常乳腺样型、人表皮生长因子受体 2(human epidermal growth factor receptor 2,HER2) 过表达型和基底细胞样型。随着分子生物学进展,临床上根据雌激素受体 ER(estrogen receptor)、孕激素受体 PR(progesterone receptor)及 HER-2 的表达情况将乳腺癌简化分为四类,其中三阴性乳腺癌(triple negative breast cancer, TNBC) 是指 ER、PR、HER-2 表达均为阴

性的乳腺癌,具有特殊的生物学行为,如侵袭性强,进展迅速,对内分泌治疗及靶向治疗不敏感,容易复发,生存时间短等特点,是临床研究的热点和难点^[4]。本研究通过检测跨膜型 TNF- α (transmembrane tumor necrosis factor-alpha, tm TNF- α)及核转录因子 kappa B(NF- κ B)在 TNBC 中的表达情况,旨在探讨其在 TNBC 发生和发展中的临床意义。

1 资料和方法

1.1 临床资料

收集我科 2009-2010 年乳腺癌患者中具有完整病理及临床资料的 52 例三阴性乳腺癌,并随机选择 30 例非三阴性乳腺

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癌作为对照。82 例均为原发性非特殊类型浸润性导管癌女性患者,年龄 20-69 岁,中位年龄 50 岁,术前均未行化疗或放疗,所有病例行乳腺癌改良根治术。

1.2 实验方法

每个病例获取两张石蜡包埋后切片,行免疫组织化学 SABC 法检测。第一抗体为 tm TNF- α ,磷酸化 NF- κ B(tm TNF- α 为实验室自行制备鼠抗人单克隆抗体,磷酸化 NF- κ B 为 cell signaling 公司购买兔抗人单克隆抗体,#3037)。免疫组化结果参照 Fromowitz 综合记分法分析阳性结果^[5]。Tm TNF- α 为胞膜和胞质阳性,磷酸化 NF- κ B 为胞核阳性(附图 1-4)。

1.3 统计学分析

应用 SPSS13.0 软件,采用组间比较及配对四格表资料的 χ^2 检验进行统计学分析,以 $P < 0.05$ 为差异有统计学意义。

2 结果与分析

在三阴性乳腺癌中,tm TNF- α 的阳性表达率为 82.7% (43/52),磷酸化 NF- κ B 的阳性表达率为 67.3%(35/52),均显著高于非三阴性乳腺癌,差异均具有统计学意义($P < 0.05$,表 1)。三阴性乳腺癌中,磷酸化 NF- κ B 和 tm TNF- α 的阳性表达率均与乳腺癌的肿瘤大小和淋巴结转移与否具有显著性相关($P < 0.05$,表 2),且 tm TNF- α 与 NF- κ B 的阳性表达率呈显著正相关性($P < 0.05$,表 3)。

表 1 TNBC 和非 TNBC 中 tm TNF- α 与磷酸化 NF- κ B 阳性表达率的比较

Table 1 Comparison of the positive rates of tm TNF- α and phosphorylated nf-kappa B between TNBC and non-TNBC

	TNBC(52cases)	non-TNBC(30cases)	P value
tm TNF- α (+)	43(82.7%)	17(56.7%)	0.01
tm TNF- α (-)	9	13	
NF- κ B(+)	35(67.3%)	13(43.3%)	0.03
NF- κ B(-)	17	17	

表 2 TNBC 中 tm TNF- α 和 NF- κ B 的阳性表达率与临床指标之间的相关性

Table 2 Correlation of the positive expression of tm TNF- α and NF- κ B with the clinical indicators of triple negative breast cancer

	tm TNF- α (+)	tm TNF- α (-)	P value	NF- κ B(+)	NF- κ B(-)	total	P value
Age							
≤ 40y	12	4	0.328	11	5	16	0.882
> 40y	31	5		24	12	36	
Tumor sizes							
≤ 2cm	11	7	0.003	6	12	18	0.0001
> 2cm	32	2		29	5	34	
Lymph node metastasis							
Yes	8	5	0.020	12	1	13	0.039
no	35	4		23	16	39	
WHO grades							
I	0	0	0.130	0	0	0	0.614
II	38	6		29	15	44	
III	5	3		6	2	8	

表 3 TNBC 中 tm TNF- α 与 NF- κ B 的阳性表达率之间的相关性

Table 3 Correlation of the positive expression of tm TNF- α with NF- κ B in triple negative breast cancer

	tm TNF- α (+)	tm TNF- α (-)	P value
NF- κ B(+)	34	1	0.002
NF- κ B(-)	9	8	

3 讨论

跨膜型 TNF- α 是分泌型 TNF- α (secretory TNF- α , s TNF- α) 的前体,首先表达在细胞膜上,经 TNF 转化酶水解,释放其胞外段而成为 s TNF- α ,两型 TNF- α 均通过 TNF 受体(TNFR)结

合发挥效应^[6]。早期研究发现^[7,8] tm TNF- α 与 TNFR 结合后,除了向受体表达细胞(靶细胞)传递“正向信号”外,也向配体表达细胞(效应细胞)传递“反向信号”。我国学者在此理论上首次证实^[9-11] tm TNF- α 通过反向信号持续性活化肿瘤细胞的 NF- κ B,诱导抗凋亡分子表达,从而抵抗 s TNF- α 引起的凋亡,

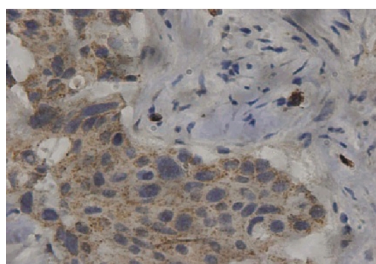
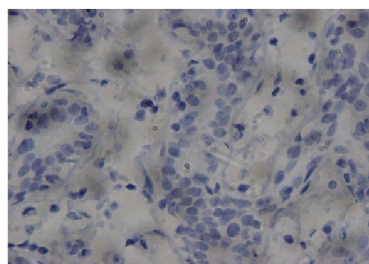
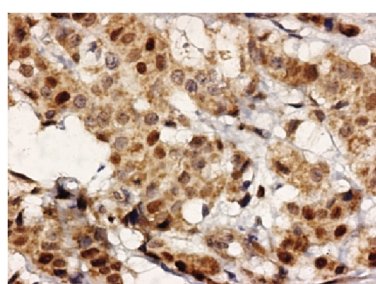
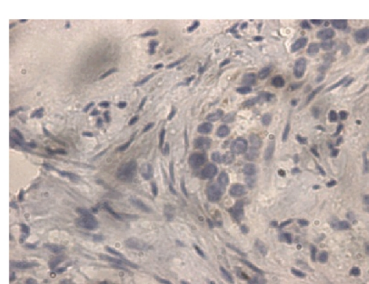
这一机制可能是肿瘤细胞耐药的关键机制。国内外的大量体外实验^[12-14]也逐步验证了 TNF- α 在乳腺肿瘤中促进增殖和侵袭进展等恶性生物学行为的关键作用。相关回顾性研究显示^[15,16], 受体阴性的乳腺癌对化疗的敏感性较受体阳性者高, 三阴性乳腺癌相比于其他类型乳腺癌而言, 对传统的化疗药的敏感性高, 但其总的预后仍然很差, 可能与其缺乏有效的靶点药物, 常规化疗后产生耐药有关。

本研究中, tm TNF- α 在 82 例乳腺癌中的阳性表达率为 73.2%, 在 TNBC 中表达率显著高于非 TNBC (82.7% vs 56.7%), 提示 tm TNF- α 可能与三阴性乳腺癌的发生有关。在进一步探讨其与 TNBC 各项临床病理特征之间的关系, 结果显示 TNBC 中 tm TNF- α 的阳性表达率与肿瘤的大小和淋巴结是否转移显著相关 ($P < 0.05$)。但不排除本组数据中淋巴结转移病例较少, 缺乏远处转移病例, 且在选择完整病理资料筛除不完整资料而造成选择偏倚。多中心研究显示肿瘤大小和淋巴结转移的情况是影响乳腺癌预后的重要因素^[17]。Tm TNF- α 有望成为 TNBC 预后的重要预测因子。

核转录因子 kappa B(NF- κ B)在细胞中通常存在两种形式, 在静息状态下, NF- κ B 与抑制蛋白 I κ B 结合以非活化状态存在于胞浆内。在 LPS 等多种因素的刺激下, I κ B 被 IKK 磷酸化而迅速降解, NF- κ B 释放并进入细胞核内与靶基因结合, 激活下

游的抗凋亡基因并且抑制促凋亡的蛋白表达从而抵抗凋亡^[18]。相关研究证实^[19,21]在 B 淋巴细胞白血病及乳腺癌等肿瘤细胞中发现持续活化的 NF- κ B, 表达活化型 NF- κ B(即磷酸化 NF- κ B)的患者对化疗的敏感性明显低于不表达活化型 NF- κ B 的患者, 通过抑制 NF- κ B 的活化, 可逆转乳腺癌细胞对蒽环类药物的敏感性, 抑制 TNF- α 介导的乳腺肿瘤细胞的迁移和侵袭。本研究结果显示, 三阴性乳腺癌中, 磷酸化 NF- κ B 的阳性表达率为 67.3%, 显著高于非三阴性乳腺癌, 且与 tm TNF- α 的表达具有显著相关性, 表明在 TNBC 中, tm TNF- α 通过反向信号激活 NF- κ B 的磷酸化, 激活下游的抗凋亡和促进增殖因子, 从而促进 TNBC 的发生和发展。进一步探讨 NF- κ B 与临床病理特征之间的关系, 结果显示 TNBC 中 NF- κ B 的阳性表达率与肿瘤的大小和淋巴结是否转移显著相关 ($P < 0.05$), 与 tm TNF- α 的研究结果类似, 再次验证了 tm TNF- α -NF- κ B 通路在 TNBC 中的重要作用, 并且为 TNBC 的预后判断提供了更多的参考因素。

综上所述, tm TNF- α 及磷酸化 NF- κ B 高表达可能预示着三阴性乳腺癌更差的化疗敏感性和预后, 有望成为三阴性乳腺癌预后的重要预测因子。跨膜型 TNF- α 通过激活磷酸化 NF- κ B 激活其下游的抗凋亡和促增殖因子, 与三阴性乳腺癌的发生发展有关。

Fig.1 tmTNF- α (Positive)Fig.2 tmTNF- α (Negative)Fig.3 NF- κ B(Positive)Fig.4 NF- κ B(Negative)

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