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研究论文

TNFα诱导STAT3信号转导的分子机制研究

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摘要 该文探讨肿瘤坏死因子α(TNFα)活化信号转导和转录激活因子3(STAT3)的分子机制。采用流式细胞术(FACS)检测TNF受体TNFR1在鼻咽癌细胞5-8F和宫颈癌细胞HeLa中的蛋白表达水平; qRT-PCR检测TNFα对其受体*TNFR1*和*TNFR2*的mRNA水平的影响; ELISA检测细胞因子白细胞介素8(IL-8)的蛋白水平; Western blot检测受体和信号转导分子的总蛋白水平及蛋白磷酸化水平。结果显示, 5-8F和HeLa细胞表达功能性的TNF受体和表皮生长因子受体(EGFR); TNFα处理细胞可诱导STAT3的活化, 且呈时间和剂量依赖性; TNFα也能活化EGFR, 用EGFR的抑制剂进行处理, 逆转了TNFα诱导的EGFR(Y1068)的磷酸化, 也逆转了STAT3的磷酸化; 进一步研究结果显示, TNFα可活化促癌酪氨酸蛋白激酶SRC, 用SRC抑制剂处理, 逆转了TNFα诱导的EGFR活化及其下游STAT3的磷酸化。总之, 在肿瘤细胞中存在TNFα-SRC-EGFR-STAT3信号转导通路, 提示EGFR可能是炎症诱导肿瘤的桥梁。

关键词 肿瘤坏死因子α; 信号转导和转录激活因子3; 表皮生长因子受体; SRC; 信号转导

The Molecular Mechanism of STAT3 Signal Transduction Induced by TNFα

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Abstract This study explored the molecular mechanism of STAT3 signal transduction induced by TNFα (tumor necrosis factor α). FACS was employed to determine the expression of TNF receptor TNFR1 in 5-8F nasopharyngeal carcinoma cells and HeLa cervical cancer cells. qRT-PCR was used to measure the mRNA levels of *TNFR1* and *TNFR2* induced by TNFα. ELISA was used to measure the protein levels of cytokine IL-8. Western blot was used to detect the total and phosphorylated protein levels of receptors and signal molecules. The results showed that 5-8F and HeLa cells expressed functional TNF receptor and EGFR. The treatment of cells with TNFα induced the activation of STAT3 in a time-dependent and dose-dependent manner. TNFα could also activate EGFR. Treatment with EGFR inhibitors reversed the phosphorylation of EGFR (Y1068) induced by TNFα and also reversed the phosphorylation of STAT3. Furthermore, TNFα activated the pro-cancer tyrosine protein kinase SRC. After treatment with SRC inhibitors, TNFα-induced EGFR activation and downstream STAT3 phosphorylation were reversed.

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In conclusion, in certain cancer cells, the signal transduction pathway TNFα-SRC-EGFR-STAT3 is existed, and EGFR may be a bridge for linking inflammation with cancer.

Keywords TNFα; STAT3; EGFR; SRC; signal transduction

肿瘤与炎症的关系一直是研究的热点问题, 研究发现: 高达20%的癌症与慢性炎症有关[1]。急性炎症转变为慢性炎症有可能导致肿瘤, 许多肿瘤与微生物的持续存在有关, 如: 鼻咽癌与EB病毒, 宫颈癌与HPV病毒。但慢性炎症引起肿瘤发生的具体信号机制尚未完全清楚。

肿瘤坏死因子α(tumor necrosis factor α, TNFα)是慢性炎症发生过程中最重要的炎症因子之一, 具有促肿瘤功能[2]。TNFα与其受体结合, 活化一系列的信号通路, 如核因子-κB(nuclear factor kappa B, NF-κB)通路、丝裂原激活的蛋白激酶(mitogen-activated protein kinase, MAPK)通路等, 参与肿瘤发生发展[3]。

信号转导和转录激活因子3(signal transducer and activator of transcription 3, STAT3)是一种参与细胞生长、凋亡、癌变等多种生命活动的信号转录蛋白[4-5], 它作为多条致癌途径的汇合点, 对肿瘤发生发展起至关重要的作用。研究表明, TNFα能直接或间接诱导STAT3激活, 促进肿瘤发生发展[3,6]。此外, 有研究表明, 表皮生长因子受体(epidermal growth factor receptor, EGFR)可诱导STAT3的激活[7]。本文研究鼻咽癌和宫颈癌中存在的重要信号转导通路, 旨在为临床治疗提供新的思路。

1 材料与方法

1.1 实验材料和试剂

重组人TNFα购自美国Peprotech公司; EGFR特异性酪氨酸激酶抑制剂AG490和AG1478、SRC酪氨酸激酶抑制剂PP2购自英国Tocris Bioscience公司; pEGFR(Y992、Y1045和Y1068)和EGFR抗体, ERK, JNK, P38, IκB-α和STAT3抗体、鼠抗人GAPDH抗体均购自美国Cell Signaling Technology公司; HeLa和5-8F细胞购自美国ATCC公司。

1.2 实验方法

1.2.1 细胞培养 复苏HeLa细胞和5-8F细胞, 用含有10% FCS和1%青–链霉素的DMEM培养基, 于37 °C、5% CO_2培养箱中培养, 待细胞生长至对数期, 用胰蛋白酶消化, 铺于24孔板中。加入各种试剂处理细胞, 收集细胞以用于后续检测。

1.2.2 Western blot分析 将收集好的细胞用100 μL细胞裂解液充分裂解后提取蛋白质, 加入5× 上样缓冲液, 100 °C煮沸5 min, 用10% SDS-PAGE凝胶分离, 将蛋白转移至PVDF膜上, 用5%的脱脂奶粉于37 °C封闭2 h, 一抗(1:1 000) 4 °C孵育过夜, 二抗(1:2 000) 37 °C孵育2 h, 用ECL化学发光法进行显色、显影。

1.2.3 qRT-PCR分析 Trizol法提取细胞总RNA, 按照试剂盒说明书将其逆转录成cDNA, 以1 μL的cDNA为模板进行PCR扩增, PCR的反应体系: 1 μL cDNA模板, 上下游引物各1 μL, 7 μL灭菌纯水, 10 μL 2× Taq Master Mix。PCR反应的条件: 94 °C预变性1 min; 95 °C变性30 s, 60 °C退火30 s, 68 °C延伸1 min, 根据需要选择扩增20~30个循环(内参基因*GAPDH*进行20个循环, 其他目的基因进行25个循环); 68 °C终末延伸10 min。PCR产物在1%的琼脂糖凝胶中电泳, 用GelRed显色并拍照。*GAPDH*引物序列如下: 上游引物5′-AAT CCC ATC ACC ATC TTC CA-3′, 下游引物5′-CCT GCT TCA CCA CCT TCT TG-3′, 扩增长度为262 bp; *TNFR1*引物序列如下: 上游引物5′-TCA GGC ACC ACA GTG CTG TT-3′, 下游引物5′-TGG AGG TGA AGG TGG AAC TG-3′, 扩增长度为265 bp; *TNFR2*引物序列如下: 上游引物5′-TGA CCA GAC AGC TCA GAT GTG-3′, 下游引物5′-ACT GCA TCC ATG CTT GCA TT-3′, 扩增长度为459 bp。

1.2.4 ELISA分析 收集细胞培养上清, 依照ELISA试剂盒说明书进行检测。主要步骤为: 取出酶标板, PBS洗3次, 设置空白对照孔、阳性对照孔、待测样品孔和标准品孔, 每孔加入100 μL样品, 4 °C过夜, PBS洗3次, 加入二抗(1:1 000), 37 °C孵育 1 h, PBS洗3次, 加入酶标试剂, 37 °C孵育30 min, PBS洗3次, 加入显色剂, 37 °C避光显色15 min, 加入终止液, 测吸光度(*D*)值, 绘制标准曲线并计算样品中IL-8的浓度。每个样品设置3个重复孔。

1.2.5 流式细胞仪检测TNFR1蛋白水平 收集细胞, 用FACS缓冲液(由5 mmol/L EDTA、0.1% NaN3和1%胎牛血清配制而成)洗涤。加入抗人TNFR1的抗体, 冰上孵育30 min, 加入FITC标记的

二抗(1:1 000), 室温放置30 min, 流式细胞仪检测荧光强度。每个样品计数10 000个细胞。

1.3 统计学分析

实验结果数据均采用均数±标准差($\bar{x}$±s)表示。作图和统计分析分别采用Excel、GraphPad Prism 8软件, 两组间的差异显著性统计使用双尾Student's t检验, Western blot条带和PCR凝胶电泳条带用Image J软件定量。P<0.05为差异具有统计学意义(对于每个qRT-PCR和ELISA实验样品, 重复数n=3)。

2 结果

2.1 5-8F和HeLa细胞表达功能性的TNF受体

鼻咽癌与EBV相关[8], 宫颈癌与HPV相关[9]。利用FACS方法, 首先检测5-8F鼻咽癌细胞和HeLa宫颈癌细胞中TNF受体的表达, 结果显示5-8F和HeLa细胞表达TNFR1(图1A); 用20 ng/mL TNFα处理细胞后, qRT-PCR结果显示在HeLa细胞中, TNFα小幅上调*TNFR1*和*TNFR2*的mRNA水平(图1B); 用不同浓度的TNFα处理细胞24 h, ELISA结果显示TNFα显著上调IL-8的蛋白水平, 并具有剂量依赖性(图1C)。这些结果表明, 5-8F和HeLa细胞表达功能性TNF受体。

2.2 TNFα激活MAPK和NF-κB信号通路

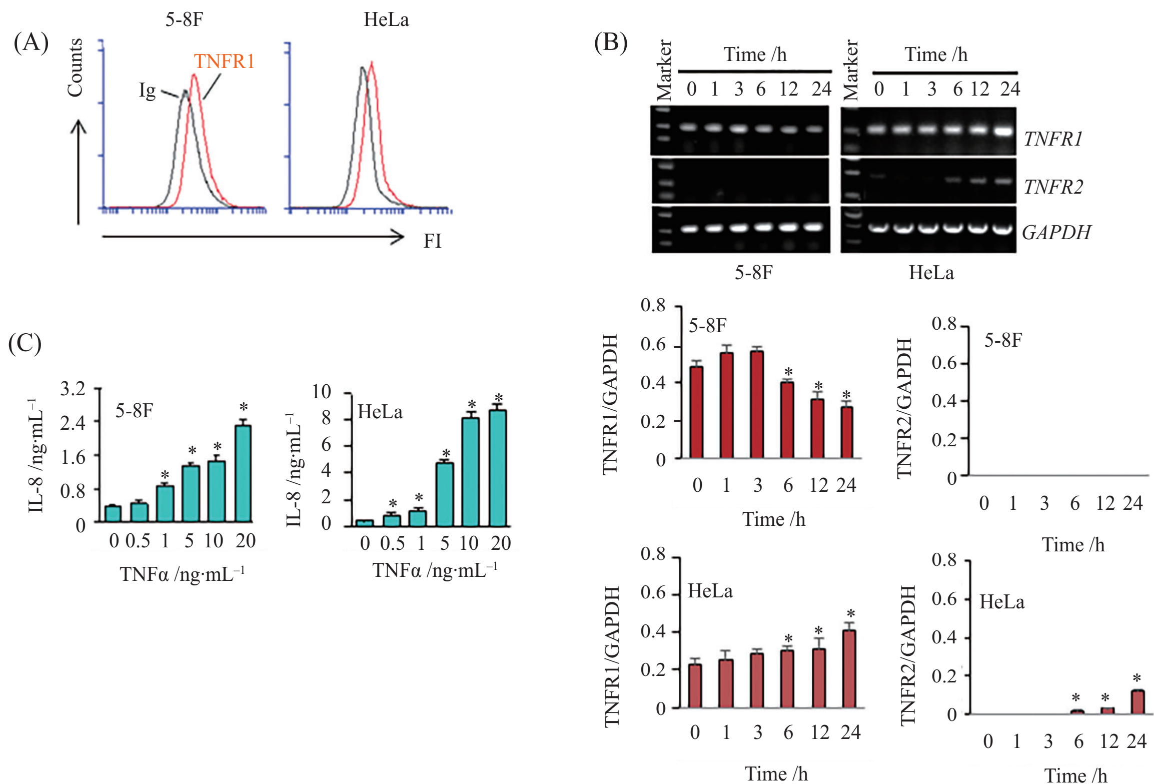
Western blot结果显示在5-8F和HeLa细胞中, TNFα可诱导ERK、JNK、P38磷酸化, 下调NF-κB抑制IκB-α的蛋白水平, 并具有时间和剂量依赖性(图2A和图2B); 这些结果表明在5-8F和HeLa中, TNFα可激活MAPK通路和NF-κB信号通路。

2.3 5-8F和HeLa细胞表达功能性EGFR

以20 ng/mL EGF处理细胞不同时间后, Western blot实验结果显示与空白组相比, EGF诱导了EGFR(Y992、Y1045和Y1068)磷酸化(图3), 表明5-8F和HeLa细胞表达功能性的EGFR。

2.4 TNFα活化STAT3

用20 ng/mL的TNFα处理细胞, Western blot

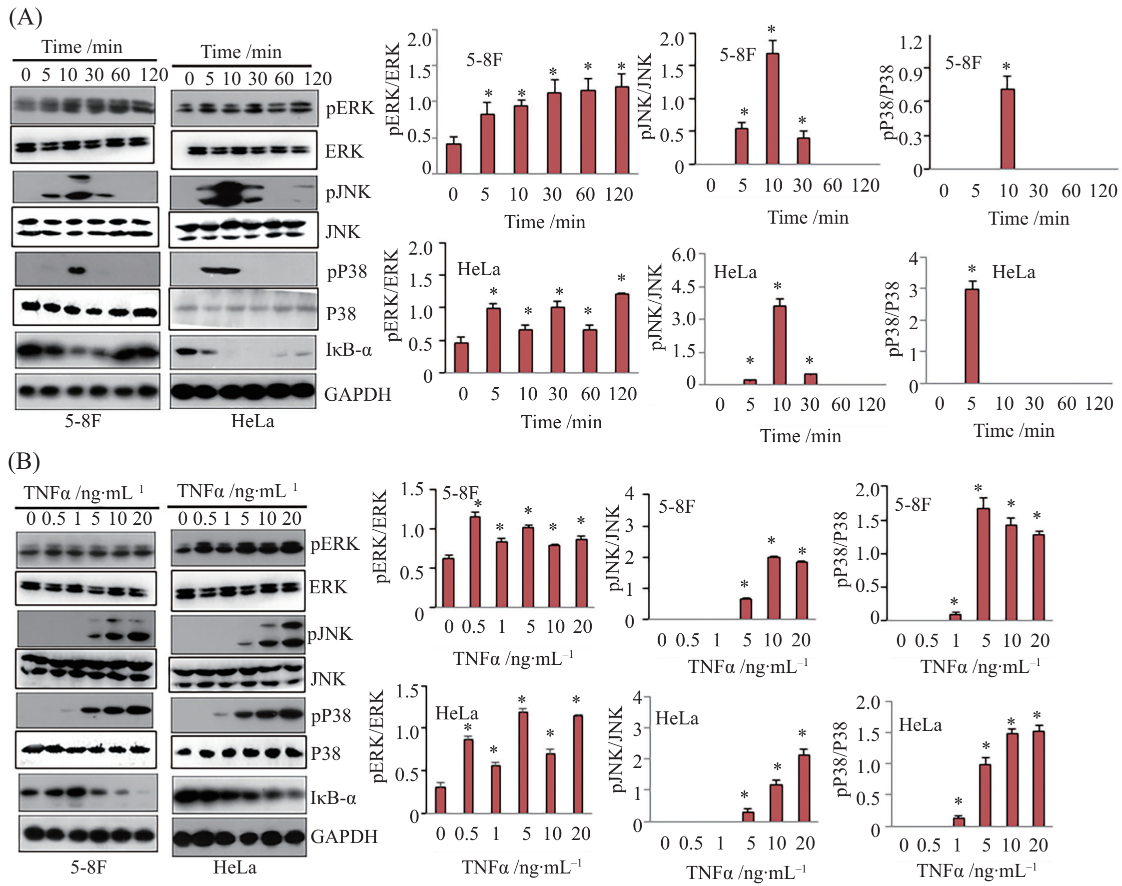


A: FACS分析5-8F和HeLa细胞中TNFR1表达情况。B: qRT-PCR检测5-8F和HeLa细胞经TNFα(20 ng/mL)处理不同时间后*TNFR1*和*TNFR2*的mRNA表达水平。C: ELISA检测5-8F和HeLa细胞经不同浓度TNFα处理24 h后IL-8的表达情况; *P<0.05, 与空白对照组相比。

A: FACS analysis of the TNFR1 expression in 5-8F and HeLa cells. B: qRT-PCR analysis of the mRNA levels of *TNFR1* and *TNFR2* in 5-8F and HeLa cells treated with 20 ng/mL TNFα for the indicated time periods. C: ELISA analysis of the IL-8 protein levels in 5-8F and HeLa cells treated with the indicated concentrations of TNFα for 24 h. *P<0.05 compared with control group.

图1 5-8F和HeLa细胞表达功能性的TNF受体

Fig.1 5-8F and HeLa cells express functional TNF receptor

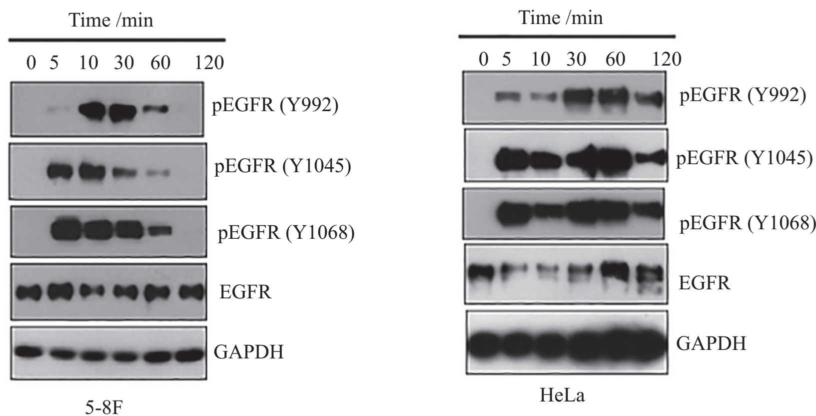


A: Western blot检测5-8F和HeLa细胞经20 ng/mL TNFα处理不同时间后的磷酸化ERK、JNK、P38, 总ERK、JNK、P38以及IκB-α的蛋白质表达水平。B: Western blot检测5-8F和HeLa细胞经不同浓度TNFα处理10 min后的磷酸化ERK、JNK、P38, 总ERK、JNK、P38以及IκB-α的蛋白质表达水平。以GAPDH总蛋白水平作为内对照。*P<0.05, 与空白对照组相比。

A: Western blot analysis of the phosphorylated and total levels of ERK, JNK, P38, and the protein levels of IκB-α in 5-8F and HeLa cells treated with 20 ng/mL TNFα for the indicated time periods. B: Western blot analysis of the phosphorylated and total levels of ERK, JNK, P38, and the protein levels of IκB-α in 5-8F and HeLa cells treated with the indicated concentrations of TNFα for 10 min. GAPDH protein levels were measured as loading controls. *P<0.05 compared with control group.

图2 TNFα激活MAPK信号通路

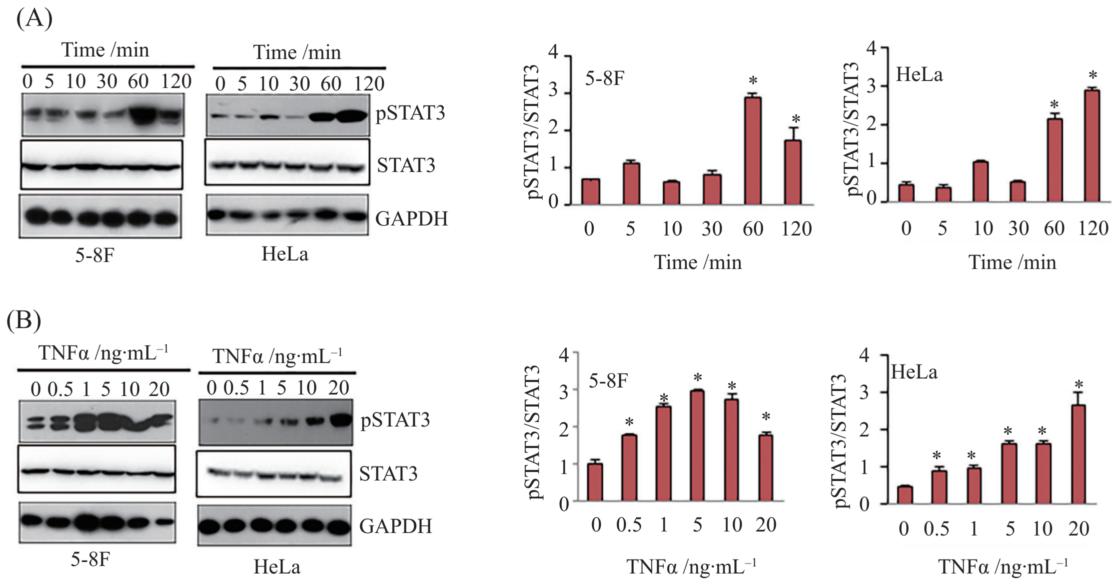
Fig.2 TNFα activates MAPK signaling pathway



Western blot检测5-8F和HeLa细胞经EGF(20 ng/mL)处理不同时间后的pEGFR(Y992、Y1045和Y1068)表达情况。检测GAPDH总蛋白水平作为内对照。
Western blot analysis of the phoshorylated levels of EGFR in 5-8F and HeLa cells treated with 20 ng/mL EGF for indicated time periods. GAPDH protein levels were measured as loading controls.

图3 5-8F和HeLa细胞表达功能性的EGFR

Fig.3 5-8F and HeLa cells express functional EGFR



A: Western blot检测5-8F和HeLa细胞经20 ng/mL TNFα处理不同时间的p-STAT3的表达水平。B: Western blot检测5-8F和HeLa细胞经不同浓度TNFα处理1 h的p-STAT3的表达水平。以GAPDH总蛋白水平作为内对照。*P<0.05, 与空白对照组相比。

A: Western blot analysis of the phosphorylated levels of STAT3 in 5-8F and HeLa cells treated with 20 ng/mL TNFα for the indicated time periods. B: Western blot analysis of the phosphorylated levels of STAT3 in 5-8F and HeLa cells treated with the indicated concentrations of TNFα for 1 h. GAPDH protein levels were measured as loading controls. *P<0.05 compared with control group.

图4 TNFα激活5-8F和HeLa细胞中的STAT3

Fig.4 TNFα activates STAT3 in 5-8F and HeLa cells

检测STAT3的磷酸化, 结果显示处理细胞60 min至120 min后, p-STAT3水平显著增加(图4A)。再用不同浓度的TNFα处理细胞60 min, 结果显示TNFα呈剂量依赖性地诱导STAT3的磷酸化(图4B), 表明TNFα可活化STAT3。

2.5 TNFα活化STAT3与EGFR有关

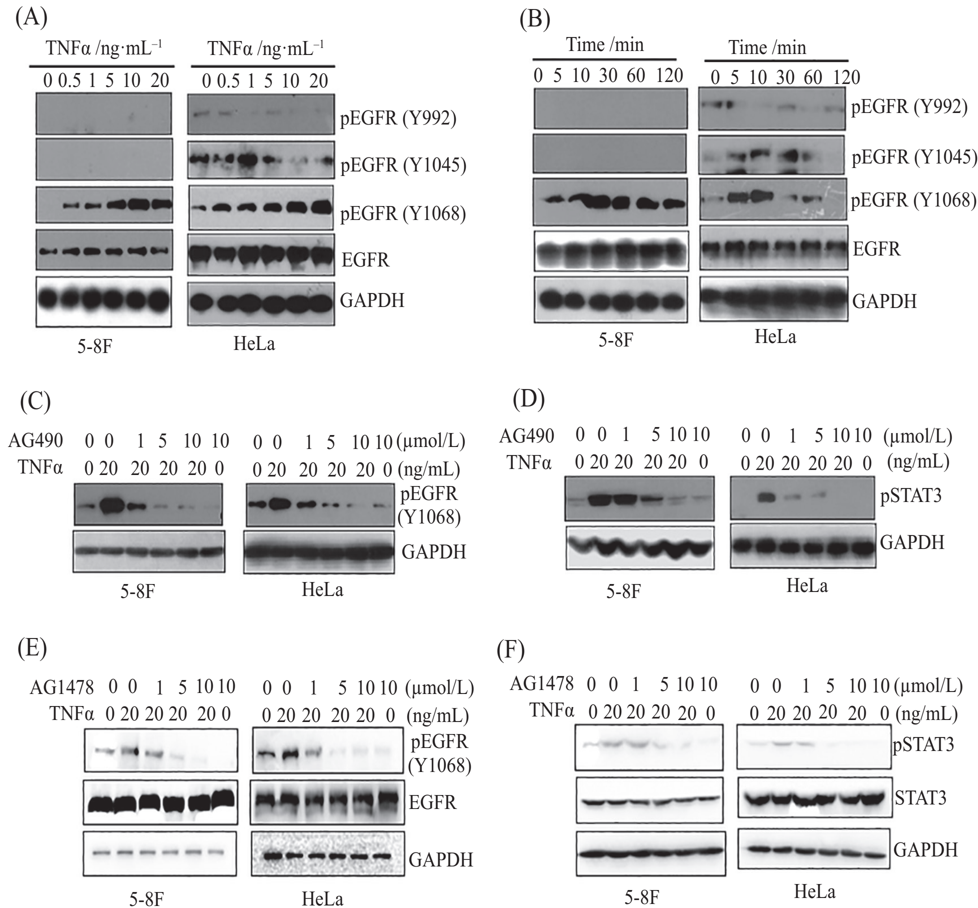
有文献报道TNFα能转移活化EGFR[10]。为了确定TNFα活化STAT3是否与EGFR有关, 先用TNFα处理细胞, 然后检测EGFR的磷酸化水平, Western blot结果显示TNFα可活化EGFR(Y1068), 且呈时间和剂量依赖性(图5A和图5B)。用EGFR的抑制剂AG490或AG1478预处理细胞1 h, 然后再加入TNFα处理细胞1 h, Western blot结果显示AG490逆转了TNFα诱导的EGFR(Y1068)的磷酸化(图5C), 也逆转了STAT3的磷酸化(图5D); 同样, 用EGFR的抑制剂AG1478预处理细胞1 h, 然后再加入TNFα处理细胞1 h, Western blot结果显示AG1478逆转了TNFα诱导的EGFR(Y1068)的磷酸化(图5E), 也逆转了STAT3的磷酸化(图5F)。这些结果表明, EGFR参与了TNFα诱导的STAT3活化。

2.6 TNFα活化STAT3与SRC有关

EL-HASHIM等[11]研究发现, SRC参与活化EGFR。用20 ng/mL的TNFα处理细胞, Western blot检测SRC的磷酸化, 结果显示TNFα呈时间依赖性地诱导SRC的活化(图6A); 用不同浓度的TNFα处理细胞10 min, Western blot检测SRC的磷酸化, 结果显示TNFα呈剂量依赖性地诱导SRC的活化(图6B); 进一步用SRC抑制剂PP2预处理细胞1 h, 再用TNFα处理细胞10 min, Western blot结果显示PP2预处理逆转了TNFα诱导的EGFR活化(图6C); 同样用SRC抑制剂PP2预处理细胞1 h, 再用TNFα处理细胞1 h, Western blot结果显示PP2预处理逆转了TNFα诱导的STAT3活化(图6D)。这些结果表明, SRC参与了TNFα活化STAT3。

3 讨论

STAT3位于17号染色体(17q21.2)上, 有24个外显子, 它包括6个功能区: N-端结构域、螺旋结构域、DNA结合域、链接区、Src同源结构2区、C-端转录激活结构域[12-13]。受体酪氨酸激酶(receptor tyrosine kinase, RTK)如EGFR、非受体酪氨酸激酶、生长因子以及干扰素(interferon, IFN)等配体均可催化STAT3的酪氨酸磷酸化。如: IL-6与IL-6的受体(IL-6R)结合, 通过一系列反应激活JAK/STAT3信号通路, 活化的STAT3与酪氨酸磷酸化位



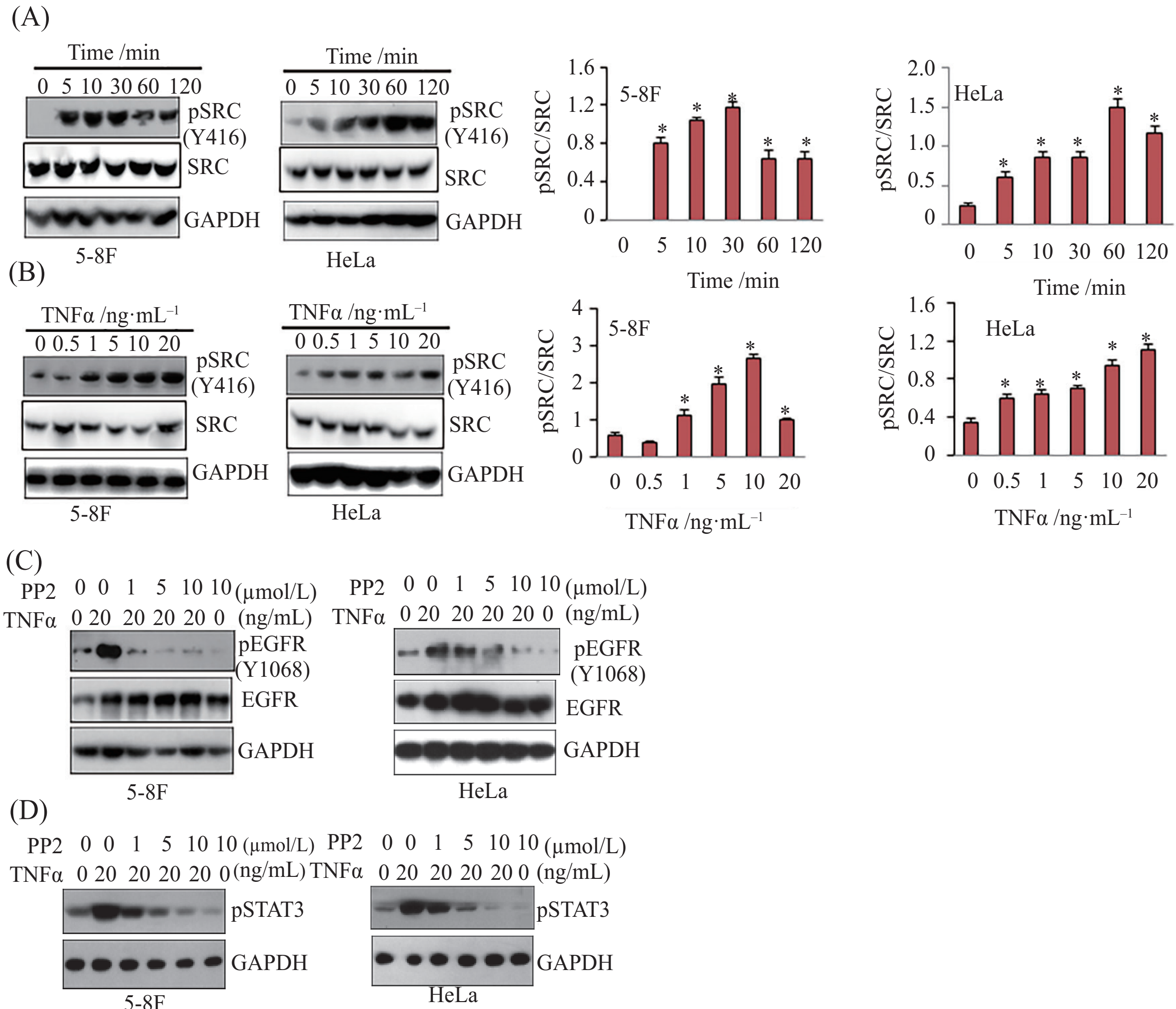
A: Western blot检测5-8F和HeLa细胞经不同浓度TNFα处理10 min后的pEGFR(Y992、Y1045和Y1068)表达情况。B: Western blot检测5-8F和HeLa细胞经20 ng/mL TNFα处理不同时间后的pEGFR(Y992、Y1045和Y1068)表达情况。C: Western blot检测5-8F和HeLa细胞经AG490预处理1 h后, 用20 ng/mL TNFα再处理1 h后的pEGFR(Y1068)表达情况。D: Western blot检测5-8F和HeLa细胞经AG490预处理1 h后, 用20 ng/mL TNFα再处理1 h后的pSTAT3表达情况。E: Western blot检测5-8F和HeLa细胞经AG1478预处理1 h后, 用20 ng/mL TNFα再处理1 h后的pEGFR(Y1068)和EFGR表达情况。F: Western blot检测5-8F和HeLa细胞经AG1478预处理1 h后, 用20 ng/mL TNFα再处理1 h后的pSTAT3和STAT3表达情况。检测GAPDH总蛋白水平作为内对照。

A: Western blot analysis of the phosphorylated levels of EGFR (Y992, Y1045, and Y1068) in 5-8F and HeLa cells treated with the indicated concentrations of TNFα for 10 min. B: Western blot analysis of the phosphorylated levels of EGFR (Y992, Y1045, and Y1068) in 5-8F and HeLa cells treated with 20 ng/mL TNFα for the indicated time periods. C: Western blot analysis of the phosphorylated levels of EGFR (Y1068) and in 5-8F and HeLa cells pretreated with the indicated concentrations of AG490 for 1 h and retreated with 20 ng/mL TNFα for 1 h. D: Western blot analysis of the phosphorylated levels of STAT3 in 5-8F and HeLa cells pretreated with the indicated concentrations of AG490 for 1 h and retreated with 20 ng/mL TNFα for 1 h. E: Western blot analysis of the phosphorylated and total levels of EGFR (Y1068) in 5-8F and HeLa cells pretreated with the indicated concentrations of AG1478 for 1 h and retreated with 20 ng/mL TNFα for 1 h. F: Western blot analysis of the phosphorylated and total levels of STAT3 in 5-8F and HeLa cells pretreated with the indicated concentrations of AG1478 for 1 h and retreated with 20 ng/mL TNFα for 1 h. GAPDH protein levels were measured as loading controls.

图5 EGFR参与TNFα诱导的STAT3活化

Fig.5 EGFR involves in the activation of STAT3 induced by TNFα

点结合形成二聚体后进入细胞核, 引起靶基因发生转录, 从而产生相应的效应[14-17], 参与调节细胞增殖、分化、凋亡, 血管生成, 炎症和免疫反应等多种生理过程。STAT3异常激活会引发致癌基因的表达, 促进肿瘤的发生发展。例如在肺癌、乳腺癌、宫颈癌和卵巢癌等肿瘤中STAT3的表达水平显著增高; STAT3激活也参与细胞增殖和生存; 如: STAT3的持续激活可诱导CyclinD1和c-Myc上调, 促进肾脏和结肠癌的细胞周期进程[18]。在肝癌中, TNFα可以通过NF-κB、JNK和STAT3等信号通路促进肿瘤的发生发展[19-20]; 在胰腺癌患者的血浆及组织中也能检测到低水平的TNFα以及激活的NF-κB通路[21]; 结肠癌患者高表达TNFα[22]; 同时有研究发现, TNFα诱导蛋白8(tumor necrosis factor-



A: Western blot检测5-8F和HeLa细胞经20 ng/mL TNFα处理不同时间后的pSRC(Y416)表达情况。B: Western blot检测5-8F和HeLa细胞经不同浓度TNFα处理10 min的pSRC(Y416)表达情况。检测GAPDH总蛋白水平作为内对照。C: Western blot检测5-8F和HeLa细胞经SRC抑制剂PP2预处理1 h后, 用20 ng/mL TNFα再处理1 h观察pEGFR(Y1068)表达情况。D: Western blot检测5-8F和HeLa细胞经SRC抑制剂PP2预处理1 h后, 用20 ng/mL TNFα再处理1 h观察pSTAT3表达情况。检测GAPDH总蛋白水平作为内对照。*$P<0.05$, 与空白对照组相比。

A: Western blot analysis of the phosphorylated levels of SRC in 5-8F and HeLa cells treated 20 ng/mL TNFα for the indicated time periods B: Western blot analysis of the phosphorylated levels of SRC in 5-8F and HeLa cells treated with the indicated concentrations of TNFα for 10 min. GAPDH protein levels were measured as loading controls.C: Western blot analysis of the phosphorylated levels of EGFR in 5-8F and HeLa cells treated with the indicated concentrations of SRC inhibitor PP2 for 1 h and retreated with 20 ng/mL TNFα for 1 h. D: Western blot analysis of the phosphorylated levels of STAT3 in 5-8F and HeLa cells pretreated with the indicated concentrations of PP2 for 1 h and retreated with 20 ng/mL TNFα for 1 h. GAPDH protein levels were measured as loading controls. *$P<0.05$ compared with control group.

图6 SRC参与TNFα诱导的STAT3激活

Fig.6 SRC involves in the activation of STAT3 induced by TNFα

alpha-induced protein 8, TNFAIP8)可促进鼻咽癌的发生[23]; YU等[24]发现, TNFα是引起鼻咽癌的原始因子, 当TNFα表达增高时, 鼻咽癌预后不良。因此, TNFα具有促进肿瘤发生发展的作用。在本研究中, 我们使用TNFα处理5-8F和HeLa细胞后发现, STAT3的磷酸化水平随着TNFα的剂量增加而上调, 提示TNFα刺激可能激活STAT3信号转导。此结果与XU等[25]在乳腺癌中的研究结果一致, 该研究发现用TNFα处理HeLa细胞能激活STAT3, 促进肿瘤发生与生长。同时我们的研究发现, 5-8F和HeLa细胞表达功能性的TNF受体, 这一结果与大量文献所述一致[26-29]。

EGFR也被称为HER1或ErbB1, 是酪氨酸激酶受体ErbB系列的第一个成员, 它是一种170 kDa的跨膜糖蛋白[25,30], 具有3个不同的结构域, 分别为: (1) 胞外区, 与配体结合; (2) 疏水性跨膜区, 参与受

体之间的二聚化; (3) 胞内酪氨酸激酶结构域, 使底物蛋白的酪氨酸磷酸化, 从而启动一系列下游信号通路, 参与调节细胞增殖、分化和存活[31]。据文献报道, EGFR在结肠癌、肺癌、乳腺癌和头颈癌等恶性肿瘤中均过表达[25]。SABBAH等[32]发现, EGFR过表达会促进癌症的发生发展。同时ESKILSSON等[33]发现, EGFR vIII促进胶质母细胞瘤增殖。CHEN等[34]发现, EGFR-PKM2信号通路促进鼻咽癌的发生。PENG等[35]发现, EGFR会促进EB病毒感染鼻咽癌细胞。此外, EGFR活化后会影响鼻咽癌细胞增殖、细胞周期、血管生成、入侵和转移等过程。由此可见, EGFR与肿瘤发生发展有关。LIU等[36]在小鼠肺组织模型中发现, EGFR突变的小细胞肺癌(non-small cell lung cancer, NSCLC)高表达TNFα。我们研究证实在宫颈癌和鼻咽癌细胞中, TNFα能激活EGFR, 表明TNFα可能通过活化EGFR发挥其促肿瘤作用。

SRC属于SRC激酶家族(Src family kinases, SFKs)。该家族由11个成员组成, 其中SRC、Yes和Fyn在哺乳动物中均表达[37]。SRC有4个不同的结构域, 分别为N-端结构域, 与细胞质膜相互作用; SH3结构域, 特异性识别富含脯氨酸序列的蛋白质; SH2结构域和SH1结构域。在肿瘤细胞中, SRC激酶过表达会促进肿瘤细胞增殖和迁移、新血管生成和转移[38]。据大量文献报道, G蛋白偶联受体可以转移活化EGFR, 而SRC是转移活化EGFR的中心环节[39-41]。我们研究发现, 抑制SRC可逆转TNFα诱导的EGFR磷酸化及其下游的STAT3磷酸化, 提示TNFα通过SRC活化EGFR进而诱导STAT3信号转导, 表明TNFα活化STAT3也需要SRC的参与。

长期感染微生物会引起慢性炎症的发生发展, 从而导致TNFα表达水平升高。有核细胞高表达TNF受体和EGFR, TNFα与受体结合可转移激活EGFR, 并活化其下游促癌分子STAT3, 导致肿瘤发生。因此, 推断EGFR是慢性炎症诱导肿瘤发生的桥梁。

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