

绿茶对体外培养的人乳腺癌细胞和血管内皮细胞 bFGF 表达的抑制作用*

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【摘要】 目的 研究绿茶对人乳腺癌细胞及人脐静脉内皮细胞碱性成纤维细胞生长因子(bFGF)表达的影响及机制。方法 ELISA 法检测 bFGF 的蛋白表达水平; mRNA 的水平用 Northern 印迹杂交法研究。结果 40 $\mu\text{g}/\text{mL}$ 的绿茶提取物(green tea extract, GTE)或绿茶主要有效成分表没食子儿茶素-3-没食子酸(epigallocatechin-3 gallate, EGCG)①能够抑制人脐静脉内皮细胞及人乳腺癌细胞株细胞内及细胞分泌入条件培养基中 bFGF 多肽水平,这种抑制作用呈剂量依赖性;②能明显抑制 MDA-MB231 细胞内的 bFGF mRNA 表达水平。结论 GTE 或 EGCG 能够在 mRNA 和蛋白水平抑制乳腺癌细胞及脐静脉内皮细胞的 bFGF 表达,绿茶可能通过该机制抑制肿瘤新生血管形成。

【关键词】 绿茶; 碱性成纤维细胞生长因子; 乳腺癌

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Inhibition Effect of Green Tea on bFGF Expression in Cultured Human Breast Cancer Cells and Endothelial Cells

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【Abstract】 Purpose To explore the role of green tea on basic fibroblast growth factor (bFGF) in human breast cancer cells and human umbilical vein endothelial cells (HUVECs) and its mechanisms. **Methods** bFGF concentrations were determined by ELISA. Northern blot hybridization was performed to detect the expression of mRNA. **Results** 40 $\mu\text{g}/\text{mL}$ GTE or EGCG could decrease the levels of bFGF peptide secreted into conditioned media as well as the bFGF peptide levels in both HUVECs and human breast cancer cells; this effect was dose dependent. 40 $\mu\text{g}/\text{mL}$ GTE and EGCG decreased the mRNA levels of bFGF in MDA-MB231 cells. **Conclusions** Green tea can inhibit bFGF expression in human breast cancer cells and human umbilical vein endothelial cells through multiple levels. This may be the partial mechanism for green tea to inhibit angiogenesis in cancers.

【Key words】 green tea; basic fibroblast growth factor; breast cancer

绿茶是人类最常饮用的健康饮品之一,有大量的文献研究表明饮用绿茶可以抑制癌前病变向浸润性癌方向发展,预防包括结直肠癌、食管癌及乳腺癌在内的一些常见肿瘤的发生^[1,2]。有报道^[3]饮用绿茶可改善乳腺癌患者的预后。肿瘤的新生血管形成能力在这一系列肿瘤形成、发展及转移过程中具有重要作用,抑制新生血管的形成是目前肿瘤治疗学

研究的热点之一。碱性成纤维细胞生长因子(basic fibroblast growth factor, bFGF)是经典的血管形成因子,在诱发肿瘤新生血管形成过程中具有重要的作用。这里我们研究了绿茶提取物(green tea extract, GTE)和其有效成分表没食子儿茶素-3-没食子酸(epigallocatechin-3 gallate, EGCG)对乳腺癌细胞和人脐静脉内皮细胞 bFGF 表达的影响。

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材料和方法

试剂 GTE 由 Pharmanex 公司提供, EGCG 购自 Sigma 公司。

细胞培养 人脐静脉内皮细胞(HUVECs, 购自 Clonetics 公司)采用内皮细胞培养液(EGM: 含 2% 的胎牛血清、50 $\mu\text{g}/\text{mL}$ 庆大霉素和 3 $\mu\text{g}/\text{mL}$ 两性霉素 B)培养。用 40 $\mu\text{g}/\text{mL}$ GTE 或 EGCG 进行干预, 观察不同时点细胞及培养基中 bFGF 变化。乳腺癌 MDA-MB-231 细胞购自 ATCC, 采用 DMEM 培养基(含 10% 胎牛血清, 100 u/mL 青霉素, 100 $\mu\text{g}/\text{mL}$ 链霉素)进行培养。

bFGF 水平的定量 用汇合状态的 HUVECs 细胞或 MDA-MB231 细胞准备条件培养基。收集 GTE 或 EGCG 治疗不同时间段的无血清 DMEM 培养基和细胞(经裂解液裂解后), 经 2 500 r/min, 5 min 离心除去沉渣, 采用 ELISA 法测定 bFGF 浓度并重复 3 次。

Northern blot 分析 用 Trizol 试剂进行细胞的总 RNA 提取。RNA(25 μg) 在 1% 的甲醛琼脂凝胶内电泳并转至尼龙膜, 在 65 $^{\circ}\text{C}$ 的快速杂交缓冲液中进行 3~4 h 预杂交, 65 $^{\circ}\text{C}$ 下过夜同 α - ^{32}P 标记的人 bFGF cDNA 探针(cDNA 由 Brygida Berse 博士馈赠)杂交后, 曝光于 X 光胶片。

统计方法 用 Student's *t* test 方法进行多因素分析, 用 Newman-Keuls test 比较不同组间差别, $P < 0.05$ 有统计学意义。

结果

GTE 或 EGCG 对条件培养基及细胞中 bFGF 蛋白浓度的影响 40 $\mu\text{g}/\text{mL}$ GTE 或 EGCG 治疗后, 在 HUVECs 细胞(图 1)和 MDA-MB231 细胞(图 2)及其各自培养基中, 不同时点均能观察到 bFGF 蛋白水平的减少。在 48 h, GTE 和 EGCG 能显著抑制 MDA-MB231 细胞和 HUVECs 中 bFGF 多肽水平($P < 0.05$)。在 72 h, GTE 和 EGCG 显著抑制 MDA-MB231 细胞及培养基中分泌 bFGF 多肽水平($P < 0.05$)。以上抑制效应表现出剂量依赖性(图 3, 4)。

GTE 或 EGCG 对 MDA-MB-231 细胞 bFGF 转录水平的抑制 用 40 $\mu\text{g}/\text{mL}$ GTE 或 EGCG 处理 MDA-MB231 细胞能够致 bFGF mRNA 水平的降低(图 5)。在处理的初期(3 和 6 h), GTE 和 EGCG 并不能影响 bFGF mRNA 表达水平(图 5A); 但延长到 24~48 h, MDA-MB-231 细胞中 bFGF mRNA 水平明显降低(图 5B)。

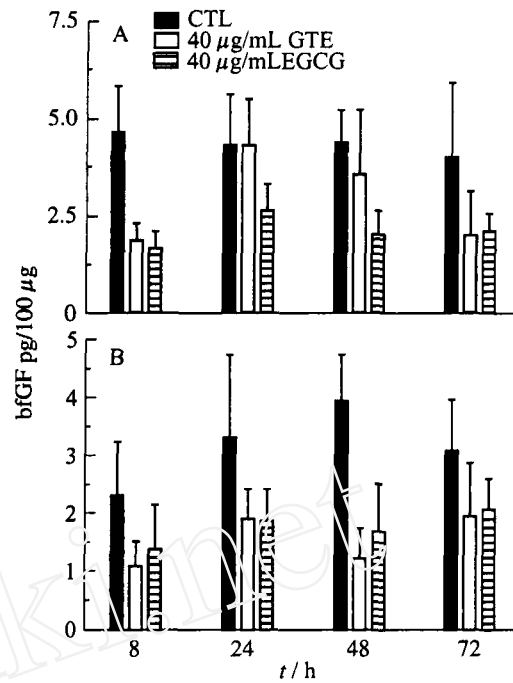


图 1 HUVECs 经 40 $\mu\text{g}/\text{mL}$ GTE 或 EGCG 处理后 bFGF 多肽水平变化

Fig 1 Levels of basic fibroblast growth factor (bFGF) peptide for HUVECs treated with 40 $\mu\text{g}/\text{mL}$ of GTE or EGCG (A) bFGF peptide content in cells; (B) Human umbilical vein endothelial cells (HUVECs) were incubated in plain media in the absence (CTL) or presence of 40 $\mu\text{g}/\text{mL}$ of either green tea extract (GTE) or epigallocatechin-3-gallate (EGCG) for 8-72 hours. bFGF levels were determined by ELISA, as detailed in Materials and Methods, and expressed as pg bFGF per 100 μg of total cell protein content. Data represents the mean \pm SE of three experiments

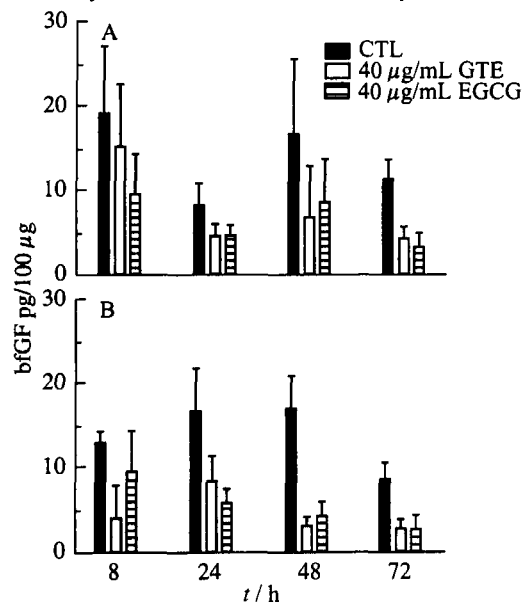


图 2 MDA-MB231 细胞经 40 $\mu\text{g}/\text{mL}$ GTE 或 EGCG 处理后 bFGF 多肽水平变化

Fig 2 Levels of basic fibroblast growth factor (bFGF) peptide for MDA-MB-435 cells treated with 40 $\mu\text{g}/\text{mL}$ of GTE or EGCG (A) bFGF peptide content in cells; (B) Human breast cancer cells MDA-MB231 were incubated in plain media in the absence (CTL) or presence of 40 $\mu\text{g}/\text{mL}$ of either green tea extract (GTE) or epigallocatechin-3-gallate (EGCG) for 8-72 hours. bFGF levels were determined by ELISA, as detailed in Materials and Methods, and expressed as pg bFGF per 100 μg of total cell protein content. Data represents the mean \pm SE of three experiments

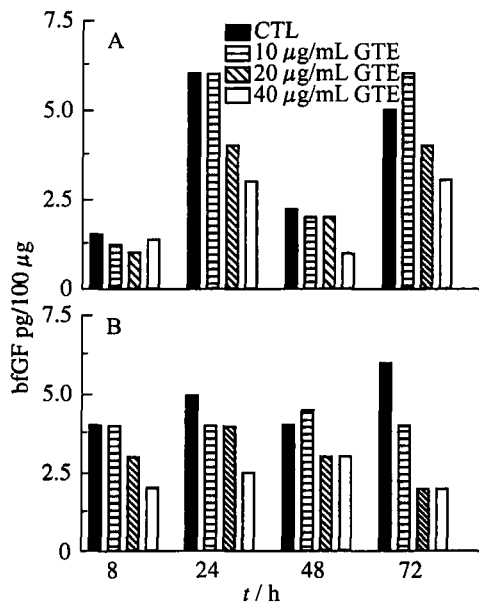


图3 HUVECs 经 10、20、40 μg/mL GTE 处理多肽水平变化
Fig 3 Levels of basic fibroblast growth factor (bFGF) peptide for HUVECs treated with 10, 20 or 40 μg/mL of GTE

(A) bFGF peptide content in cells; (B) Human umbilical vein endothelial cells (HUVECs) were incubated in plain media in the absence (CTL) or presence of 10–40 μg/mL of green tea extract (GTE) for 8–72 hours. bFGF levels were determined by ELISA, as detailed in Materials and Methods, and expressed as pg bFGF per 100 μg of total cell protein content. Data represents the mean ± SE of three experiments

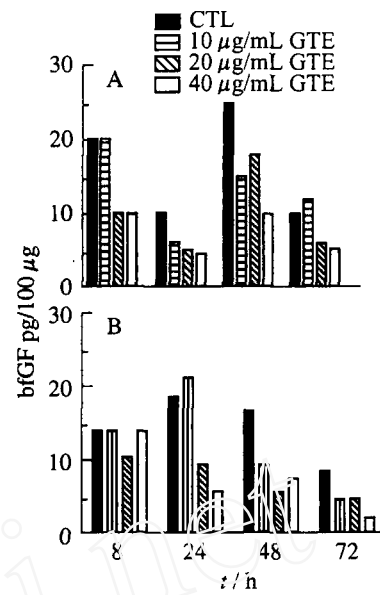


图4 MDA-MB231 经 10、20、40 μg/mL GTE 处理多肽水平变化
Fig 4 Levels of basic fibroblast growth factor (bFGF) peptide for MDA-MB-435 cells treated with 10, 20 or 40 μg/mL of GTE

(A) bFGF peptide content in cells; (B) Human breast cancer cells MDA-MB231 were incubated in plain media in the absence (CTL) or presence of 10–40 μg/mL of green tea extract (GTE) for 8–72 hours. bFGF levels were determined by ELISA, as detailed in Materials and Methods, and expressed as pg bFGF per 100 μg of total cell protein content. Data represents the mean ± SE of three experiments

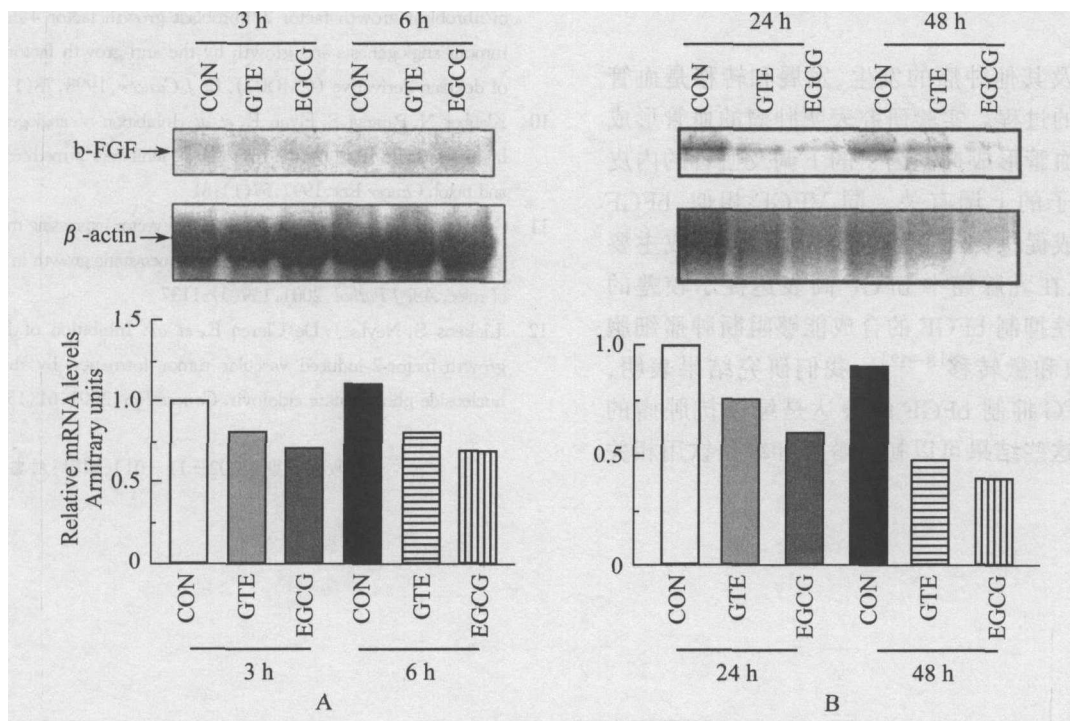


图5 Northern blots 研究碱性成纤维细胞生长因子 (bFGF) 的转录

Fig 5 Northern analysis of basic fibroblast growth factor (bFGF) transcript

Human breast cancer cells MDA-MB231 were cultured in the absence (CON) or presence of 40 μg/mL of either green tea extract (GTE) or epigallocatechin-3-gallate (EGCG) for 3–6 hours (A) 24–48 hours (B) Twenty five μg of RNA was hybridized with bFGF and β-actin cDNA probes. Data represents the results of one representative experiment out of three

讨 论

流行病学研究表明饮用绿茶可以抑制癌前病变向浸润性癌方向发展,对结直肠癌、食管癌及乳腺癌在内的一些常见肿瘤有化学预防的作用。在啮齿类动物模型的研究中,人们发现绿茶能够抑制皮肤、肺、乳腺和胃肠道等肿瘤的生长^[4]。但绿茶抑制肿瘤发生的机制目前尚不确定且鲜有报道。有人认为绿茶还可抑制多种细胞色素酶的催化活性,包括 P-4501A 和 2B1,而这些酶可能和癌症发生的启动阶段有关^[5],另有报道绿茶可阻断和抑制乳腺癌细胞的雌激素受体表达^[6]。我们的前期研究表明无论是绿茶提取物还是其有效成分—多酚类化合物均显示出抑制乳腺癌细胞和血管内皮细胞增殖的能力,在小鼠的活体实验中有人发现,绿茶提取物能够抑制移植瘤的生长、减低肿瘤血管密度^[7]。我们近期的研究表明 40 $\mu\text{g}/\text{mL}$ 的 GTE 或绿茶主要有效成分 EGCG 能够明显减少人乳腺癌细胞内及细胞分泌入条件培养基中血管内皮生长因子(VEGF)蛋白的水平,这种抑制作用为剂量依赖性,发生在转录调控水平,同时 VEGF 启动子的活性也降低。本研究进一步表明了 40 $\mu\text{g}/\text{mL}$ GTE 或 EGCG 可显著降低人乳腺癌细胞及培养基中 bFGF 蛋白及 bFGF mRNA 水平。

乳腺癌及其他肿瘤的发生、发展和转移是血管形成依赖性的过程。实验研究表明肿瘤的血管形成活性可能和血管形成抑制因子的下调及血管的内皮细胞生长因子的上调有关。同 VEGF 相似,bFGF 作为血管生成促进因子,是肿瘤相关血管生成主要的调节因子,在乳腺癌中 bFGF 高表达提示较差的预后。特异性抑制 bFGF 的合成能够阻断肿瘤细胞的转化、增殖和微转移^[8~12]。我们研究结果表明,GTE 或 EGCG 抑制 bFGF 的表达是绿茶抗肿瘤的机制之一。这些结果可以初步解释和绿茶饮用相关

的抗肿瘤现象。绿茶茶多酚抗肿瘤效应的分子机制的研究,对于进一步将绿茶用于肿瘤的治疗或预防有着重要意义。

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