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ErbB2 通过 FAK - Src - MAPK 信号通路诱导细胞转化和移动侵袭*

何 强, 梁力建, 彭宝岗, 李绍强, 汤 地, 阿力亚
(中山大学附属第一医院肝胆外科,广东 广州 510080)

[摘 要] **目的**:探讨表皮生长因子受体2(ErbB2)诱导肿瘤转化和侵袭的分子机制。**方法**:用表达 ErbB2 逆病毒颗粒感染 $FAK^{+/+}$ 细胞,Western 印迹检测 ErbB2 在 $FAK^{+/+}$ 细胞中的表达,免疫沉淀检测 ErbB2 的功能。用 Src 阻断剂 - PP2 阻断 Src,用 MAPK 阻断剂 - UO126 阻断 MAPK,观察 Src 或 MAPK 被阻断后对 ErbB2 诱导的细胞移动和细胞转化的影响。**结果**:感染后 ErbB2 在 $FAK^{+/+}$ 细胞中稳定表达和激活。PP2 抑制 ErbB2 诱导的 FAK 磷酸化以及 ErbB2 诱导的细胞移动。UO126 阻断 ErbB2 诱导的 MAPK 磷酸化以及 ErbB2 诱导的细胞锚定依赖性生存 - 细胞转化。**结论**:ErbB2 通过 FAK - Src - MAPK 信号转导通路诱导 $FAK^{+/+}$ 细胞转化和移动。

[关键词] 受体,表皮生长因子; FAK - Src - MAPK 通路; 肿瘤侵润
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Oncogenic transformation, migration and invasiveness of cells induced by ErbB2 are mediated via FAK - Src - MAPK signaling pathway

HE Qiang, LIANG Li - jian, PENG Bao - gang, LI Shao - qiang, TANG Di, A Li - ya
(*Department of Hepatobiliary Surgery, The First Affiliated Hospital, Sun Yat - sen University, Guangzhou* 510080, *China*. *E - mail: lheqiang@ hotmail. com*)

[**ABSTRACT**] **AIM**: To explore the possibility that ErbB2 - induced oncogenic transformation and invasion involve FAK - Src - MAPK signaling pathway. **METHODS**: Parental $FAK^{+/+}$ cells were infected by retro - vector particles expressing ErbB2. Expression of ErbB2 and its function were assayed by Western blotting and immunopreciptation, respectively. Src inhibitor PP2 or MAPK inhibitor UO126 was used to detect Src or MAPK function on ErbB2 - induced cell oncogenic transformation and migration. **RESULTS**: ErbB2 was overexpressed and functionally activated in $FAK^{+/+}$ cells. The phosphorylation of FAK induced by ErbB2 was inhibited by PP2, and the inhibition of FAK by PP2 was associated with impaired cell migration and invasion. UO126 blocked phosphorylation of MAPK induced by ErbB2, and was responsible to impaired anchorage - dependent cell survival in soft agar. **CONCLUSION**: Cell oncogenic transformation, migration, and invasion induced by ErbB2 are mediated via FAK - Src - MAPK signaling pathway.

[**KEY WORDS**] Receptor, epidermal growth factor; FAK - Src - MAPK pathway; Neoplasm invasiveness

表皮生长因子受体2(ErbB2)属于酪氨酸激酶受体。过量表达 ErbB2 与人体肿瘤的高侵袭性和预后不良有密切关系[1]。焦点黏链激酶(focal adhesion kinase,FAK)是一个主要的焦点黏连蛋白,在细胞生存和迁徙过程中起重要的作用[2,3]。我们以前的研究表明,FAK 在调节 ErbB2 诱导的细胞生存、肿瘤形成和侵袭等方面起着重要的作用,其机制可能涉及到 FAK - Src - MAPK 转导途径[4]。本实验使用 Src、MAPK 阻断剂分别阻断 FAK 蛋白作用分子 Src 和 MAPK,通过观察 Src 和 MAPK 信号对于细胞转化和移动的影响而阐明 ErbB2 诱导的细胞转化和移动侵袭是否通过 FAK - Src - MAPK 信号通路。

材 料 和 方 法

1 细胞株和细胞培养

小鼠胚胎纤维原细胞 $FAK^{+/+}$ 细胞培养于 DMEM,该介质含有 10% 胎牛血清(FBS)、1 mmol/L 丙酮酸、1% 非必需氨基酸以及青/链霉素。

2 抗体和试剂

Anti - ErbB - 2 (Ab - 3, clone 3B5)购自 Oncogene;anti - FAK (A - 17, C - 20)购自 Santa Cruz;anti - phosphotyrosine antibody (4G10)和 antiphospho

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E - mail: lheqiang@ hotmail. com

－MAPK(42/44)以及 MAPK(42/44)购自 Upstate；Heregulin(HRG)购自 Neomarkers。

3 细胞转染

应用表达 ErbB2 受体并带有强化绿荧光蛋白(enhanced green fluorescent protein，EGFP)的双顺反子逆载体方法稳定转染 293 包装细胞，产生重组逆病毒颗粒。用 AP2－ErbB2 逆病毒颗粒重组体转导细胞。于 24 孔培养板中每孔植入 2×10^4 细胞，加入浓缩 100 倍的逆病毒，连续感染 3 d。稳定转导细胞的进行扩增。

4 Western 印迹和免疫沉淀(immunoprecipitation，IP)

细胞长至 70% 汇合度，改良 RIPA 液裂解细胞，提取蛋白和测定蛋白浓度。取等量蛋白，加特异性抗体，4 ℃下孵育 90 min，加蛋白－A 或蛋白－G 琼脂珠 4 ℃下孵育 60 min。免疫复合物进行聚丙烯酰氨胶电泳(SDS－PAGE)分析，并转至硝酸纤维膜。5% 脱脂牛奶室温下封闭 1 h，加Ⅰ抗 4 ℃下孵育 4 h，加Ⅱ抗室温下孵育 40 min。使用 ECL 显影系统进行荧光显影，胶片曝光、冲洗。膜用 Strip Buffer 于 50 ℃下作用 30 min，封闭后再分别加入Ⅰ抗、Ⅱ抗孵育，ECL 显影、胶片曝光。

5 锚定依赖性细胞生存

细胞生长至 70% 汇合度，trypsin 消化，用全细胞培养液悬浮。将 5 000 个细胞重新悬浮于 0.3% SeaPlaque 琼脂糖中(用全细胞培养液稀释)，并倒在 0.7% SeaPlaque 琼脂糖层。细胞置于 37 ℃培养箱中，每 3 d 换含有或不含有 UO126 的全细胞培养液(含有或不含有 HRG，20 μg/L)。4 周后计算细胞克隆数，克隆直径大于 20 μm 者为阳性。

6 体外细胞侵袭实验

使用 8 μm 裹有 matrigel 的多孔单元进行细胞移动和侵袭实验。在单元的上部加入或不加入 PP2，下部加入 20 μg/L HRG 作为趋化物，连续培养以让细胞通过 matrigel，至 48 h 时对 matrigel 下层的侵袭细胞进行固定、染色和计数。

7 统计学处理

实验重复 3 次，取 3 次的平均值，数据用均数±标准差($\bar{x}\pm s$)表示。两组间比较用 t 检验。

结　果

1 ErbB2 受体在 $FAK^{+/+}$ 细胞中稳定表达和激活

Western blotting 显示，野生型(wild－type，WT)$FAK^{+/+}$ 细胞中 ErbB2 表达水平低。感染 ErbB2 逆病毒颗粒后，ErbB2 呈现过量表达，见图 1A。

免疫沉淀以及 Western 印迹分析显示，无论有无 HRG 的刺激，ErbB2 都表现为激活状态，见图 1B。

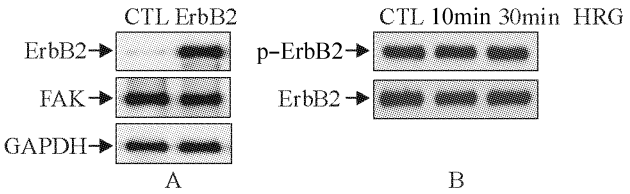

Fig 1 ErbB2 was overexpressed and activated in $FAK^{+/+}$ cells. A: $FAK^{+/+}$ cells were infected by retrovectors particles expressing ErbB2. Cells that stably expressed ErbB2 were cloned and expanded. Western blotting showed that ErbB2 was overexpressed. B: ErbB2 was activated in $FAK^{+/+}$ cells. Cells at 70% of confluence were starved in serum－free media for 16 h, and then treated with HRG (20 μg/L) for 10 min, 30 min, respectively, or kept untreated (control, CTL). Immunoprecipitation and Western blotting showed ErbB2 was activated in the presence or absence of HRG.

图 1 ErbB2 在 $FAK^{+/+}$ 细胞中过量表达和激活

2 PP2 阻断 Src、抑制 FAK 磷酸化，进而阻断 ErbB2 诱导的细胞移动和侵袭

免疫沉淀以及 Western 印迹分析显示，HRG 刺激 10 min，FAK 呈现磷酸化改变，而 ErbB2 诱导的 FAK 磷酸化可以被 Src 阻断剂 PP2 所抑制，见图 2A。

细胞移动侵袭实验显示，HRG 促进细胞的移动和侵袭(54.3333 ± 13.8684 *vs* 21.6667 ± 7.7675，$P<0.05$)，而这一作用可以被 Src 阻断剂 PP2 所抑制(8.3333 ± 3.5119 *vs* 54.3333 ± 13.8684，$P<0.01$)，见图 2B。

3 UO126 抑制 MAPK 磷酸化、阻断 ErbB2 诱导的细胞生存

免疫沉淀以及 Western 印迹分析显示，HRG 刺激 10 min，MAPK 呈现磷酸化改变，而 MAPK 这一磷酸化可以被 UO126 所抑制，见图 3A。

软琼脂细胞锚定依赖性生存实验显示，HRG 促进细胞锚定依赖性生存(73.1667 ± 16.3146 *vs* 32.3333 ± 13.9666，$P<0.01$)，而这一作用可以被 UO126 所抑制(6.5000 ± 2.6646 *vs* 73.1667 ± 16.3146，$P<0.05$)，见图 3B。

讨　论

ErbB 受体促进肿瘤生长和侵袭，其机制与细胞分子信号转导放大有关。体外实验显示，在 FAK 富含细胞中过量表达 ErbB2 促进细胞的移动和侵袭。相反，在 $FAK^{-/-}$ 细胞中过量表达 ErbB2，细胞不能在软琼脂中生存，在小鼠体内也不能成活并形成实体瘤，细胞也缺乏移动和侵袭功能。这些结果提示 ErbB2 诱导的细胞转化和侵袭依赖于 FAK 功能；FAK 在调节 ErbB2 诱导的细胞生存、肿瘤形成和侵袭等方面起着重要的作用[4]。

ErbB2 可以与多种信号分子发生作用，也可以被 FAK 所召集。FAK 的激活可以引发多种信号分子的激活，其中包括 Src 激酶和 Grab2。激活的 ErbB2 和

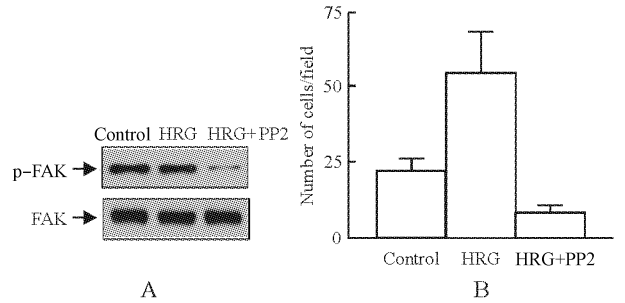

Fig 2 PP2 blocked phosphorylation of FAK and cell migration and invasion induced by ErbB2. A: cells at 70% of confluence were starved in serum - free media for 16 h, and then pre - treated with or without PP2 (50 nmol/L) for 30 min, followed by the treatment with HRG(20 μg/L) for 10 min. Cells were lysed with modified RIPA and subjected to immunoprecipitation and Western blotting. The figure showed phosphorylation of FAK induced by ErbB2 was inhibited by PP2. B: cell migration and invasion assay showed significantly decreased invasive cells seen in PP2 group compared with HRG group (8.3333 ± 3.5119 *vs* 54.3333 ± 13.8684, $n = 3$, $P < 0.01$), suggesting cell migration and invasion were inhibited by PP2.

图 2 PP2 阻断 FAKK 的磷酸化,进而阻断 ErbB2 介导的细胞移动和侵袭

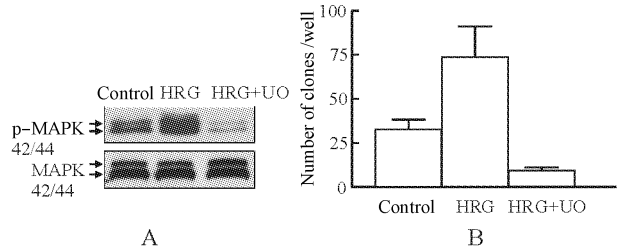

Fig 3 UO blocked phosphorylation of MAPK and oncogenic transformation of cells induced by ErbB2. A: cells at 70% of confluence were serum - starved for 16 h, and then pre - treated with or without UO(10 μmol/L), followed by the treatment with HRG (20 μg/L) for 10 min. Cells were lysed and subjected Western blotting. The figure showed that phosphorylation of MAPK induced by ErbB2 was inhibited by UO. B: the number of cell clones was significantly decreased in UO group compared with HRG group as shown by the assay of anchorage dependent survival of cells in soft agar(6.5000 ± 2.6646 *vs* 73.1667 ± 16.3146, $n = 6$, $P < 0.05$), suggesting cell oncogenic transformation induced by ErbB2 was inhibited by UO.

图 3 UO126(UO) 阻断 ErbB2 诱导的 MAPK 磷酸化以及细胞转化

FAK 均可以与 Src 产生作用。c - Src 通过 FAK 的 Tyr - 397 与其发生作用[5]。HRG 可以上调 c - Src 位于 215 区域的酪氨酸磷酸化,增强 Src 激酶的活性,而 Src 活性增加与细胞之间黏附的减少有关[6],并与肿瘤转移有关[7]。ErbB2 诱导的细胞生存、肿瘤形成和侵袭依赖于 FAK 功能,这可能与 FAK 下游信号通路有关。我们实验显示,使用阻断 Src 阻断剂 - PP2,可以抑制 FAK 的磷酸化,并且抑制 ErbB2 诱导的细胞移动和侵袭,提示 ErbB2 - FAK - Src 信号通路与细胞移动和侵袭功能有关。这一结果与文献报道相似。Schlaepfer 等[8] 和 Westhoff 等[9] 研究提示 v - Src 诱导的肿瘤形成可能通过 FAK - Src - MAPK 信号通道而实现。Olayioye 等[10] 研究显示,FAK 是 MAPK 的激活过程中一个重要的中间体。我们实验显示,使用 MAPK 阻断剂 UO126 可以阻断 MAPK 的磷酸化,进而阻断了 ErbB2 诱导的细胞转化,提示 MAPK 可能是 ErbB2/FAK 下游信号分子,可以被 FAK 所召集,并转导信号。因此,ErbB2 诱导的细胞转化可能涉及到 ErbB2 - FAK - MAPK 信号转导通路。

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