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整合素连接激酶对鼻咽癌细胞系迁移及上皮-间充质转化的影响

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[摘要] 目的: 探讨整合素连接激酶(integrin-linked kinase, ILK)对鼻咽癌细胞迁移及上皮-间充质转化(epithelial-mesenchymal transition, EMT)转化的影响及其作用机制。方法: 通过脂质体介导将ILK SiRNA转染CNE2鼻咽癌细胞系, 细胞分为siILK组、siGAPDH阳性对照组及siControl阴性对照组。RT-PCR和Western印迹检测鼻咽癌细胞系CNE2中ILK的RNA水平和蛋白水平表达的变化。Transwell实验和划痕试验观察ILK SiRNA对细胞迁移能力及侵袭能力的影响, RT-PCR法检测了与EMT过程中的相关基因mRNA的表达水平。结果: 转染ILK SiRNA后, CNE2细胞中ILK mRNA和蛋白表达水平均明显下降; ILK SiRNA可明显抑制CNE2细胞的迁移及侵袭能力; 上调E-cadherin的表达, 下调OCLN, Vimentin, Twist1, FN1的表达, 抑制EMT过程的发生。结论: ILK在鼻咽癌细胞系CNE2中高表达, 降低ILK表达可诱导鼻咽癌细胞间充质-上皮细胞转型的发生, 并因此而显著降低其侵袭和转移潜力。

[关键词] 整合素连接激酶; 鼻咽癌; 生物标志物; 转移; 细胞迁移; 上皮-间充质转化

Effects of ILK regulating epithelial-mesenchymal transition on invasion and migration in nasopharyngeal cancer

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Abstract **Objective:** To investigate the effect of integrin-linked kinase (ILK) on migration and epithelial-mesenchymal transition of nasopharyngeal carcinoma cells and its mechanism. **Methods:** ILK SiRNA was transfected into CNE2 nasopharyngeal carcinoma cell line by liposome. The cells were divided into siILK group, positive control

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group siGAPDH and siControl negative control group. RT-PCR and Western blot were used to detect the changes of ILK RNA and protein levels in nasopharyngeal carcinoma cell line CNE2. The effects of ILK siRNA on cell migration and invasion were observed by Transwell assay and scratch assay. The expression level of related Marker in epithelial-mesenchymal transition (EMT) was detected by RT-PCR. **Results:** After transfection of ILK siRNA, the expression of ILK mRNA and protein in CNE2 cells decreased significantly. ILK siRNA could significantly inhibit the migration and invasion of CNE2 cells; up-regulate the expression of E-cadherin and down-regulate the expression of OCLN, Vimentin, Twist1 and FN1, inhibit the occurrence of epithelial-mesenchymal transition process. **Conclusion:** ILK is highly expressed in the nasopharyngeal carcinoma cell line CNE2. Decreasing ILK expression induces the development of mesenchymal-epithelial cell transition in nasopharyngeal carcinoma cells, and thus significantly reduces its invasion and metastatic potential.

Keywords integrin-linked kinase; nasopharyngeal carcinoma; biomarkers; metastasis; cell migration; epithelial-mesenchymal transition

鼻咽癌是我国最常见的恶性肿瘤之一, 发病具有明显的地区聚集性, 其高发区主要集中在东南亚和中国南方, 在中国南方部分地区可高达15~50/10万^[1]。由于鼻咽部的位置较隐蔽, 早期症状不典型, 70%患者确诊时已是晚期。此外, 鼻咽癌大部分属于低分化或未分化鳞状细胞癌, 其恶性程度较高, 容易出现远处转移。单纯放疗或同步放化疗是鼻咽癌重要的治疗手段^[2], 尽管对放化疗敏感, 但鼻咽癌的治疗失败率仍然较高, 失败的主要原因是局部复发和转移。晚期鼻咽癌的远处转移率仍高达30%^[3-4]。发生远处转移的鼻咽癌患者中位生存期仅19~21个月^[5]。鼻咽癌局部复发和转移是导致患者预后差的最主要原因, 因此探索鼻咽癌进展和转移的机制对于制定更有效的治疗方案十分必要。

整合素连接激酶(integrin-linked kinase, ILK)是一种Ser/Thr蛋白激酶。在胚胎发育中发挥重要作用, 且广泛表达于黑色素瘤, 结肠癌, 前列腺癌, 卵巢癌, 非小细胞肺癌和头颈部鳞状细胞癌等多种肿瘤^[6-9]。研究^[10-11]表明: 下调口腔鳞癌和卵巢癌等多种恶性肿瘤中ILK基因的表达可阻滞上皮-间充质转化(epithelial-mesenchymal transition, EMT)的发生, 从而抑制肿瘤细胞侵袭和迁移。然而, ILK在鼻咽癌中是否也参与EMT的发生, 影响肿瘤细胞侵袭和迁移, 目前尚无相关报道。

本研究应用RNA干扰技术, 将特异性靶向ILK基因的siRNA(即ILK siRNA)转染至鼻咽癌细胞中, 通过抑制ILK基因表达, 探究ILK表达对鼻咽癌细胞EMT及迁移侵袭的影响, 从而为鼻咽癌的

靶向治疗提供更多理论依据。

1 材料与方法

1.1 材料

CNE2细胞系由中南大学湘雅二医院实验室赠送。细胞培养用RPMI 1640和胎牛血清(fetal bovine serum, FBS)购自美国Gibco公司, 0.25 g/L胰酶、PBS购自美国Invitrogen公司。siRNA及转染试剂购自广州市锐博生物科技有限公司, Transwell小室购自德国默克密理博公司, RNA提取试剂盒、反转录试剂盒购自天根生化科技(北京)有限公司, RT-PCR试剂盒购自德国Qiagen公司, 结晶紫溶液购自上海碧云天生物技术有限公司。Western印迹法所用抗体购自美国Abcam公司。细胞培养耗材和基质胶购自美国Corning公司。ILK正向引物: 5'-GACGAAGCTCAACGAGAA-3', 反向引物: 5'-AGTCCCTGCTCTTCCTTGT-3'; E-CADHERIN正向引物: 5'-GTTCCACCATTAACAGGAAC-3', 反向引物: 5'-ACCTTGAAGGTCAGCAGC-3'; OCCLUDIN正向引物: 5'-GAGTGAACCCAACTGCTC-3', 反向引物: 5'-TACTGATCCACGTAGAGTC-3'; Vimentin正向引物: 5'-CTCGCTGGCCGACGCCATCA-3', 反向引物: 5'-CGATGTAGTTGGCGAAGCGGTC-3'; FN1正向引物: 5'-CGTCAGGGAGAAAATGGCCAG-3', 反向引物: 5'-ATCATCATAACACGTTGCCTCA-3'; Twist1正向引物: 5'-ACGAGCTGGACTCCAAGATGG-3', 反向引物: 5'-ATCCTCCAGACCGAGAAGGCG-3'; BCATENIN正向引物: 5'-GTCTTACCTGGACTCTGGAATC-3', 反向

引物: 5'-AGGTATCCACATCCTCTTC-3'。

1.2 细胞培养及转染

CNE2细胞系接种于含有10%胎牛血清的RPMI 1640培养基, 置于37℃, 5%CO₂培养箱常规培养, 隔天换液, 细胞融合度达到90%时进行传代。12孔板细胞融合度达到70%时进行转染, 每孔24 μL转染试剂, 10 μL siRNA, 设置阳性对照组siGAPDH和阴性对照组, 转染48 h后进行后续实验。

1.3 RT-PCR

试剂盒法提取细胞总RNA, 取1 μg总RNA, 随机引物, 20 μL体系逆转录成cDNA。稀释50倍作为PCR模板。以GAPDH作为内参, SYBR发光法检测目的基因相对表达水平。

1.4 划痕试验

取转染后24 h后的各组细胞接种于6孔板, 待贴壁后用10 μL无菌枪头画一直线, PBS冲洗3次后, 加入无血清RPMI1640培养基培养, 24 h拍照1次。

1.5 Transwell 细胞侵袭试验

在24孔板内放置预先用Matrigel处理过的Transwell小室。取转染后24 h的人鼻咽癌细胞至无血清的RPMI1640培养基中制成细胞悬液, 将200 μL体积含有10万个细胞的细胞悬液置于上室, 下室

加入500 μL含有10% FBS的培养基。培养16 h后取出小室, 4%多聚甲醛固定20 min, 结晶紫溶液染色15 min, PBS冲洗, 低倍镜下随机4个视野计数拍照, 每组设置3个重复。

1.6 Transwell 细胞迁移试验

除不用加Matrigel外, 其余步骤同侵袭试验。

1.7 Western 印迹法

RIPA法提取总蛋白, 煮沸变性后每孔30 μg总蛋白进行垂直电泳, 蛋白湿转至PVDF膜上, 脱脂奶粉封闭1 h, 加入一抗孵育2 h, 二抗1 h, ECL法显影。Image Lab软件进行分析。

1.8 统计学处理

采用SPSS 16.0统计软件进行数据分析, 组间比较采用t检验, $P < 0.05$ 为差异有统计学意义。

2 结果

2.1 在鼻咽癌细胞系 CNE2 中成功敲减 ILK 表达量

分别在RNA水平和蛋白水平对鼻咽癌细胞系CNE2中的ILK表达情况进行了检测, Si01, si02和si03三条序列对鼻咽癌细胞系中的ILK都有很好的抑制作用, 表明通过转染siRNA能够在鼻咽癌细胞内下调ILK的表达量, 选取Si01序列作为实验序列(图1)。

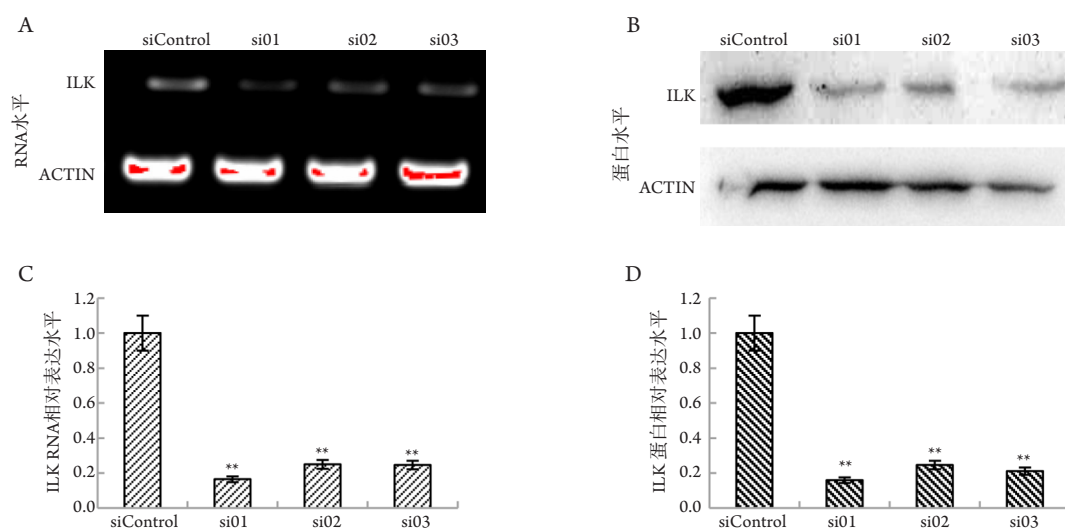


图1 RNA水平(A, C)和蛋白水平(B, D)检测鼻咽癌细胞系CNE2中敲低ILK的表达效果

Figure 1 Expression of ILK in nasopharyngeal carcinoma cell line CNE2 by RT-PCR (A) and Western blotting (B) analysis with siControl group, ** $P < 0.001$.

Compared with the siControl group, ** $P < 0.001$.

2.2 下调 ILK 表达能抑制鼻咽癌细胞迁移能力

划痕试验显示：与对照组比较，下调了ILK的鼻咽癌细胞划痕愈合度更低，细胞迁移速度更慢，证明抑制ILK表达能够降低鼻咽癌细胞的运动能力(图2)。Transwell试验显示：在鼻咽癌细胞中抑制ILK的表达能够显著减少穿过小室的细胞数量，表明抑制ILK基因能够降低鼻咽癌细胞的迁移

能力(图3A, 3B)。

2.3 下调 ILK 表达能抑制鼻咽癌细胞的侵袭能力

Transwell试验显示：在鼻咽癌细胞中抑制ILK的表达能够显著减少穿过小室的细胞数量，表明抑制ILK基因能够降低鼻咽癌细胞的侵袭能力(图3C, 3D)。

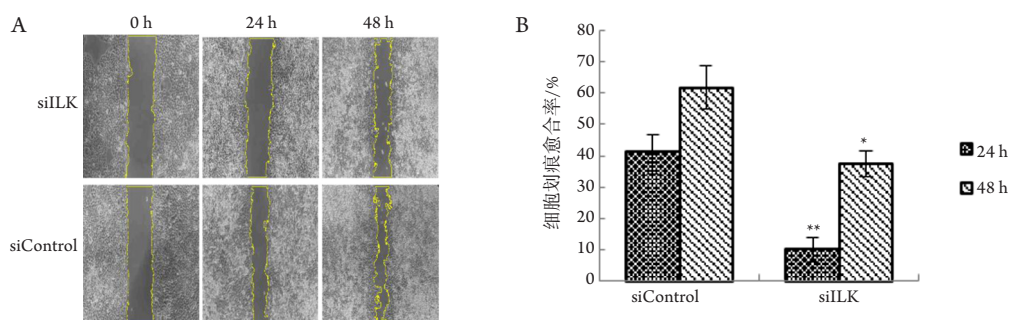


图2 划痕试验分析敲低ILK表达抑制鼻咽癌细胞迁移

Figure 2 Low-expression of ILK inhibit the migration of nasopharyngeal carcinoma cell by scratch-wound assay

(A) siILK组与siControl组细胞划痕试验；(B) siILK组与siControl组细胞划痕愈合率比较。与siControl组相比，* $P < 0.05$ ，** $P < 0.001$ 。

(A) Comparison of wound scratch assay between the siILK group and the siControl group; (B) Comparison of scar healing rate between the siILK group and the siControl group. Compared with the siControl group, * $P < 0.05$, ** $P < 0.001$.

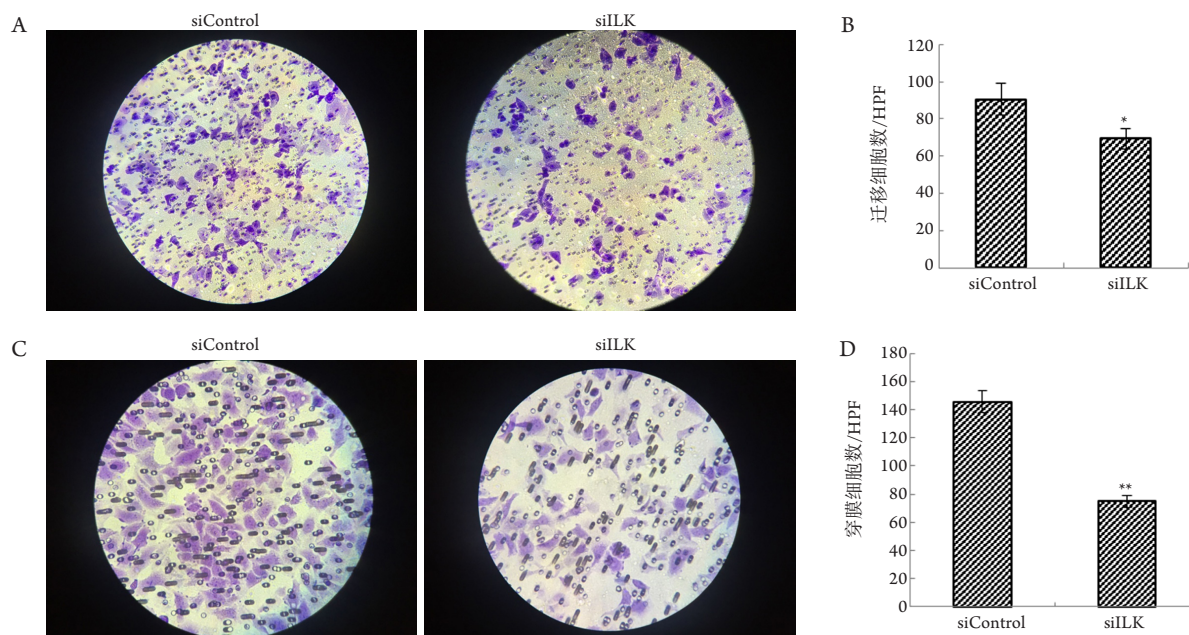


图3 Transwell试验分析ILK SiRNA对鼻咽癌细胞迁移(A, B)和侵袭(C, D)的影响

Figure 3 Effects of ILK SiRNA on the migration (A, B) and invasion (C, D) of nasopharyngeal carcinoma cells by Transwell experiments

与siControl组相比，* $P < 0.05$ ，** $P < 0.01$ 。

Compared with the siControl group, * $P < 0.05$, ** $P < 0.01$.

2.4 下调 ILK 表达对 EMT 相关基因表达量的影响

用RT-PCR法检测了与EMT过程中相关标志物的表达水平,结果显示:在干扰了ILK表达后,上皮细胞标志物E-cadherin的表达增强,间充质细胞标志物OCLN, Vimentin, Twist1等表达量降低,同时与细胞运动相关的FN1表达量降低,提示ILK可能通过抑制EMT途径来影响鼻咽癌细胞的迁移和浸润能力(图4)。

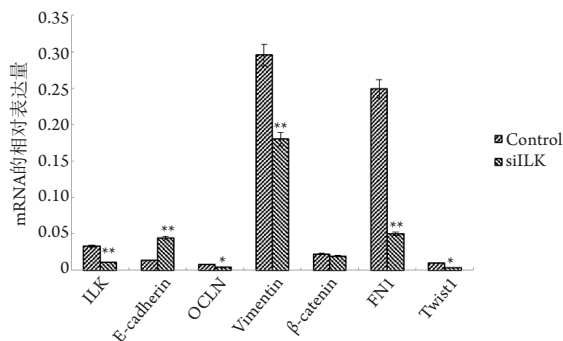


图4 RT-PCR检测ILK SiRNA对鼻咽癌细胞EMT相关因子mRNA表达的影响

Figure 4 Effects of ILK SiRNA on the mRNA expression of EMT markers in nasopharyngeal carcinoma cells by RT-PCR

与siControl组相比, * $P < 0.05$, ** $P < 0.01$ 。

Compared with the siControl group, * $P < 0.05$, ** $P < 0.01$.

3 讨论

ILK是一个独特的具有衔接蛋白和激酶活性的细胞内信号蛋白,ILK能与整合素 $\beta 1$ 和 $\beta 3$ 亚单位相互作用连接整合素和肌动蛋白细胞骨架,被整合素或生长因子以依赖于PI3K的方式激活,进而作用于下游的PKB/AKT(protein Kinase B)和GSK-3(glycogen synthase-3),协调细胞外基质和生长因子的信号转导,调控细胞的生存、增殖、分化、黏附、迁移、侵袭和血管形成等过程^[6,12-14]。ILK在多种肿瘤中都存在过量表达,例如黑素瘤、结肠癌、前列腺癌、卵巢癌、非小细胞肺癌和头颈部鳞状细胞癌^[6-9]。

笔者前期研究发现:与正常鼻咽上皮细胞相比,鼻咽癌细胞中ILK的水平更高。最近的研究^[9]表明:ILK过表达与口腔鳞癌患者的肿瘤浸润分级、组织学分级,临床分期和淋巴结受累之间存在显著相关性,与之前关于人类结肠癌的临床研究^[15]结果一致,提示ILK可能通过促进鼻咽癌细胞的侵袭和转移在鼻咽癌进展中发挥重要作用。

越来越多的证据表明ILK可调节癌细胞存活和肿瘤进展的几种信号通路,诱导细胞EMT,进而促进肿瘤侵袭转移。EMT是上皮细胞失去其上皮形态并获得间充质表型的关键过程,其特征在于上皮蛋白如钙黏蛋白E-cadherin的减少,以及间充质蛋白如波形蛋白Vimentin的增加。已经鉴定了几种转录因子通过抑制E-cadherin来诱导EMT过程,例如Twist, ZEB1, Snail和Slug^[6,16-17]。人们普遍认为EMT在肿瘤侵袭和转移过程中起重要作用,侵袭性癌细胞通常表现为上皮特征丧失并获得间充质表型^[18-19]。

为探索ILK在鼻咽癌细胞迁移和侵袭中如何起作用,本研究进行了体外实验,研究结果表明:敲低ILK的表达显著降低了鼻咽癌细胞迁移和侵袭的能力;在干扰ILK表达后,上皮细胞标志物E-cadherin的表达增强,间充质细胞标志物OCLN, Vimentin, Twist1等表达量降低,同时与细胞运动相关的FN1表达量降低,提示ILK可能通过抑制EMT途径来影响鼻咽癌细胞的迁移和浸润能力。

ILK作为一个重要的信号整合器在人类癌症发展过程的EMT过程中起重要的作用,但其机制尚不完全清楚。ILK能通过磷酸化PKB和GSK-3 β 参与到相应的信号通路。磷酸化的PKB生物活性降低,使得细胞凋亡受抑制,使肿瘤细胞存活时间延长;GSK-3 β 的磷酸化导致细胞周期蛋白D1(cyclinD1)等基因的表达上调并影响细胞周期,促进细胞增殖;ILK的过量表达还能够通过磷酸化PKB而诱导转录因子Snail和ZEB1的表达,从而抑制E-cadherin的表达,促进肿瘤的侵袭和迁移;与此同时,ILK也能刺激MMP-9, MMP-2的表达,促进细胞的侵袭和迁移。ILK可调配细胞外基质及生长因子的信号传导,调控细胞的生长、分化、迁移^[6,13-14,20]。

综上,本研究表明,ILK的表达在鼻咽癌细胞系中显著增加,ILK表达显著调节体外细胞迁移和侵袭以及EMT相关基因的表达。

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