



· 论 著 ·

乌司他丁抑制CCL17/CCL22-CCR4介导的小鼠乳腺癌肝转移

李霞, 印国兵, 程熙, 潘倍倍, 李顺波, 代朦, 郭丹
重庆医科大学附属第二医院乳腺甲状腺外科, 重庆 400010

[摘要] 背景与目的: 越来越多的研究表明趋化因子及其受体在癌症的发生、发展和转移中起关键作用。本文研究了乌司他丁 (ulinastatin) 对胸腺和活化调节趋化因子 (thymus and activation-regulated chemokine, TARC) 即CCL17/巨噬细胞来源的趋化因子 (macrophage-derived chemokine, MDC) 即CCL22-CC族趋化因子受体4 (CC chemokine receptor 4, CCR4) 信号通路介导的乳腺癌肝转移的影响及其作用机制。方法: 通过小鼠乳腺脂肪垫 (mfp) 接种4T1乳腺癌细胞的方式构建小鼠乳腺癌模型, 15 d后取小鼠乳腺肿瘤, 并记录乳腺肿瘤的质量; 采用免疫组织化学法检测乳腺肿瘤组织中CCR4及肝脏转移瘤中CCL22、CCL17蛋白的表达; 采用慢病毒转染的方式抑制4T1乳腺癌细胞CCR4基因, 采用蛋白质印迹法 (Western blot) 检测抑制效果, 并以同样方式进行小鼠乳腺成瘤并观察肿瘤生长情况; 用三种不同浓度乌司他丁处理4T1细胞荷瘤小鼠, 15 d后采用免疫组织化学法检测乳腺肿瘤组织中CCR4及肝脏组织中CCL22及CCL17的表达, 采用Western blot和实时荧光定量聚合酶链反应 (real-time fluorescent quantitative polymerase chain reaction, RTFQ-PCR) 分别检测肝脏组织中TGF- β 的表达以及microRNA-34a和microRNA-31的含量, 并进行TGF- β 和microRNA-34a、microRNA-31、CCL22以及CCL17的相关性分析。结果: 小鼠乳腺癌组织高表达CCR4, 肝脏转移瘤高表达CCL22和CCL17; 沉默4T1乳腺癌细胞CCR4的表达, 可抑制乳腺癌成瘤性; 乌司他丁抑制CCR4、TGF- β 、CCL22、microRNA-31和CCL17的表达, 促进microRNA-34a的表达。结论: 乌司他丁具有抑制乳腺癌肝转移的作用, 其具体机制可能与乌司他丁通过TGF- β -microRNA-34a-CCL22轴及microRNA-31-TGF- β -CCL17轴抑制肝脏组织中与乳腺癌肝转移密切相关的CCL17/CCL22-CCR4信号通路有关。

[关键词] 乳腺癌; 肝转移; 乌司他丁; CCR4; CCL22; CCL17

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Ulinastatin inhibits liver metastasis from breast cancer in mice through CCL17/CCL22-CCR4 pathway LI Xia, YIN Guobing, CHENG Xi, PAN Beibei, LI Shunbo, DAI Meng, GUO Dan (Department of Breast and Thyroid Surgery, the Second Affiliated Hospital of Chongqing Medical University, Chongqing 400010, China)

Correspondence to: GUO Dan E-mail: guodan-e1973814@126.com

[Abstract] **Background and purpose:** Increasing evidences have shown the key roles of chemokines in the formation and metastasis of cancers. In this study, we investigated the molecular mechanisms of liver metastasis from breast cancer and the effects of ulinastatin on liver metastasis from breast cancer mediated by CCL17/CCL22-CCR4 pathway. **Methods:** A mouse xenograft model and corresponding control were established by subcutaneously inoculating 4T1 breast cancer cells into mouse mammary fat pad. Fifteen days later, the mice were sacrificed, and the breast tumors were weighed. Immunohistochemistry was performed to detect CCR4 protein expression in breast tumors and CCL22 and CCL17 protein expressions in liver metastasis. The CCR4 gene was found to be inhibited by lentiviral transduction in 4T1 breast cancer cells. Western blot was used to examine the inhibitory effect. The same method was

used to induce breast tumorigenesis and to examine tumor growth. Three different concentrations of ulinastatin were used to treat 4T1 tumor-bearing mice. Fifteen days later, immunohistochemistry was used to assess the expression of CCR4 in breast tumors and the expression of CCL22 and CCL17 in liver tissues. Western blot and real-time fluorescent quantitative polymerase chain reaction (RTFQ-PCR) were used to detect the expression of TGF- β , microRNA-34a and microRNA-31. We also analyzed the correlation between TGF- β and microRNA-34a, microRNA-31, CCL22, and CCL17. **Results:** Our results showed that CCR4 was highly expressed in mouse breast tumors. CCL22 and CCL17 were highly expressed in the liver metastasis. CCR4 expression was silenced in the 4T1 breast cancer cells and the *in vivo* growth of breast cancer xenograft tumor was inhibited. Ulinastatin significantly inhibited CCR4, TGF- β , CCL22, microRNA-31 and CCL17, but upregulated microRNA-34a. **Conclusion:** These results showed that ulinastatin can inhibit liver metastasis of breast cancer. The specific mechanism may involve ulinastatin acting on TGF- β -microRNA-34a-CCL22 and microRNA-31-TGF- β -CCL17 axes to inhibit CCL17/CCL22-CCR4 signaling pathway in liver tissues.

[**Key words**] Breast cancer; Liver metastasis; Ulinastatin; CCR4; CCL22; CCL17

CC族趋化因子受体4 (CC chemokine receptor 4, CCR4) 是肿瘤转移中常见的趋化因子受体^[1]。CCL22被称为巨噬细胞来源的趋化因子(macrophage-derived chemokine, MDC), CCL17也被称为胸腺和活化调节的趋化因子(thymus and activation-regulated chemokine, TARC), 这两者又是CCR4的主要配体^[2-3]。研究表明, CCR4-CCL17轴以及CCR4-CCL22轴与前列腺癌细胞的侵袭以及肺癌细胞的侵袭等密切相关^[4]。乳腺癌细胞高表达CCR4促进乳腺癌肺转移, 其具体机制是乳腺癌高表达CCR4通过远程促进CCL22和CCL17在肺组织中的表达进而促进乳腺癌肺转移。通过抑制乳腺肿瘤细胞CCR4的表达可以显著抑制乳腺癌肺转移^[4]。乌司他丁 (ulinastatin) 作为一种广谱酶抑制剂, 在增强巨噬细胞吞噬功能以及维持机体免疫力方面具有重要作用^[5], 目前在治疗急性、慢性胰腺炎中应用较为广泛。本课题组前期研究发现乌司他丁能抑制乳腺癌CCR4和TGF- β 的表达^[6], 肿瘤分泌CCL22和CCL17时存在TGF- β -microRNA-34a-CCL22轴^[7]和microRNA-31-TGF- β -CCL17轴的激活^[8]。肝脏是乳腺癌晚期容易转移的部位之一, 乳腺癌肝转移严重影响患者预后。本研究旨在探寻CCL17/CCL22-CCR4在乳腺癌肝转移中的作用, 以及乌司他丁对乳腺癌肝转移的影响及其分子机制, 为进一步探寻乳腺癌肝转移的治疗提供理论基础。

1 材料和方法

1.1 材料

4T1乳腺癌细胞株和HC11小鼠正常乳腺上皮细胞株均购自中国科学院上海生命科学研究院生物化学与细胞生物学研究所细胞库, SPF级雌性BALB/c小鼠(20 g/只)购自重庆医科大学实验动物中心(合格证号: 0005818), RPMI-1640培养基和胎牛血清购自美国Gibco公司, 乌司他丁购自广东天普生化医药股份有限公司, 免疫组织化学试剂盒及DAB显色试剂盒购自北京中杉金桥生物技术有限公司, 羊抗小鼠CCR4单克隆抗体(100 μ g/mL, ab1669)、兔抗小鼠CCL17多克隆抗体(100 μ g/mL, ab182793)和兔抗小鼠CCL22单克隆抗体(150 μ g/mL, EPR1362)购自美国Abcam公司, 所有PCR引物序列由重庆金麦生物技术有限公司合成, CCR4干扰慢病毒购自汉恒生物科技(上海)有限公司。

1.2 方法

1.2.1 细胞培养

将4T1乳腺癌细胞和HC11正常小鼠乳腺上皮细胞在体积分数CO₂为5%、37 °C的条件下培养于含10%胎牛血清、1%的青霉素-链霉素双抗的RPMI-1640高糖培养基中。

1.2.2 慢病毒转染

将生长状态良好的4T1细胞以每孔 1×10^5 个接

种在24孔板中,待细胞贴壁后更换培养基,继续培养。24 h后,待细胞生长至每孔 2×10^5 个,更换新鲜培养基,加入适量沉默CCR4基因的慢病毒及阴性慢病毒悬液,37 °C温育24 h,用新鲜培养基更换含病毒的培养基继续培养。96 h后用荧光显微镜观察慢病毒的转染效率,用流式细胞术检查慢病毒的转染率,并用蛋白质印迹法(Western blot)进行验证。

1.2.3 实验动物分组及处理

将20只BALB/c小鼠随机分为乳腺癌荷瘤组及对照组,每组10只,分别给予50 μ L约 1×10^6 个的4T1细胞悬液和等量HC11细胞悬液于BALB/c小鼠第4对乳腺脂肪垫(mfp)接种;将16只BALB/c小鼠随机分为CCR4抑制组(Transfection)和对照组(Control),每组各8只,分别给予50 μ L约 1×10^6 个转染慢病毒的4T1细胞和未转染慢病毒的4T1细胞;将32只乳腺癌荷瘤模型构建成功的小鼠随机分为4组,即对照组(Control)和乌司他丁低浓度组(Low)、中浓度组(Medium)及高浓度组(High),每组8只,分别每日腹腔注射200 μ L(只·d)0.9%氯化钠溶液及400、800和1 600 U乌司他丁,给药周期为15 d。本实验所涉及的步骤经重庆医科大学动物伦理委员会评价及批准。

1.2.4 采用免疫组织化学法检测小鼠乳腺肿瘤CCR4及肝脏CCL22和CCL17的表达

以颈椎脱臼法处死各组小鼠,取乳腺肿瘤及肝脏组织,用4%多聚甲醛固定48 h,石蜡包埋切片,切片厚度3~5 μ m,切片常规脱蜡,微波蒸煮法抗原修复,采用3%的H₂O₂阻断内源性过氧化物酶活性,山羊血清封闭1 h,滴加CCR4、CCL22及CCL17抗体(1:1 000),4 °C过夜,滴加生物素标记二抗,于室温温育20 min,滴加辣根过氧化物酶标记的链霉卵白素工作液,室温温育1 h,DAB显色,显微镜下观察显色程度,1~3 min终止显色,苏木精复染30~60 s,脱水、透明、封片。运用Image Pro Plus图像分析软件分析免疫组织化学图片,按照公式:阳性指数(positive index, PI)=阳性平均吸光度(D)值 \times 阳性面积百分率计算PI值。

1.2.5 采用Western blot法检测各组小鼠肝脏组织TGF- β 的蛋白水平

以颈椎脱臼法处死各组小鼠,取肝脏组织液氮保存,取大小为3 mm \times 3 mm \times 3 mm的转移瘤组织或对照组组织置于研钵中研磨,研磨后的组织粉末加入细胞裂解液作用30 min,12 000 \times g高速离心10 min,对上清液采用BCA法进行蛋白质含量测定。等量蛋白经SDS-PAGE分离后,转移到PVDF膜上。用5%脱脂奶粉封闭60 min,加入TGF- β 抗体(浓度均为1:1 000),4 °C作用过夜,然后用TBST洗5 min/3次,加入二抗稀释液室温温育60 min,然后再用TBST洗3次,每次5 min。ECL发光液将PVDF膜显色,暗室曝光到X线片上,凝胶成像系统扫描分析结果。实验重复3次。

1.2.6 实时荧光定量聚合酶链反应(real-time fluorescent quantitative polymerase chain reaction, RTFQ-PCR)法检测肝脏组织中TGF- β 、microRNA-34a以及microRNA-31的表达

内参U6基因上游引物序列为5'-CTCGCTTCGGCAGCACCA-3',下游引物序列为5'-AACGCTTCCACGAATTTGCGT-3';microRNA-34a上游引物序列为5'-GGCAGTGTCTTAGCTGGTTG-3',下游引物序列为5'-AAGAGCTTCCGAAGTCCTGG-3';microRNA-31上游引物序列为5'-CGCGGAGGCAAGATGCT-3',下游引物序列为5'-CAGTGCAGGGTCCGAGGTATT-3';TGF- β 上游引物序列为5'-CTGGTTCACGTGACTGATGG-3',下游引物序列为5'-CTTCCCAGGAGCTCTTTCT-3'。具体方法见参考文献[8],采用 $2^{-\Delta\Delta Ct}$ 法计算相对表达。

1.3 统计学处理

使用SPSS 22.0统计分析软件对数据进行处理,所得数值均以 $\bar{x} \pm s$ 表示,多样本均数比较,若方差齐并满足正态分布则用单因素方差分析,两两比较采用LSD-*t*检验法,否则采用秩合检验, $P < 0.05$ 为差异有统计学意义。

2 结 果

2.1 小鼠乳腺癌高表达CCR4并在肝转移时促进肝脏转移瘤表达CCL22和CCL17

CCR4是肿瘤转移常见的趋化因子受体^[1]，CCL22以及CCL17是其主要的配体^[2-3]。为了研究CCR4以及CCL22和CCL17在小鼠乳腺癌肝转移中的作用，我们通过小鼠乳腺脂肪垫注射4T1乳腺癌细胞株以及HC11小鼠正常乳腺上皮细胞株悬液的方式，分别构建荷瘤组小鼠乳腺癌模型和对照组模型，每组各10只小鼠，成瘤15 d

后，摘取两组小鼠乳腺肿瘤、肝脏转移瘤以及转移瘤的癌旁组织，采用免疫组织化学染色法分别检测乳腺肿瘤组织中CCR4以及肝脏转移瘤中CCL22和CCL17的表达。其中，CCR4在荷瘤组（4T1）小鼠乳腺肿瘤组织中呈高表达，在对照组（HC11）小鼠乳腺良性肿块中表达较低。H-E染色法检测到荷瘤组小鼠出现明显的肝转移病灶，对照组肝脏无明显的变化；荷瘤组小鼠乳腺癌出现肝转移后，CCL22和CCL17在转移的肝脏肿瘤组织中的表达较转移瘤癌旁组织显著升高（图1）。

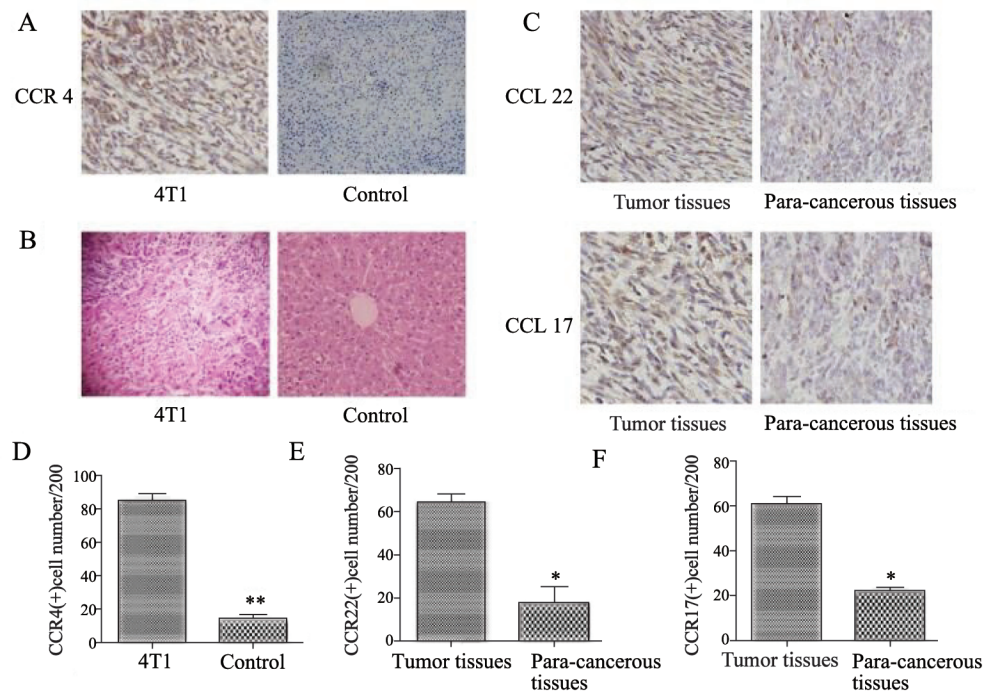


图1 CCR4以及CCL22和CCL17在小鼠乳腺癌肝转移中的表达

Fig. 1 The expression of CCR4, CCL22 and CCL17 in breast cancer with liver metastasis

A: The expression of CCR4 was detected by immunohistochemistry in breast tissues of 4T1 group and HC11 group ($\times 400$); B: The liver tissues of the two groups were detected by H-E staining ($\times 400$); C: The expression of CCL22 and CCL17 was detected by immunohistochemistry in liver metastasis tumor tissues and para-cancerous tissues ($\times 400$); D: CCR4 (+) cell number in 4T1 group and HC11 group; E: CCL22(+) cell number in liver metastasis tumor tissues and para-cancerous tissues; F: CCL17(+) cell number in liver metastasis tumor tissues and para-cancerous tissues. **: $P < 0.01$; *: $P < 0.05$

2.2 沉默乳腺癌细胞CCR4的表达抑制乳腺癌移植瘤的生长

为了进一步研究CCR4对乳腺癌生长的作用，我们通过慢病毒转染的方式抑制4T1细胞CCR4的表达，将未转染（Control）和转染（Transfection）慢病毒的4T1细胞同样以乳腺脂肪垫注射细胞的方式构建乳腺癌模型，每组

各8只小鼠。结果显示，慢病毒转染率较高，达58.4%。慢病毒转染后CCR4的抑制效果较明显。小鼠皮下注射细胞后，继续喂养15 d，取下乳腺肿瘤并称其质量，发现抑制组乳腺肿瘤质量明显低于对照组（图2）。综上所述，沉默4T1乳腺癌细胞CCR4的表达可显著抑制乳腺癌移植瘤的生长。

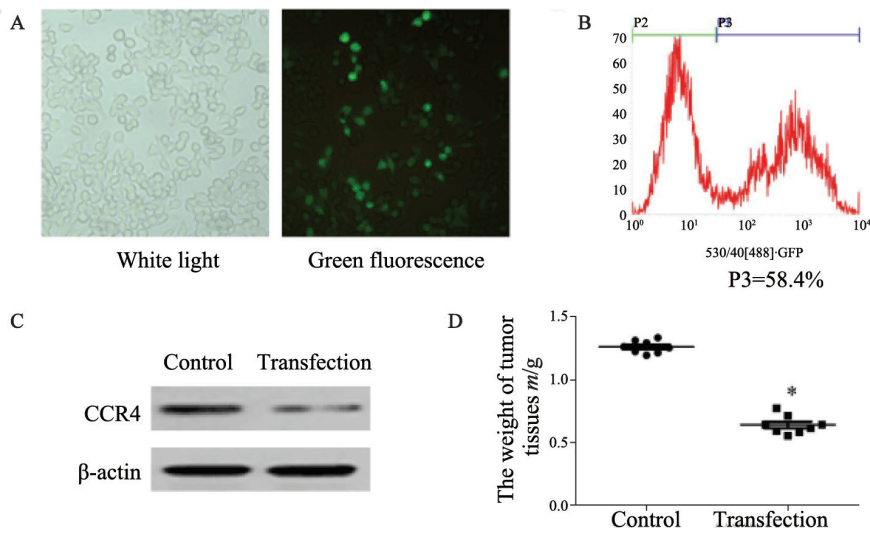


图2 沉默乳腺癌细胞CCR4的表达抑制乳腺癌的生长

Fig. 2 Silencing of CCR4 in breast cancer cells inhibited the growth of breast cancer

A: Lentivirus transfection; B: Flow cytometry was used to detect the rate of lentivirus transfection; C: Lentivirus silence was detected by Western blot; D: The weight of tumor was measured; *: $P < 0.05$

2.3 乌司他丁抑制小鼠乳腺癌肝转移

2.3.1 乌司他丁抑制乳腺癌CCR4的表达和乳腺癌肝转移

随着乌司他丁给药浓度增高, 乳腺癌组织中CCR4表达降低, 乳腺癌肿瘤质量减轻, 并且肝脏转移瘤的质量减轻(图3)。

2.3.2 乌司他丁通过抑制TGF-β-microRNA-34a-CCL22轴及microRNA-31-TGF-β-CCL17轴抑制转移瘤CCL22和CCL17的表达

结果显示, 乌司他丁抑制肝脏转移瘤TGF-β的表达(图4A, 4B); 乌司他丁促进肝脏转移瘤microRNA-34a的表达, 并抑制microRNA-31的表达(图4C); 乌司他丁抑制肝脏转移瘤CCL22和CCL17的表达(图4D); 相关性分析发现TGF-β的表达水平和microRNA-34a、microRNA-31、CCL22以及CCL17的表达高度相关(图4E)。

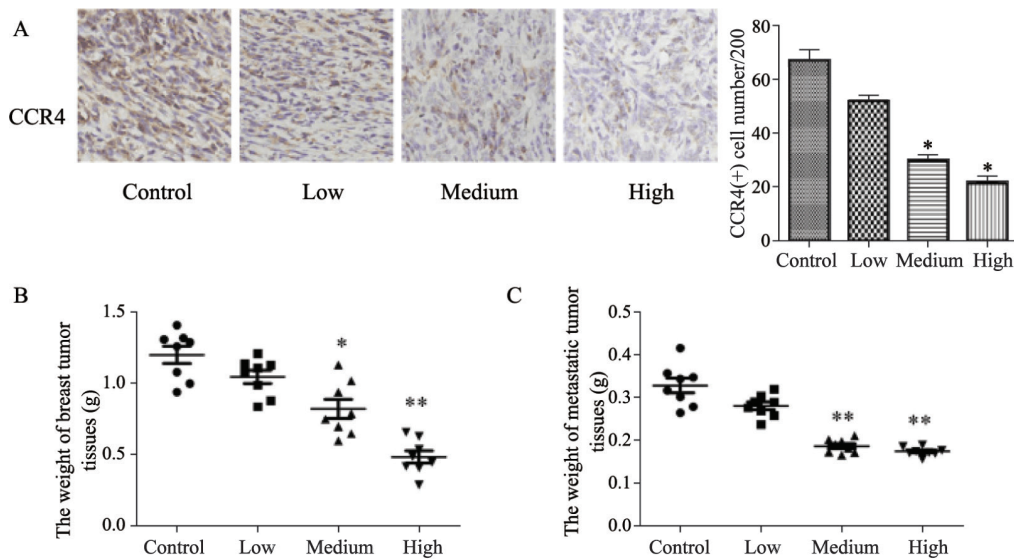


图3 乌司他丁抑制乳腺癌CCR4的表达和乳腺癌的生长并抑制乳腺癌肝转移

Fig. 3 Ulinastatin inhibited the expression of CCR4 in breast tumor tissues, the growth of breast tumor and metastatic tumor

A: The expression of CCR4 was detected by immunohistochemistry in breast tissues after treatment with ulinastatin for 15 d ($\times 400$); B: The weight of breast tumor was measured; C: The weight of metastatic tumor was measured, **: $P < 0.01$; *: $P < 0.05$

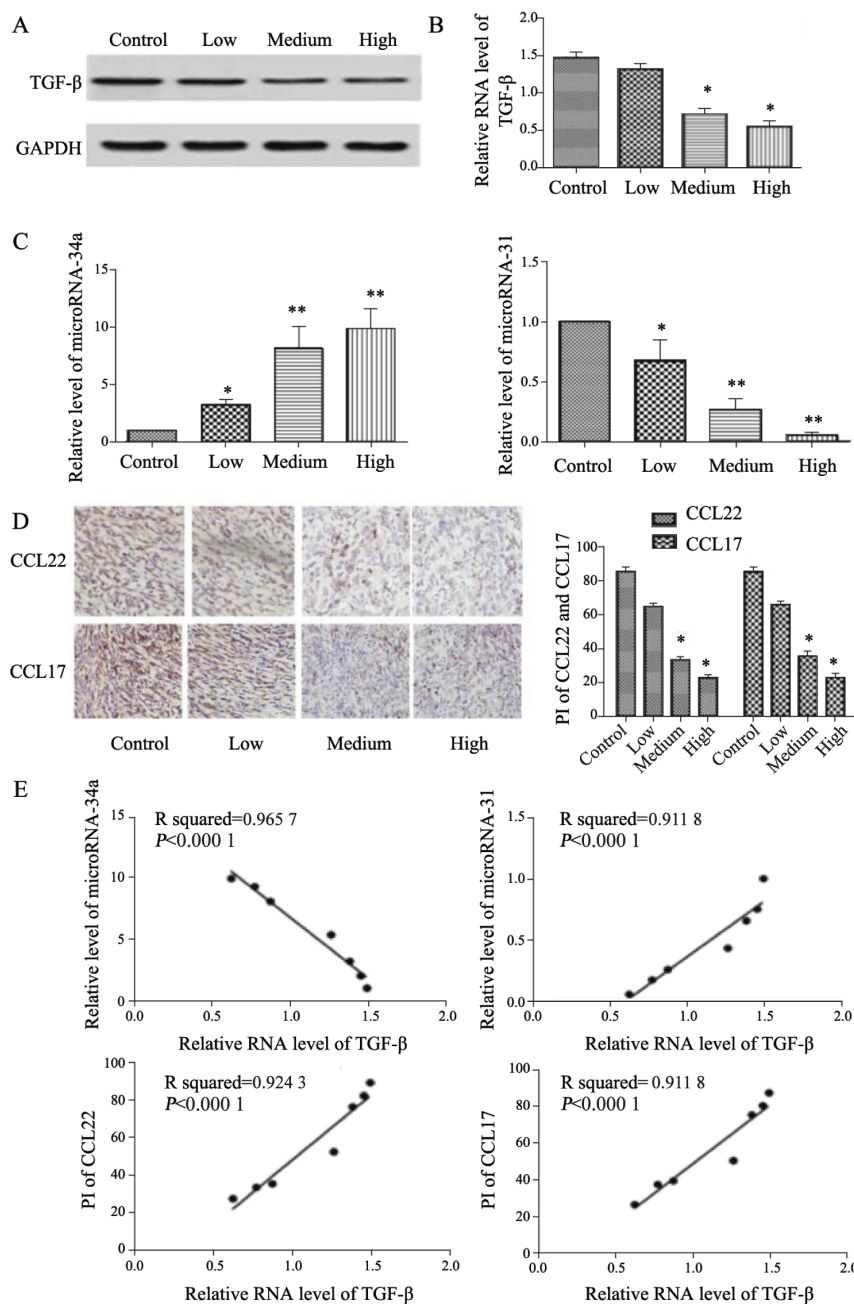


图4 乌司他丁通过抑制TGF-β-microRNA-34a-CCL22轴及microRNA-31-TGF-β-CCL17轴抑制转移癌CCL22和CCL17的表达

Fig. 4 Ulinastatin inhibited the expression of CCL22 and CCL17 in liver tissues via TGF-β-microRNA-34a-CCL22 and microRNA-31-TGF-β-CCL17 axes

A: The expression of TGF-β in liver metastasis tissues was detected by Western blot; B: RTFQ-PCR was used to detect the mRNA expression of TGF-β in liver metastasis tissues; C: RTFQ-PCR was used to detect the mRNA expression of microRNA-34a and microRNA-31 in liver metastasis tissues; D: The expression of CCL22 and CCL17 in liver metastasis tissues was detected by immunohistochemistry (×400); E: Correlation analysis showed that the expression level of TGF-β was highly related to the expression of microRNA-34a, microRNA-31, CCL22 and CCL17. *: $P < 0.01$; **: $P < 0.05$

3 讨 论

乳腺癌细胞具有高度的侵袭性，肝脏是晚期乳腺癌转移的器官之一。研究表明，肝脏微环境

和肝血窦结构是乳腺癌在肝脏内转移侵袭至关重要的环节^[9-12]。进一步研究乳腺癌肝转移的机制具有重要意义。CCR4是肿瘤转移中常见的趋化因子受体^[1]，CCL22和CCL17是CCR4主要的配体^[2-3]。研究表明，乳腺癌细胞高表达CCR4

与乳腺癌细胞的恶性生物学行为密切相关^[13]。高表达CCR4的乳腺癌细胞促进乳腺癌肺转移,其具体机制是乳腺癌高表达CCR4通过远程促进CCL22和CCL17在肺组织中的表达进而促进乳腺癌肺转移^[4]。乌司他丁作为一种广谱酶抑制剂,在增强巨噬细胞吞噬功能以及维持机体免疫力方面具有重要作用^[5],目前主要用于急性、慢性胰腺炎以及休克的治疗。近年来关于乌司他丁在肿瘤治疗中的作用也有很多研究,也取得了一定的成果。Shen等^[14]研究表明乌司他丁和姜黄素协同作用可以有效地抑制小鼠结肠癌远处转移;Gao等^[6]研究发现乌司他丁与多西他赛联合用药可以抑制乳腺癌细胞的侵袭。然而乌司他丁在乳腺癌肝转移中的作用目前未见相关报道。

本课题组前期研究发现乌司他丁能抑制乳腺癌CCR4和TGF- β 的表达^[6,15,16],而研究表明肿瘤细胞分泌CCL22和CCL17时存在TGF- β -microRNA-34a-CCL22轴^[7]和microRNA-31-TGF- β -CCL17轴的激活^[8],TGF- β -microRNA-34a-CCL22轴调节CCL22的分泌^[7],内源性的microRNA-31可以正向调控TGF- β 介导的内皮间质转化(endothelial-mesenchymal transition, EMT),进而诱导CCL17的表达^[8],两条通路与肿瘤的转移密切相关。但是,CCR4及其配体CCL22和CCL17与乳腺癌肝转移是否相关以及乌司他丁对乳腺癌肝转移的影响及其相关机制目前尚不清楚。

本研究通过构建乳腺癌小鼠荷瘤模型同样发现乳腺癌肝转移时乳腺原发肿瘤中CCR4呈高表达,与肿瘤转移密切相关的CCL22和CCL17在转移的肝脏肿瘤组织中的表达也显著升高,沉默乳腺癌细胞CCR4的表达可抑制乳腺癌的生长。乌司他丁可以明显抑制乳腺癌CCR4的表达和乳腺肿瘤的生长,并抑制乳腺癌肝转移,随着乌司他丁浓度的升高,抑制效果越明显。此外,我们通过Western blot检测到乌司他丁处理组的小鼠肝脏转移瘤TGF- β 呈低表达;RTQ-PCR检测发现乌司他丁高浓度组肝脏转移瘤microRNA-34a mRNA表达量较其他组增加,而microRNA-31 mRNA表达量较其他组减少。相关

性分析发现TGF- β 的蛋白表达和microRNA-34a、microRNA-31、CCL22和CCL17表达高度相关。以上研究提示乌司他丁可能通过抑制细胞TGF- β -microRNA-34a-CCL22轴及microRNA-31-TGF- β -CCL17轴进而抑制乳腺癌肝转移肝脏组织中CCL12和CCL17的表达。

综上所述,本研究证实乳腺癌肝转移时,CCL22和CCL17在转移的肝脏组织中呈高表达;乌司他丁通过抑制TGF- β -microRNA-34a-CCL22轴以及microRNA-31-TGF- β -CCL17轴下调肝脏组织CCL22和CCL17的表达,进而抑制由CCL17/CCL22-CCR4介导的小鼠乳腺癌的肝转移。乌司他丁是临床成熟用药,且不良反应较小,此研究为临床治疗乳腺癌肝转移提供了实验基础^[17]。当然,本研究也存在不足,没有深入地探索乌司他丁抑制TGF- β 的相关机制,也没有对乳腺癌肝转移的临床样本进行相关因子的检测,这需要在后续的研究中进一步深入探讨。

[参 考 文 献]

- [1] COLLART M A. The Ccr4-Not complex is a key regulator of eukaryotic gene expression [J]. Wiley Interdiscip Rev RNA, 2016, 7(4): 438-454.
- [2] JAFARZADEH A, ARABI Z, AHANGAR-PARVIN R, et al. Ginger extract modulates the expression of chemokines CCL20 and CCL22 and their receptors (CCR6 and CCR4) in the central nervous system of mice with experimental autoimmune encephalomyelitis [J]. Drug Res (Stuttg), 2017, 67(11): 632-639.
- [3] HIYOSHI M, OKUMA K, TATEYAMA S, et al. Furin-dependent CCL17-fused recombinant toxin controls HTLV-1 infection by targeting and eliminating infected CCR4-expressing cells *in vivo* and *in vitro* [J]. Retrovirology, 2015, 20(12): 73-87.
- [4] OLKHANUD P B, BAATAR D, BODOGAI M, et al. Breast cancer lung metastasis requires expression of chemokine receptor CCR4 and regulatory T cells [J]. Cancer Res, 2009, 69(14): 5996-6004.
- [5] WEI F, LIU S, LUO L, et al. Anti-inflammatory mechanism of ulinastatin: inhibiting the hyperpermeability of vascular endothelial cells induced by TNF- α via the RhoA/ROCK signal pathway [J]. Int Immunopharmacol, 2017, 5(46): 220-227.
- [6] GAO F, SUN Z J, SUN X, et al. Ulinastatin exerts synergistic effects with taxotere and inhibits invasion and metastasis of breast cancer by blocking angiogenesis and the epithelial-mesenchymal transition [J]. Cancer Biother Radiopharm,

- 2013, 28(3): 218–225.
- [7] MAOLAKE A, IZUMI K, SHIGEHARA K, et al. Tumor-associated macrophages promote prostate cancer migration through activation of the CCL22–CCR4 axis [J] . *Oncotarget*, 2017, 8(6): 9739–9751.
- [8] KATSURA A, SUZUKI H I, UENO T, et al. MicroRNA–31 is a positive modulator of endothelial–mesenchymal transition and associated secretory phenotype induced by TGF- β [J] . *Genes Cells*, 2016, 21(1): 99–116.
- [9] GYAWALI B, NIRLAULA S. Duration of adjuvant trastuzumab in HER–2 positive breast cancer: overall and disease free survival results from meta–analyses of randomized controlled trials [J] . *Cancer Treat Rev*, 2017, 12(60): 18–23.
- [10] BOROS M, GEORGESCU R, PODOLEANU C, et al. Review on practical approach in multiple breast carcinomas: does each focus matter? [J] . *Chirurgia (Bucur)*, 2017, 112(4): 418–428.
- [11] GRUBER–ROUH T, LANGENBACH M, NAGUIB N N N, et al. Trans–arterial chemoperfusion for the treatment of liver metastases of breast cancer and colorectal cancer: clinical results in palliative care patients [J] . *World J Clin Oncol*, 2017, 8(4): 343–350.
- [12] PIEROBON M, RAMOS C, WONG S, et al. Enrichment of PI3K–AKT–mTOR pathway activation in hepatic metastases from breast cancer [J] . *Clin Cancer Res*, 2017, 23(16): 4919–4928.
- [13] MACHEREY S, MALLMANN P, MALTER W, et al. Lung metastasectomy for pulmonary metastatic breast carcinoma [J] . *Geburtshilfe Frauenheilkd*, 2017, 77(6): 645–650.
- [14] SHEN F, CAI W S, LI J L, et al. Synergism from the combination of ulinastatin and curcumin offers greater inhibition against colorectal cancer liver metastases via modulating matrix metalloproteinase–9 and E–cadherin expression [J] . *Onco Targets Ther*, 2014, 18(7): 305–314.
- [15] SUN Z J, YU T, CHEN J S, et al. Effects of ulinastatin and cyclophosphamide on the growth of xenograft breast cancer and expression of CXCL chemokine receptor 4 and matrix metalloproteinase–9 in cancers [J] . *J Int Med Res*, 2010, 38(3): 967–976.
- [16] 宋晓丹, 谭 令, 崔晓江, 等. 乌司他丁联合多西他赛对乳腺癌免疫微环境的影响 [J] . *重庆医科大学学报*, 2014, 39(12): 1794–1799.
- [17] LIU D, YU Z, YIN J, et al. Effect of ulinastatin combined with thymosin alpha1 on sepsis: a systematic review and meta–analysis of Chinese and Indian patients [J] . *J Crit Care*, 2017, 39(6): 259–266.

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