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ROR1 激活 AKT/FOXO1 信号介导非小细胞肺癌吉非替尼耐药

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摘要 EGFR-TKI 靶向治疗在非小细胞肺癌(non-small cell lung cancer, NSCLC)综合治疗中显示出重要作用;然而,耐药性却极大限制其临床治疗效果。受体酪氨酸激酶样孤儿受体(receptor tyrosine kinase-like orphan receptor 1, ROR1)是 I 型受体酪氨酸激酶家族中的成员,在肿瘤发生发展中发挥重要作用。本研究拟探讨 ROR1 介导非小细胞肺癌吉非替尼耐药的作用及机制。采用吉非替尼反复诱导非小细胞肺癌 HCC827 细胞,建立吉非替尼耐药细胞株 HCC827/GR。应用荧光定量 PCR 和 Western 印迹检测 HCC827/GR 内 ROR1 的表达。采用 shRNA 的方法体外检测 *ROR*1 敲除前后 HCC827/GR 对吉非替尼耐药的变化,采用体外检测 ROR1 过表达前后 HCC827 对吉非替尼耐药的变化。体内检测 *ROR*1 敲除前后 HCC827/GR 对吉非替尼耐药的变化。Western 印迹检测 HCC827/GR 内 ROR1 下游信号分子的活化。实时荧光定量 PCR 及 Western 印迹结果显示,HCC827/GR 耐药细胞中的 ROR1 mRNA 和蛋白质表达水平显著高于 HCC827 敏感细胞。体外干扰 ROR1 表达,可明显增强 HCC827/GR 耐药细胞对吉非替尼的敏感性(IC_{50} 15.3±3.69 *vs.* 4.2±1.38),增加吉非替尼诱导的细胞凋亡(20.5±2.52 *vs.* 41.8±3.74)。体外过表达 ROR1 显著增强 HCC827 敏感细胞对吉非替尼的耐药性(IC_{50} 0.8±0.52 *vs.* 2.2±0.87)。体内裸鼠移植瘤实验同样发现,干扰 ROR1 能增强 HCC827/GR 移植瘤对吉非替尼的敏感性。进一步研究发现,AKT/FOXO1 信号在 HCC827/GR 耐药细胞中异常活化,而干扰 ROR1 能够抑制 AKT 的磷酸化,并上调 FOXO1 的表达。上述结果表明,ROR1 参与非小细胞肺癌吉非替尼耐药,抑制 ROR1 能够逆转吉非替尼耐药,其机制与 ROR1 调控 AKT/FOXO1 信号有关。

关键词 非小细胞肺癌; 受体酪氨酸激酶样孤儿受体; 吉非替尼耐药; AKT; Forkhead box O1
中图分类号 R73

ROR1-mediated Gefitinib Resistance of Non-small Cell Lung Cancer by Activating AKT/FOXO1

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Abstract EGFR-TKI targeted therapy has been playing an important role in the treatment of non-small cell lung cancer (NSCLC). However, unavoidable therapeutic resistance significantly limits the clinical efficacy of TKI. Receptor tyrosine kinase-like orphan receptor 1 (ROR1) is a member of the type I receptor tyrosine kinase family and plays an important role in cancer development and progression. The aim of this work is to investigate the effect and molecular mechanism of ROR1 on gefitinib resistance in NSCLC. EGFR-TKI-resistant HCC827/GR cells were established from parental HCC827 cells by continuous exposure to gefitinib. ROR1 expression on the mRNA and protein levels was detected by qRT-

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PCR and Western blot respectively. Influence of ROR1 to gefitinib resistance in HCC827/GR cells was assayed by ROR1 shRNA in vitro. Influence of ROR1 to gefitinib resistance was assayed by ROR1 overexpression in HCC827 cells in vitro or by ROR1 shRNA in HCC827/GR cells in xenograft mouse model. ROR1 downstream signal molecules were detected by Western blot. We found that the mRNA and protein levels of ROR1 are significantly increased in HCC827/GR. Knockdown of ROR1 significantly increased gefitinib sensitivity of HCC827/GR cells and gefitinib-induced cell apoptosis in vitro. Overexpression of ROR1 significantly increased gefitinib resistance of HCC827 cells in vitro. Moreover, in vivo assays also showed that knockdown of ROR1 significantly increased gefitinib sensitivity ($P < 0.05$). Furthermore, we found that abnormal activation of AKT/FOXO1 signaling was detected in HCC827/GR cells, and knockdown of ROR1 significantly inhibited the phosphorylation of AKT, but increased the expression of FOXO1. Taken together, these results indicated that ROR1 could increase gefitinib resistance in HCC827 cells, by which mechanism is related to activation of AKT/FOXO1 signaling.

Key words non-small cell lung cancer; receptor tyrosine kinase-like orphan receptor 1 (ROR1); gefitinib resistance; AKT; forkhead box O1 (FOXO1)

肺癌是中国发病率和死亡率最高的恶性肿瘤[1]。肺癌依据其组织形态和临床特征可分为小细胞肺癌(small cell lung cancer, SCLC)和非小细胞肺癌(non-small cell lung cancer, NSCLC)。NSCLC包括腺癌、鳞癌和腺鳞癌等,占肺癌的80%左右[2]。NSCLC临床治疗方法包括手术、放化疗和靶向治疗等。其中,靶向治疗以其选择性作用强,疗效显著,毒副作用小等优势,在临床NSCLC治疗中展现了不可替代的作用,已逐渐成为NSCLC临床标准治疗的一部分[3]。众多研究发现,NSCLC组织存在大量与肿瘤发生发展、生长增殖、凋亡及生存密切相关的驱动基因(driver oncogene)突变(driver mutation)活化。如 *EGFR*(epidermal growth factor receptor)、*KRAS*、*MET*、*HER*2、*ALK*、*ROS*、*RET*、P53、*PTEN* 等基因的点突变、扩增、重排和缺失等[4,5]。表皮生长因子受体(EGFR)是具有细胞增殖和凋亡调控功能的跨膜受体酪氨酸激酶(RTK),是肺癌最重要的驱动基因[6]。近年来,针对EGFR靶向治疗越来越受到人们的重视,以吉非替尼(gefitinib)、厄洛替尼(erlotinib)为代表的EGFR-酪氨酸激酶抑制剂(tyrosine kinase inhibitor, TKI)正在临床用于NSCLC,获得普遍优于传统化疗的疗效[7,8]。临床治疗和研究发现,几乎所有初始EGFR靶向治疗有效的NSCLC患者,经过治疗后不可避免地出现耐药,致使肺癌总的生存率难以获得明显提高[9]。因此,深入研究EGFR靶向治疗耐药的分子机制,对于肺癌临床治疗具有重要意义。

受体酪氨酸激酶样孤儿受体(receptor tyrosine kinase-like orphan receptor 1, ROR1)是I型受体酪氨酸激酶家族中的重要成员,参与细胞间信号交流、胞内信号转导等过程,调节细胞增殖、分化和转移[10]。新近研究表明,ROR1在肺癌组织中高表达,且与患者临床分期、淋巴结转移和预后不良相关[11,12]。Karachaliou等[13]研究发现,ROR1高表达与非小细胞肺癌TKI靶向治疗患者无进展生存期显著负相关,提示ROR1可能与NSCLC靶向治疗耐药有关。然而,迄今对于ROR1在NSCLC靶向治疗耐药中的作用及机制尚不明确。本研究拟探讨ROR1介导非小细胞肺癌HCC827吉非替尼耐药的作用及可能机制。

1 材料与方法

1.1 材料

RPMI-1640培养基,胎牛血清,胰蛋白酶购自Gibco公司; ROR1, FOXO1, FOXO3a, FOXO4, p-AKT, AKT抗体购自CST公司;HRP标记山羊抗鼠IgG、山羊抗兔IgG、β-肌动蛋白购自Santa cruz公司,预染蛋白质标记物购自Fermentas公司;Annexin V-FITC细胞凋亡检测试剂盒、RIPA裂解液购自碧云天生物技术公司; ECL化学发光底物试剂盒购自Pierce公司;Quick Start Bradford蛋白定量试剂购自Bio-Rad公司;反转录试剂盒、real time RT-PCR试剂盒购自TaKaRa公司;PCR引物由上海生工公司合成;gefitinib、嘌呤霉素购自Sigma公司,其余试剂均为国产分析纯以上。

1.2 细胞及其培养

吉非替尼敏感细胞系HCC827及其他肺癌细胞株A549、PC9、H1650等均购自中科院上海细胞所。HCC827及H1650细胞用RPMI-1640培养基,A549及PC9细胞用DMEM培养基,(两种培养基均含

10% 胎牛血清、100 U/mL 青霉素、100 U/mL 链霉素),于 37 ℃、5% CO_2 的培养箱内饱和湿度培养。利用低浓度吉非替尼(0.5 mmol/L)持续诱导 HCC827 细胞 6 个月,期间逐渐提升诱导药物浓度至 3 mmol/L,建立吉非替尼耐药细胞系 HCC827/GR。

1.3　ROR1 稳定干扰细胞系的筛选

利用慢病毒系统在 293T 细胞中包装 ROR1 shRNA 病毒,接种 HCC827/GR 耐药细胞或者 H1650 细胞于 6 孔细胞培养板,待细胞达到 70% 汇合度,去除培养基,每孔加入 2 mL 10 mg/mL polybrene,2 mL 新鲜培养基,0.3 mL 病毒颗粒。37 ℃培养 24 h,采用嘌呤霉素筛选稳定细胞株。收集细胞用于 qRT-PCR 及免疫印迹检测干扰效率。

1.4　MTS 检测

以 2×10^4/mL 接种细胞到 96 孔板中,100 mL/孔,培养过夜使细胞贴壁。向对应试验孔加入不同浓度(0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mmol/L)的吉非替尼,继续培养 72 h,吸去培养基,加入 100 mL 含 0.5 mg/mL MTS 的 RPMI-1640,继续培养 4 h。最后用酶标仪测定 490 nm 波长下的 A 值,并计算 IC_{50} 值。

1.5　克隆形成实验

取对数期生长细胞消化计数并制成细胞悬液,每种细胞在六孔板每孔同时接种 200 个/孔,每孔中加入 2 mL 的 RPMI-1640 培养基,置于培养箱中培养 15 d,PBS 清洗 3 次,固定液固定细胞 30 min,之后加入 0.1% 的结晶紫染色 2 h,清水冲洗干净后晾干,拍照。

1.6　Annexin V/PI 细胞凋亡检测

以 3×10^5/孔接种细胞到 6 孔板中,采用不同浓度的吉非替尼处理细胞 48 h。收集细胞,按照 Annexin V/PI 凋亡检测试剂盒的说明书操作,先后加入 Annexin V 和 PI,避光、室温孵育 10 min,流式细胞仪检测细胞凋亡。

1.7　实时荧光定量 PCR

按照 Trizol(Invitrogen)说明书方法提取细胞总 RNA,按 Primescript RT reagent Kit 反转录试剂盒(TaKaRa)说明书操作,将 RNA 逆转录为 cDNA。引物由华大基因设计并合成,引物序列见 Table 1。Real-time PCR 反应体系参照 SYBR Premix Ex TapTM 试剂盒(TaKaRa);反应条件如下:95 ℃ 30 s; 95 ℃ 5 s; 60 ℃ 30 s,40 个循环。$2^{-\triangle\triangle CT}$ 法计算 mRNA 的相对表达量,以 GAPDH 作为内参。每个实验组重复 3 次。

Table 1　The primers used in real-time RT-PCR

Name	Sequences (5′-3′)
ROR1-F	AATGCAGAGTAACGTGGAAGTGGTC
ROR1-R	TGGTCGCTCAATCTCCAGGTC
GAPDH-F	GCACCGTCAAGGCTGAGAAC
GAPDH-R	TGGTGAAGACGCCAGTGGA

1.8　Western 印迹分析

PBS 洗涤细胞 2 次,加入 RIPA 裂解液冰上放置 30 min,收集裂解液于 4℃ 12 000 r/min 离心 20 min,取上清,采用 BCA 法测定蛋白质浓度。12% 的 SDS-PAGE 分离蛋白质,恒流 200 mA 湿转 100 min 至 PVDF 膜上,5% 脱脂奶粉室温封闭 2 h,加入一抗 ROR1(1∶1 000 稀释),β-肌动蛋白(1∶1 000 稀释),4℃孵育过夜,PBST 洗膜 3 次,每次 10 min,加入 HRP 标记的特异性二抗(1∶5 000 稀释),室温孵育 2 h,PBST 洗膜,采用 ECL 化学发光试剂对 X 光片显影,扫描图片。

1.9　过表达载体构建及转染

LV-ROR1 慢病毒表达载体及对照载体 LV-Control 购自于吉凯基因公司。接种 HCC827 细胞于 6 孔细胞培养板,待细胞达到 70% 汇合度,转染 ROR1 过表达慢病毒载体及对照载体 48 h,qRT-PCR 检测转染效果。

1.10　裸鼠移植瘤检测

按照每只裸鼠 1×10^6 个细胞/200 mL 的体积,接种于稳定干扰 ROR1 的 HCC827/GR 细胞(HCC827/GR/shROR1)和对照细胞(HCC827/GR/shCon)至裸鼠左侧臀部皮下。待移植瘤长至约 30 mm^3,按 30 mg/kg/d 腹腔注射吉非替尼。游标卡尺测定肿瘤的长径 a 和短径 b,按 $v = 1/2ab^2$ 计算肿瘤体积,每 3 d 测量 1 次,绘制肿瘤体积变化曲线。于给药后第 24 d 采用颈椎脱臼法处死裸鼠,解剖裸鼠,剥离移植瘤。

1.11　统计学方法

所有实验均重复 3 次,所有数据采用 Graphpad Prim 软件分析。数据以平均数 ± 标准差(Mean ± SD)表示,组间差异比较采用 t 检验分析,$P < 0.05$ 具有显著性统计学差异。

2　结果

2.1　构建吉非替尼耐药 HCC837/GR 细胞株

采用浓度梯度的吉非替尼诱导该药耐药性亚克隆形成,建立吉非替尼耐药细胞株 HCC827/

GR。在倒置显微镜下 HCC827 呈铺路石状上皮细胞形态,而 HCC827/GR 细胞呈现梭形改变(Fig. 1 A)。采用不同浓度梯度的吉非替尼分别处理 HCC827 和 HCC827/GR 细胞,结果显示,吉非替尼能够有效抑制 HCC827 细胞增殖,而对 HCC827/GR 耐药细胞的增殖抑制作用较弱,$P < 0.05$(Fig. 1B);吉非替尼对 HCC827 和 HCC827/GR 细胞的 IC_{50} 分别为 0.86 ± 2.38 mmol/L 和 15.23 ± 3.65 mmol/L。结果表明,成功建立吉非替尼耐药细胞株 HCC827/GR。

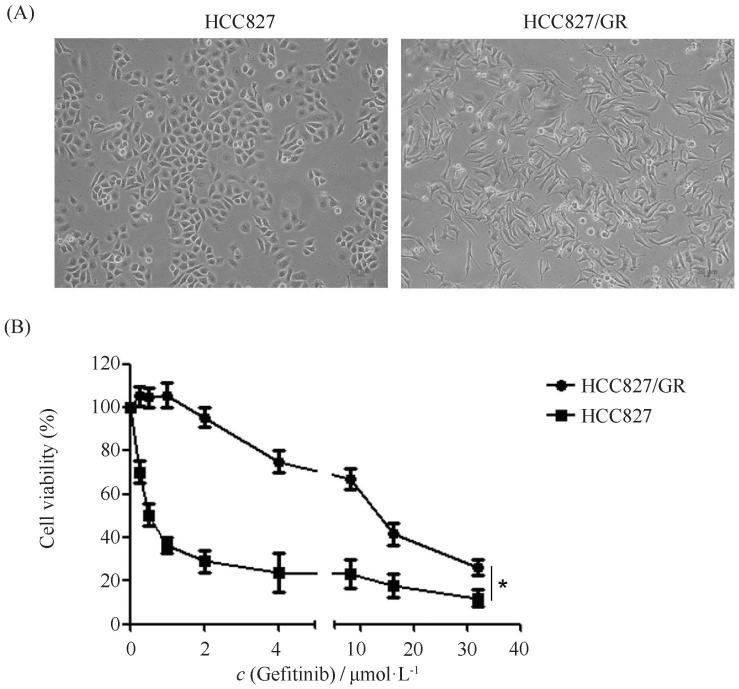

Fig. 1 Establishment of gefitinib resistant non-small cell lung cancer cell line, HCC827/GR (A) Cell morphology of HCC827 and HCC827/GR cells, scale bar: 20 μm. (B) HCC827 and HCC827/GR cells were treated with gefitinib at the indicated concentration (0, 0.25, 0.5, 1, 2, 4, 8, 16, 32 mmol/L) for 48 hours, and cell viability was measured by MTS assay

2.2 ROR1 在 HCC827/GR 耐药细胞中高表达

为明确 ROR1 在细胞中表达水平,采用实时荧光定量 PCR 检测 ROR1 在 HCC827 细胞和耐药细胞 HCC827/GR 中的表达量。结果显示,ROR1 mRNA 在 HCC827/GR 细胞内的表达显著高于 HCC827 细胞(1 ± 0.05 *vs.* 3.2 ± 0.8)(Fig. 2 A)。进一步采用 Western 印迹检测肺癌细胞 A549、PC9、H1650、HCC827 和耐药细胞 HCC827/GR 中 ROR1 的表达,发现 ROR1 蛋白在 H1650 和耐药细胞 HCC827/GR 中的表达水平明显高于其在 HCC827 细胞、A549 和 PC9 细胞中的表达(Fig. 2B)。

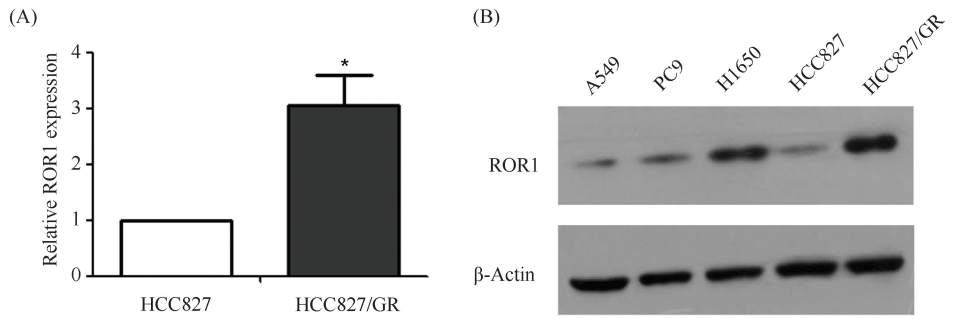

Fig. 2 ROR1 is over-expressed in gefitinib-resistance HCC827/GR cells (A) The expression of ROR1 in HCC827 and HCC827/GR cells was analyzed by qRT-PCR. b-Actin was employed as an internal control. * $P < 0.05$. (B) The expression of ROR1 in A549, PC9, H1650, HCC827 and HCC827/GR cells was analyzed by Western blotting

2.3 *ROR1* 基因沉默增强耐药细胞对吉非替尼的敏感性

为考察 ROR1 是否介导吉非替尼耐药,本文采用 ROR1 shRNA 稳定干扰 HCC827/GR 耐药细胞中 ROR1 的表达(1 ± 0.01 *vs.* 0.35 ± 0.1)(Fig. 3A)。MTS 结果显示,干扰 ROR1 能明显增加 HCC827/GR

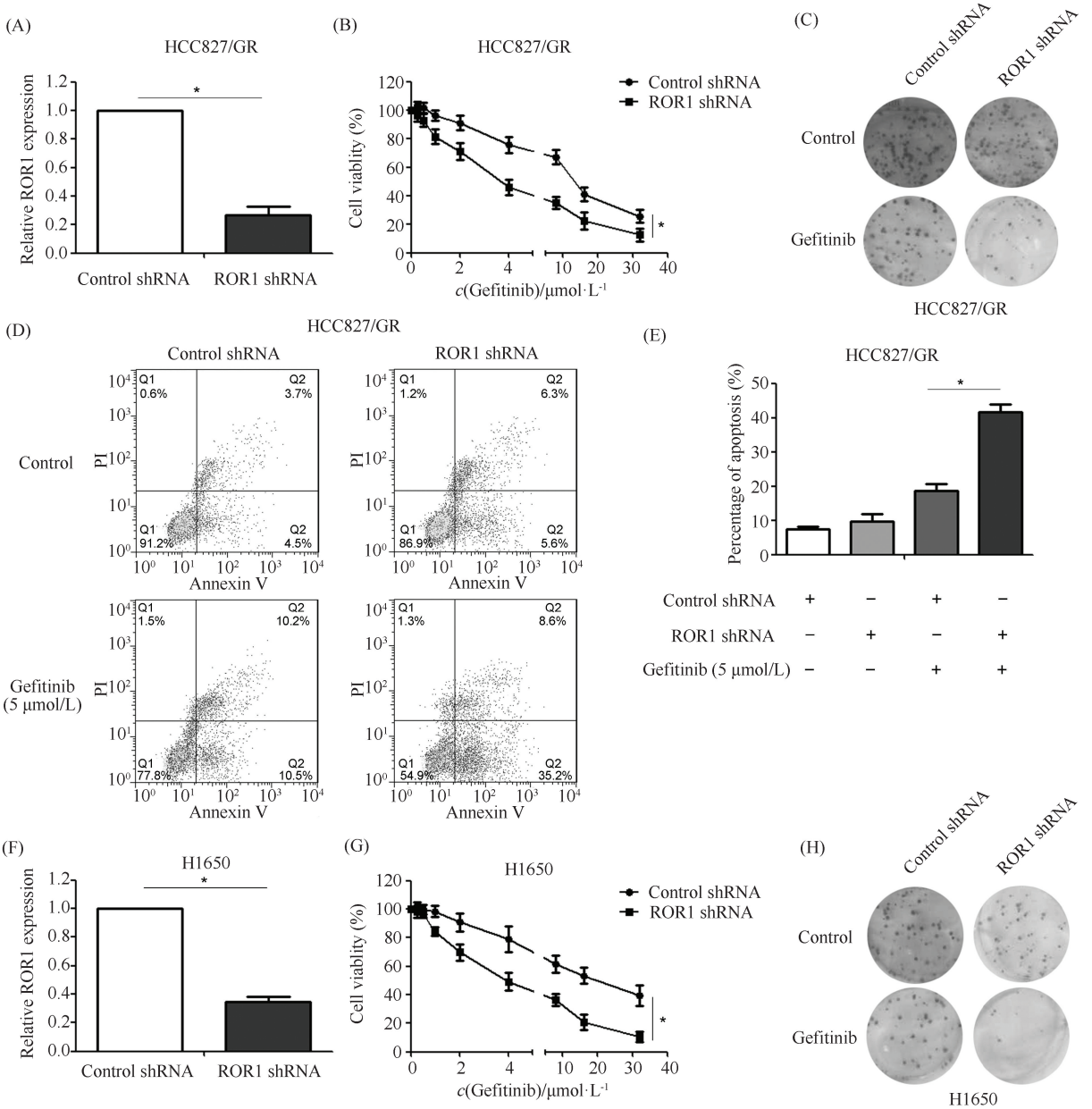

Fig. 3　Knockdown of ROR1 increased gefitinib sensitivity *in vitro*　(A) The expression of ROR1 in HCC827/GR cells transfected with Control shRNA or ROR1 shRNA was analyzed by qRT-PCR. β-Actin was employed as an internal control. $^{*}P < 0.05$. (B) HCC827/GR cells transfected with Control shRNA or ROR1 shRNA were treated with gefitinib at the indicated concentrations for 48 hours, and cell viability was measured by MTS assay. (C) HCC827/GR cells transfected with Control shRNA or ROR1 shRNA were treated with 5 mmol/L gefitinib for 14 days, and colonies were fixed with acetic acid-methanol (1∶4) and stained with crystal violet. (D) HCC827/GR cells transfected with Control shRNA or ROR1 shRNA were treated with 5 mmol/L gefitinib for 48 hours, and cells were stained with Annexin V-FITC and propidium iodide. The cell apoptosis was analyzed by flow cytometry. (E) The rates of cell apoptosis were calculated. $^{*}P < 0.05$. (F) The expression of ROR1 in H1650 cells transfected with Control shRNA or ROR1 shRNA was analyzed by qRT-PCR. b-Actin was employed as an internal control. $^{*}P < 0.05$. (G) H1650 cells transfected with Control shRNA or ROR1 shRNA were treated with gefitinib at the indicated concentrations for 48 hours, and cell viability was measured by MTS assay. (H) H1650 cells transfected with Control shRNA or ROR1 shRNA were treated with 5 mmol/L gefitinib for 14 days, and colonies were fixed with acetic acid-methanol (1∶4) and stained with crystal violet

耐药细胞对吉非替尼的敏感性（IC_{50} 15.3 ± 3.69 *vs.* 4.2 ± 1.38）（Fig. 3B）。克隆形成实验同样显示，ROR1 干扰组的克隆形成数明显少于对照组（55 ± 15 *vs.* 32 ± 8）（Fig. 3C）。进一步采用凋亡检测干扰 ROR1 对吉非替尼诱导 HCC827/GR 细胞凋亡的影响，结果表明，干扰 ROR1 能够显著增加吉非替尼诱导的细胞凋亡比率（20.5 ± 2.52 *vs.* 41.8 ± 3.74）（Fig. 3D，E）。采用 ROR1 shRNA 稳定干扰高表达 ROR1 细胞 H1650 中 ROR1 的表达（Fig. 3F），MTS 结果表明，干扰 ROR1 能明显增加 H1650 细胞对吉非替尼的敏感性（IC_{50} 23.4 ± 2.15 *vs.* 4.1 ± 0.56）（Fig. 3G）。克隆形成实验同样显示，ROR1 干扰组的克隆形成数明显少于对照组（35 ± 17 *vs.* 8 ± 5）（Fig. 3H）。结果表明，干扰 ROR1 可增强肿瘤细胞对吉非替尼的敏感性。

2.4 过表达 ROR1 增强敏感细胞对吉非替尼的耐药性

为考察 ROR1 是否介导吉非替尼耐药，本文在 HCC827 吉非替尼敏感细胞株中过表达 ROR1（Fig. 4 A）。MTS 分析的结果显示，过表达 ROR1 显著增强 HCC827 敏感细胞对吉非替尼耐药（IC_{50} 0.8 ± 0.52 *vs.* 2.2 ± 0.87）（Fig. 4B）。克隆形成实验也显示，过表达 ROR1 组的克隆形成数明显多于对照组（8 ± 6 *vs.* 40 ± 12）（Fig. 4C）。

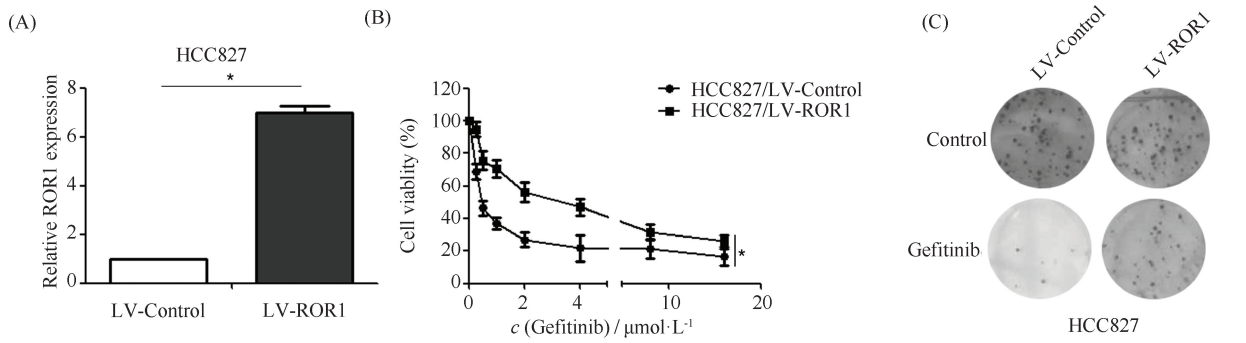

Fig. 4 Overexpression of ROR1 increased Gefitinib resistance *in vitro* (A) The expression of ROR1 in HCC827 cells transfected with LV-Control or LV-ROR1 was analyzed by qRT-PCR. b-Actin was employed as an internal control. * $P<0.05$. (B) HCC827 cells transfected with LV-Control or LV-ROR1 were treated with gefitinib at the indicated concentrations for 48 hours, and cell viability was measured by MTS assay. * $P<0.05$. (C) HCC827 cells transfected with LV-Control or LV-ROR1 were treated with 5 mmol/L gefitinib for 14 days, and colonies were fixed with acetic acid-methanol (1∶4) and stained with crystal violet

2.5 ROR1 基因沉默增强 HCC827/GR 移植瘤对吉非替尼的敏感性

进一步采用小鼠移植瘤实验探讨干扰 ROR1 对吉非替尼耐药性的影响。皮下接种 ROR1 稳定干扰的 HCC827/GR 细胞（HCC827/GR/shROR1）和对照细胞（HCC827/GR/shCon）至裸鼠，待肿瘤长至约 30 mm^3，采用腹腔注射吉非替尼（30 mg/kg/d）处理裸鼠。用游标卡尺测量肿瘤的大小并计算肿瘤体积。Fig. 5 A 为移植瘤的图片。Fig. 5B 为肿瘤大小的生长曲线。与 HCC827/GR/shCon 移植瘤相比，HCC827/GR/shROR1 移植瘤的生长缓慢（Fig. 5 A），HCC827/GR/shROR1 移植瘤大小（Fig. 5B）和体积（Fig. 5C）均小于 HCC827/GR/shCon 组的移植瘤，差异具有统计学意义。结果表明，干扰 ROR1 能增强 HCC827/GR 移植瘤对吉非替尼的敏感性。

2.6 *ROR1* 基因沉默抑制 AKT/FOXO1 信号

研究表明，AKT 信号活化是 EGFR-TKI 获得性耐药的重要机制。本文采用 Western 印迹检测发现，HCC827/GR 耐药细胞 AKT 的磷酸化水平明显高于 HCC827 敏感细胞（Fig. 6 A）。同时发现，AKT 下游肿瘤抑制因子 FOXO1 在 HCC827/GR 耐药细胞的表达显著下调，而 FOXO3a、FOXO4 的表达未见明显变化（Fig. 6 A）。为了进一步探讨 ROR1 是否通过调控 AKT/FOXO1 信号介导 HCC827 细胞吉非替尼耐药。结果发现，在 HCC827/GR 细胞中干扰 ROR1 能够明显抑制 AKT 的磷酸化，促进 FOXO1 的表达（Fig. 6B）。结果表明，干扰 ROR1 能够抑制 AKT/FOXO1 信号。

3 讨论

EGFR-TKI 靶向治疗以其选择性作用强，疗效显著，毒副作用小等优势在临床 NSCLC 治疗中越来越被重视，然而对其产生耐药性已成为 NSCLC 治疗的主要障碍[6-9]。TKI 耐药是多因素参与的复杂过程，其分子机制尚未明确。因此，本研究首先采用体外浓度梯度递增的诱导方法处理 HCC827 细胞，建

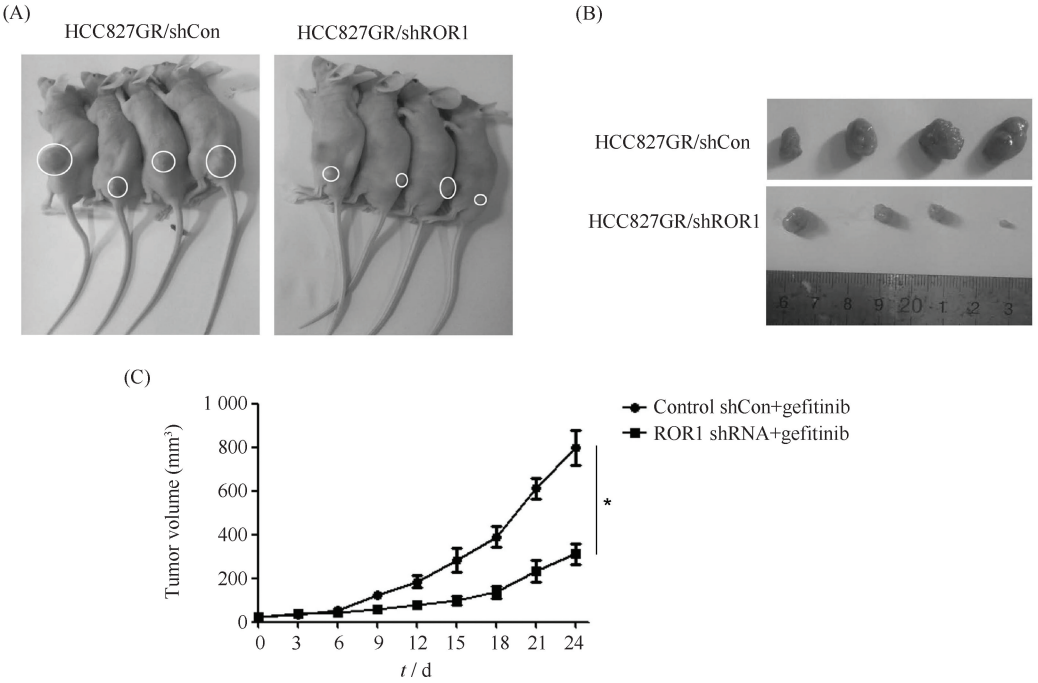

Fig. 5　Knockdown of ROR1 increased gefitinib sensitivity *in vivo*　(A) HCC827/GR cells transfected with Control shRNA or ROR1 shRNA were injected to the left buttocks of nude mice. Tumor-bearing mice were intraperitoneally injected with gefitinib (30 mg/kg/day) for 4 weeks. (B) At the end of treatment, tumors were excised. (C) Tumor sizes were measured at every 3 days with calipers, and the volumes of tumor were determined using the following formula: volume = length × width^2 × 0.5. $^*P<0.05$

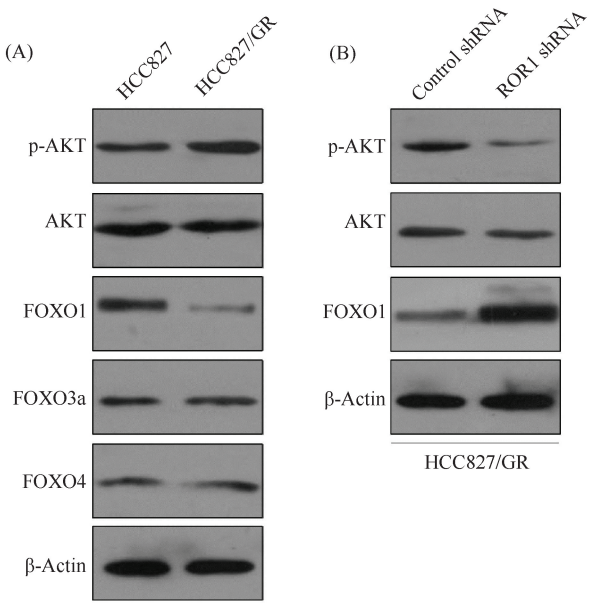

Fig. 6　Knockdown of ROR1 increased gefitinib sensitivity via activation of AKT/FOXO1 signaling
(A) The expression of p-AKT, AKT, FOXO1, FOXO3a, and FOXO4 in HCC827 and HCC827/GR cells was analyzed by Western blotting. (B) HCC827/GR cells were transfected with Control shRNA or ROR1 shRNA, and the expression of p-AKT, AKT, FOXO1 was analyzed by Western blotting

立了吉非替尼获得性耐药细胞株 HCC827/GR,为进一步探索 NSCLC 耐药机制的关键分子,寻求耐药逆转剂来克服耐药提供细胞模型。ROR1 是 I 型受体酪氨酸激酶家族中的重要成员[10]。近年的研究发现,ROR1 在多种恶性肿瘤中异常表达,其肿瘤发生发展中的作用已引起广泛关注[11,14,15]。MacKeigan 等最先发现 ROR1 具有原癌基因的作用[16]。随后,多项研究表明 ROR1 能够体内外促进肿瘤生长,在肿瘤恶性进展中发挥着促进作用[17-19]。研究表明,ROR1 是 Wnt5a 的重要受体,Wnt5a 通过与 ROR1 直接结合而激活 NF-κB 信号[20];Wnt5a 重组蛋白刺激能促进 ROR1 与酪蛋白激酶 1 (casein kinase1, CK1)结合,进而激活 PI3K/AKT 信号[21]。新近研究发现,Wnt5a 介导多种癌症类型的化疗耐药作用[22,23]。然而,作为 Wnt5a 的重要受体,ROR1 是否介导肿瘤药物耐受尚无报道。本研究发现,ROR1 在 HCC827/GR 耐药细胞中的表达显著高于亲本 HCC827 细胞,体内外研究发现,干扰 ROR1 能够增加 HCC827/GR 耐药细胞的药物敏感性。

研究发现 EGFR-TKI 耐药的主要机制包括:(1) *EGFR* 基因 20 外显子 T790 M 突变是 EGFR-TKI 耐药的最常见机制,在约 60% 的继发耐药 NSCLC 患者中可见 20 外显子 T790 M 突变[24,25]。(2)原癌基因 *Met* 扩增是 EGFR-TKI 获得性耐药的另一机

制,并发现22%的EGFR-TKI获得性耐药患者肿瘤组织中存在 *Met* 基因扩增[26]。后续的研究表明,*Met* 基因扩增主要通过激活PI3K/AKT信号导致EGFR-TKI耐药[27]。前期研究中,我们在HCC827/GR耐药细胞中未检测到T790 M突变,却发现HCC827/GR耐药细胞中AKT磷酸化水平显著上调,而其下游的重要肿瘤抑制因子FOXO1则表达下调,结果表明,AKT信号在HCC827/GR耐药细胞中异常活化。Gentile等研究发现,ROR1是介导Met驱动肿瘤发生的的关键分子[28],干扰ROR1能够抑制Met下游信号活化[29]。本文进一步研究发现,干扰ROR1能够抑制HCC827/GR耐药细胞中AKT的磷酸化,进而促进FOXO1的表达。因此,ROR1可能参与Met对AKT信号通路的调控作用,从而促进NSCLC吉非替尼耐药。

总之,本文研究发现,ROR1可能通过激活AKT/FOXO1信号在NSCLC吉非替尼耐药中发挥重要作用,是逆转NSCLC吉非替尼耐药的有效靶点,但其具体功能和作用机制仍需进一步研究。随着ROR1逆转肿瘤耐药的分子机制的进一步的研究,针对ROR1的靶向干预为NSCLC靶向治疗耐药机制的研究提出了新的思路,在NSCLC预防与治疗中必将发挥重要作用。

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