TBL1XR1 介导上皮-间质转化和干细胞特性调控头颈部鳞状细胞癌细胞迁移和侵袭

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摘　要：［目的］阐明转导素 β1X 连锁受体蛋白 1（transducin β-like 1 X-linked receptor 1，TBL1XR1）可介导上皮-间质转化和干细胞特性，从而调控头颈鳞癌细胞的迁移、侵袭。［方法］利用 UALCAN 数据库网站搜索 TBL1XR1 在头颈鳞癌组织及癌旁组织中的表达情况，应用 Cbioprotal 在线分析平台分析癌症基因组图集（The Cancer Genome Atlas，TCGA）数据库中 523 例头颈鳞癌 RNA-SEQ 数据。将慢病毒介导的 TBL1XR1 过表达载体、沉默载体和对应空白载体分别转染头颈鳞癌细胞 Tu686。划痕愈合实验、Transwell 迁移和侵袭实验评价 Tu686 细胞的迁移、侵袭能力；Western blot、qRT-PCR 检测上皮-间质转化标志物表达；肿瘤球形成实验、qRT-PCR 检测干细胞特性；抑制 Wnt/β-catenin 信号通路后检测 TBL1XR1 过表达引起的细胞迁移、侵袭、上皮-间质转化和干细胞特性。［结果］TBL1XR1 mRNA 在头颈鳞癌组织中的表达明显高于相应癌旁组织（P<0.001）。过表达 TBL1XR1 的 Tu686 细胞划痕愈合能力（90%±4% *vs* 53%±6%）、Transwell 迁移（0.68±0.04 *vs* 0.32±0.03）、侵袭能力（0.59±0.04 *vs* 0.26±0.04）较对照组显著性增强（P 均<0.05）。TBL1XR1 表达被抑制时，细胞划痕愈合能力（70%±4% *vs* 96%±5%）、Transwell 迁移（0.32±0.06 *vs* 0.63±0.08）、侵袭能力（0.24±0.04 *vs* 0.52±0.05）较对照组显著性降低（P 均<0.05）。TBL1XR1 过表达后，Tu686 细胞的间质细胞标志物 Vimentin、N-cadherin 表达升高，上皮细胞标志物 E-cadherin 表达降低；肿瘤球形成数较对照组明显增多（41±9 *vs* 13±4，P<0.05），肿瘤干细胞标志物 ALDH1、CD44、CD133 表达上调。抑制 Wnt/β-catenin 信号通路可部分逆转 TBL1XR1 过表达引起的迁移、侵袭、上皮-间质转化及干细胞特性改变。［结论］TBL1XR1 可在体外介导上皮-间质转化和干细胞特性，进而促进头颈鳞癌细胞迁移、侵袭，其调控机制可能与 Wnt/β-catenin 信号通路相关。

主题词：头颈部肿瘤；癌，鳞状细胞；转导素 β1X 连锁受体蛋白 1；上皮-间质转化；肿瘤干细胞；Wnt/β-catenin
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TBL1XR1 Regulates Migration and Invasion of Head and Neck Squamous Cell Carcinoma Cells *via* Inducing Epithelial-mesenchymal Transition and Stemness

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Abstract: [Objective] To investigate the regulatory effect of transducin β-like 1 X-linked receptor 1 (TBL1XR1) on the migration and invasion of head and neck squamous cell carcinoma (HNSCC) cell and its mechanism. [Methods] The TBL1XR1 gene expression level in HNSCC and paracancerous tissues was obtained from UALCAN database. Data of 523 patients with HNSCC in the TCGA database were analyzed by cbioprotal online analysis platform. The relationship between the TBL1XR1 genetic alteration and lymph node stage was analyzed. HNSCC Tu686 cells were transfected with lentivirus mediated TBL1XR1 overexpression vector, downregulation vector and control vector. The wound healing assay, transwell migration and invasion assays were employed to measure the effects of TBL1XR1 on the migratory and invasive abilities of Tu686 cells. Western blot, qRT-PCR were used to investigate the effects of TBL1XR1 on epithelial-mesenchymal transition makers. The tumor sphere formation assay and qRT-PCR assay were performed to investigate the stemness of Tu686 cells. The inhibitor was used to block the activation of Wnt/β-catenin signaling pathway in Tu686 cells , then the changes of migration and invasion abilities, epithelial-mesenchymal transition, stemness were measured. [Results] The expression of TBL1XR1 in HNSCC was higher than those in paracancerous tissues (P<0.001). The abilities of wound healing (90%±4% *vs* 53%±6%), transwell migration (0.68±0.04 *vs* 0.32±0.03) and invasion (0.59±0.04 *vs* 0.26±0.04) of TBL1XR1-overexpression Tu686 cells were significantly enhanced compared with control cells (all P<0.05). On the contrary, significantly decreased wound healing (70%±4% *vs* 96%±5%), migration (0.32±0.06 *vs* 0.63±0.08) and invasive abilities (0.24±0.04 *vs* 0.52±0.05) were observed when TBL1XR1 expression was inhibited (all P<0.05). After TBL1XR1 was up-regulated, the expression of mesenchymal markers Vimentin, N-cadherin were elevated, the expression of epithelial maker E-cadherin was reduced, the number of tumor sphere formation(41±9 *vs* 13±4, P<0.05) was significantly ele-

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vated, the expression of cancer stem cell makers ALDH1, CD44, CD133 were significantly upregulated. The blocking of Wnt/β-catenin signaling pathway partially abrogated TBL1XR1-induced migration, invision, epithelial-mesenchymal transition and stemness. [Conclusion] TBL1XR1 can promote the migration and invasion of HNSCC cells via inducing epithelial-mesenchymal transition and stemness *in vitro*, which may be related to Wnt/β-catenin signaling pathway.

Subject words: head and neck neoplasms; carcinoma, squamous cell; transducin β-like 1 X-linked receptor 1; epithelial-mesenchymal transition; neoplastic stem cell; Wnt/β-catenin

头颈部鳞状细胞癌(简称头颈鳞癌)占头颈部恶性肿瘤的90%以上,每年新发病例达60万,对人类健康造成极大威胁[1-2]。转移在头颈鳞癌患者的死亡中起着至关重要的作用,并为确定治疗方案提供重要信息[3-4]。头颈鳞癌侵袭、转移的分子机制仍不完全清楚。因此,迫切需要探索新的肿瘤标志物以更好地了解头颈鳞癌侵袭转移的分子机制。转导素β1X连锁受体蛋白1(transducin β-like 1 X-linked receptor 1,TBL1XR1)是核受体抑制因子(nuclear receptor corepressor,NCoR)和维甲酸与甲状腺激素受体(silencing mediator for retinoid and thyroid hormone receptors,SMRT)复合物的重要组成部分,在多种人类实体肿瘤中过表达,并参与肝癌、胃癌、胰腺癌和非小细胞肺癌等肿瘤的发生和进展[5-8]。研究表明,TBL1XR1在肿瘤细胞的增殖、凋亡、侵袭、迁移、耐药等多种功能中发挥重要作用[6-7,9]。TBL1XR1在恶性肿瘤中的异常表达和功能障碍提示TBL1XR1可能是一个潜在的分子生物标志物和治疗靶点。研究发现,TBL1XR1在鼻咽癌细胞及组织中表达上调,高水平TBL1XR1可通过激活NF-κB通路抑制鼻咽癌细胞对顺铂的敏感性[10]。然而,TBL1XR1在头颈鳞癌中的确切作用仍不十分清楚。本研究探讨TBL1XR1在头颈鳞癌细胞迁移、侵袭中的作用及相关机制。

1 材料与方法

1.1 实验材料

DMEM/F12细胞培养基(美国Hyclone),特级胎牛血清(杭州四季青生物工程材料有限公司),双抗(青霉素-链霉素,美国Gibco),TBL1XR1过表达载体pCMV6-Entry-TBL1XR1和空白载体pCMV6-Entry vector(美国Origene),TBL1XR1沉默载体Lenti-TBL1XR1-shRNA (sc-106601-V)和空白载体(sc-108080)(美国Santa Cruz Biotechnology),Lipofectamine 2000脂质体转染试剂盒(美国Invitrogen),嘌呤霉素(美国Santa Cruz Biotechnology),小鼠抗人TBL1XR1单克隆抗体(美国Sigma),小鼠抗人E-cadherin单克隆抗体(美国Santa Cruz Biotechnology),小鼠抗人N-cadherin单克隆抗体(美国Santa Cruz Biotechnology),小鼠抗人Vimentin单克隆抗体(美国Santa Cruz Biotechnology),小鼠抗人β-actin抗体(上海碧云天公司),TRIzol总RNA提取试剂盒(美国Invitrogen),RNA反转录试剂盒(美国Applied Biosystems),SYBR® Green PCR Master Mix(美国Applied Biosystems),iCRT14(美国Sigma)。

1.2 方 法

1.2.1 TBL1XR1在头颈鳞癌中的表达量分析

利用UALCAN数据库网站(http://ualcan.path.uab.edu/index.htmL)搜索TBL1XR1在520例头颈鳞癌组织及44例癌旁组织中的表达情况并进行比较。

1.2.2 TBL1XR1与头颈鳞癌患者临床参数分析

通过在线的癌症基因组图集数据库(TCGA)可视化平台Cbioportal网站(www. cbioportal. org)获得TCGA数据库中的523例头颈鳞癌样本,提取患者相关资料分析TBL1XR1表达与临床参数的关系。

1.2.3 细胞培养

人头颈鳞癌细胞株Tu686由美国爱默尔大学Winship肿瘤研究所馈赠。该细胞在37 ℃、5% CO_2、饱和湿度条件下,采用含有10%特级胎牛血清、100 U/mL青霉素和1 μg/mL链霉素的DMEM/F12(1∶1)细胞培养基培养。实验用细胞均为对数生长期细胞。

1.2.4 细胞稳定转染

将状态良好的Tu686细胞按约1×10^6个接种于6孔板中,用无抗生素细胞培养基培养24 h,使转染

时细胞融合率达 80%~85%。转染按照 Lipofectamine 2000 转染说明书进行。将 TBL1XR1 过表达载体 pCMV6-Entry-TBL1XR1 和空白载体 pCMV6-Entry vector、TBL1XR1 沉默载体 Lenti-TBL1XR1-shRNA(sc-106601-V)和空白载体(sc-108080)分别转染头颈鳞癌细胞 Tu686。使用 300 μg/mL G418 或 5 μg/mL 嘌呤霉素选择过表达 TBL1XR1 或沉默 TBL1XR1 的稳定细胞系。通过 Western blot 检测 TBL1XR1 过表达和沉默效果。

1.2.5 实时荧光定量聚合酶链反应

采用 TRIzol 法提取细胞总 RNA,紫外分光光度仪测定 RNA 纯度及浓度,按照 RNA 反转录试剂盒说明合成 cDNA。qRT-PCR 检测各指标的相对表达水平。引物序列如下:TBL1XR1:F:5′-TGAAGTTGGGTTCAGGAGTGT-3′;R:5′-CACAGGATATAACCCATTGGCAG-3′;E-cadherin:F:5′-GCTGGACCGAGAGAGTTTCC-3′;R:5′-CAAAATCCAAGCCCGTGGTG-3′;Vimentin:F:5′-TGTCCAAATCGATGTGGATGTTTC-3′;R:5′-TTGTACCATTCTTCTGCCTCCTG-3′;N-cadherin:F:5′-TGGGAAATGGAAACTTGATGGC-3′;R:5′-AGTTGCTAAACTTCACTGAAAGGA-3′;ALDH1:F:5′-TGAAGTTGGGTTCAGGAGTGT-3′;R:5′-CACAGGATATAACCCATTGGCAG-3′;CD44:F:5′-TGAAGTTGGGTTCAGGAGTGT-3′;R:5′-CACAGGATATAACCCATTGGCAG-3′;CD133:F:5′-TGAAGTTGGGTTCAGGAGTGT-3′;R:5′-CACAGGATATAACCCATTGGCAG-3′;GAPDH,F:5′-TCCAAAATCAAGTGGGGCGA-3′,R:5′-AGTAGAGGCAGG GATGATGT-3′。采用 $2^{-\Delta\Delta Ct}$ 法计算相对表达量。实验重复 3 次。

1.2.6 Western blot

提取细胞总蛋白,采用二喹啉甲酸法(bicinchoninic acid,BCA)测定细胞总蛋白浓度。取 50 μg 总蛋白进行变性处理,进行 10%~12%十二烷基硫酸钠–聚丙烯酰胺凝胶(SDS-PAGE)电泳。蛋白电泳分离后转至 PVDF 膜,5%脱脂牛奶室温下封闭 2 h。加入小鼠抗人 TBL1XR1 单克隆抗体(1:800)、小鼠抗人 E-cadherin 单克隆抗体(1:400)、小鼠抗人 N-cadherin 单克隆抗体(1:400)、小鼠抗人 Vimentin 单克隆抗体(1:400)、小鼠抗人 β-actin 抗体(1:1 000)于 4 ℃下孵育过夜。洗膜后,于辣根过氧化物酶标记的二抗(1:1 000)室温下孵育 1 h。洗膜后,经化学发光剂显影后,拍照并分析。实验重复 3 次。

1.2.7 划痕愈合实验

按 2×10^5 个细胞/孔接种六孔板,培养过夜,换无血清培养基,继续培养 24 h 使细胞呈现出单层贴壁生长状态并达到 100%融合率。用 200 μL 移液器头给培养皿中的细胞划痕,以 PBS 洗去刮下的漂浮细胞,加入不含血清的 DMEM/F12(1:1)细胞培养基。分别于划痕后 0、36、60 h 给细胞拍照,观察划痕愈合能力。实验重复 3 次。

1.2.8 Transwell 迁移、侵袭实验

迁移实验:无血清 Tu686 细胞(3×10^4~5×10^4)置于孔径为 6.5 mm Transwell 聚碳脂膜上,下室加入含 10%胎牛血清的 DMEM/F12(1:1)细胞培养基。孵育 48 h 后,用棉签轻轻擦拭膜上表面的细胞,结晶紫染色 10 min,用水冲洗。在 10%醋酸中脱色,560 nm 处用酶标仪测量光密度(OD)值定量穿膜细胞数[11-12]。

侵袭实验:无血清 Tu686 细胞(5×10^4 ~ 6×10^4)接种在铺有基质胶的 Transwell 小室,其余步骤与迁移实验相同。实验重复 3 次。

1.2.9 肿瘤球形成实验

将 2×10^3 个 Tu686 细胞接种到低黏附 24 孔细胞培养板,在含有 1×B27、20 ng/mL EGF、20 ng/mL bFGF 的无血清 DMEM/F12(1:1)培养基中培养 7~10 d。每 3 d 加 1 次培养液。倒置显微镜下计数直径 >75 μm 的微球。实验重复 3 次。

1.3 统计学处理

统计分析均采用 SPSS 软件(17.0 版)进行。计量资料以均数±标准差($\bar{x}\pm s$)表示,采用独立样本 t 检验进行两样本均数比较。检验均为双侧检验,$P<0.05$ 为差异有统计学意义。

2 结 果

2.1 TBL1XR1 在头颈鳞癌组织中的表达及与淋巴结分级的关系

利用 UALCAN 数据库网站搜索到 TBL1XR1 mRNA 在头颈鳞癌组织中的表达明显高于对应癌旁组织($P<0.001$)(Figure 1)。通过在线的 TCGA 可视化平台 Cbioportal 网站获得 TBL1XR1 与头颈鳞癌患者临床参数的关系,其中 TBL1XR1 与淋巴结分级相关($P=0.023\ 8$)。

2.2 过表达或沉默 TBL1XR1 对 Tu686 细胞迁移和侵袭能力的影响

TBL1XR1 上调后,Tu686 细胞的划痕愈合速度明显快于对照组细胞（90%±4% *vs* 53%±6%,t=−9.820,P<0.001)(Figure 2)。Transwell 迁移和侵袭实验表明，与对照组细胞相比，TBL1XR1 过表达的 Tu686 细胞迁移和侵袭能力较对照组显著性增强（迁移:0.68±0.04 *vs* 0.32±0.03,t=−12.274,P<0.001;侵袭:0.59±0.04 *vs* 0.26±0.04,t=−10.847,P<0.001)(Figure 2)。相反,当 TBL1XR1 表达下调后,Tu686 细胞的划痕愈合能力较对照组受抑制（70%±4% *vs* 96%±5%,t=8.345,P<0.001)(Figure 3)。Transwell 迁移和侵袭能力亦较对照组显著性降低(迁移:0.32±0.06 *vs* 0.63±0.08,t=7.134,P<0.001;侵袭:0.24±0.04 *vs* 0.52±0.05,t=9.949,P<0.001)(Figure 3)。

2.3 过表达 TBL1XR1 对 Tu686 细胞上皮−间质转化标志物表达的影响

qRT-PCR 和 Western blot 分析显示，TBL1XR1 过表达的 Tu686 细胞间质细胞标志物 Vimentin、N-cadherin 表达明显升高；相反，上皮细胞标志物 E-cadherin 的表达大幅降低(Figure 4)。

2.4 过表达 TBL1XR1 对 Tu686 细胞干细胞特性的影响

头颈鳞癌细胞 Tu686 的成球能力结果显示,TBL1XR1 过表达组的肿瘤球形成数较对照组明显增多(41±9 *vs* 13±4,t=−5.615,P=0.001)(Figure 5)。进一步 qRT-PCR 结果显示,肿瘤干细胞标志物 ALDH1、CD44、CD133 在过表达 TBL1XR1 的 Tu686 细胞中表达显著性上调(Figure 5)。

2.5 阻断 Wnt/β-catenin 信号通路对 TBL1XR1 诱导的 Tu686 细胞迁移、侵袭的影响

Tanswell 实验表明,抑制 Wnt/β-catenin 信号通路可显著性降低细胞的迁移和侵袭能力(迁移:0.68±0.04 *vs* 0.41±0.06,t=6.786,P=0.002;侵袭:0.59±0.04 *vs* 0.36±0.04,t=8.030,P=0.001)(Figure 6)。信号通路阻断后,间质细胞标志物 Vimentin、N-cadherin 水平显著性降低,上皮细胞标志物 E-cadherin 水平显著性升高;同时,肿瘤干细胞标志物 ALDH1、CD44、CD133 表达也明显下调(Figure 6)。

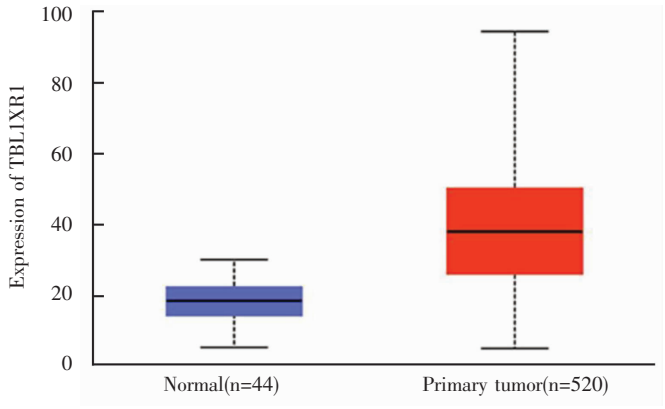
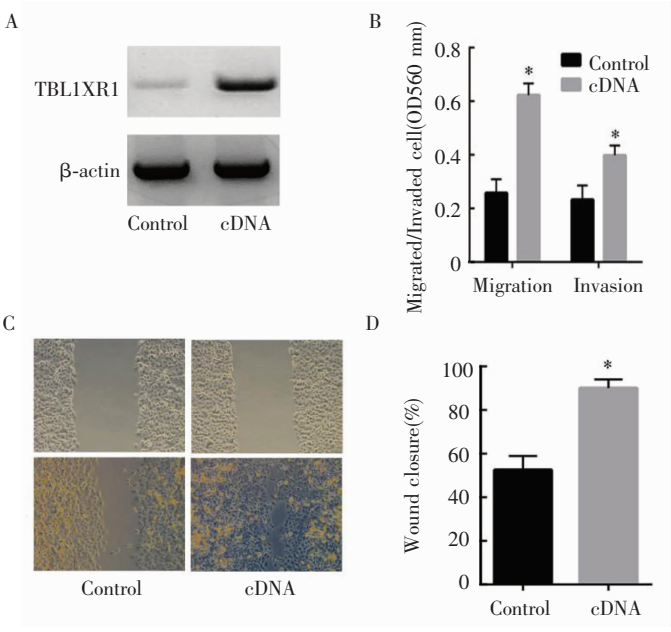


Figure 1 TBL1XR1 was overexpressed in head and neck squamous cell carcinoma

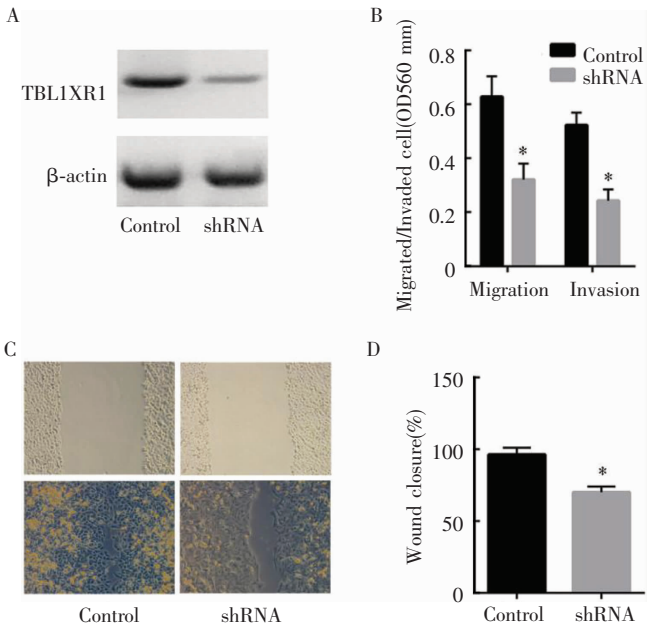


Notes: A: Western blot were performed to detect the overexpressing effect of TBL1XR1 in Tu686 cell line. B: Transwell assays were conducted to determine the migration and invasion ability of Tu686 cells. C~D: Wound healing assays were employed to determine the migration of Tu686 cells (magnification×100). (*:P<0.05 *vs* Control. Control:Tu686 cells transfected with empty lentivirus. cDNA:Tu686 cells transfected by lentivirus mediated TBL1XR1 cDNA)

Figure 2 TBL1XR1 promoted Tu686 cell migration and invasion

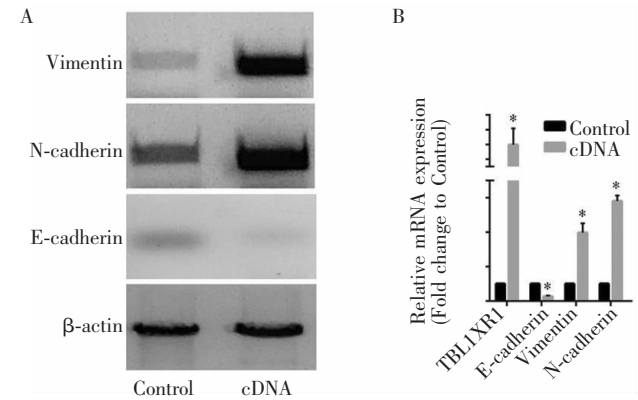
3 讨 论

TBL1XR1 是一种转录辅助因子，在转录调控中对基因抑制与激活之间的精确切换起着重要作用。研究发现，



Notes: A: Western blot were performed to detect the silencing effect of TBL1XR1 in Tu686 cell line. B: Transwell assays were conducted to determine the migration and invasion ability of Tu686 cells. C~D: Wound healing assays were employed to determine the migration of Tu686 cells (magnification ×100).(*: $P<0.05$ *vs* Control. Control: Tu686 cells transfected with empty lentivirus. shRNA: Tu686 cells transfected by lentivirus mediated TBL1XR1 shRNA)

Figure 3 Inhibition TBL1XR1 attenuated the migration and invasion capacity of Tu686 cell



Notes: Western blot(A) and qRT-PCR(B) assays were performed to check the expression of epithelial-mesenchymal transition markers including E-cadherin, Vimentin and N-cadherin.(*: $P<0.05$ *vs* Control. Control: Tu686 cells transfected with empty lentivirus. cDNA: Tu686 cells transfected by lentivirus mediated TBL1XR1 cDNA)

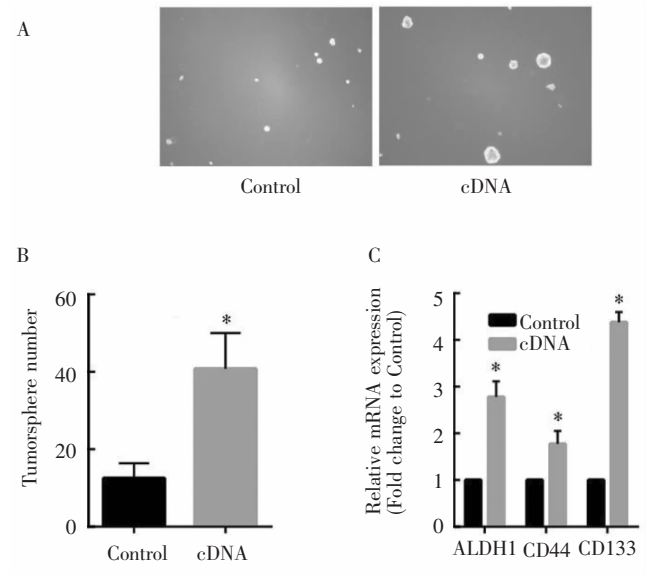
Figure 4 TBL1XR1 induced epithelial-mesenchymal transition of Tu686 cell

TBL1XR1 在乳腺癌、肝癌、胃癌、宫颈癌等多种恶性肿瘤中高表达，与临床晚期、转移能力增强、生存期明显降低的恶性肿瘤高度相关[8,13-14]。研究表明，TBL1XR1 是多种人类癌症的潜在预后标志物和有价值的治疗靶点。已有研究发现，TBL1XR1 在鼻咽癌细胞及组织中表达上调，高水平 TBL1XR1 通过激活 NF-κB 通路抑制顺铂敏感性[10]。在本研究中，通过 TBL1XR1 在头颈鳞癌细胞中的功能获得和功能缺失研究发现，TBL1XR1 在体外调节细胞迁移和侵袭，提示 TBL1XR1 也是头颈鳞癌的转移相关分子。

上皮-间质转化是一个复杂的过程。在这个过程中，上皮细胞失去了特有的特征，并获得了一种间充质样表型，已经被证明在多种恶性肿瘤中促进迁移和侵袭[15-16]。上皮-间质转化与头颈鳞癌转移的相关性在我们之前的报道和其他文献中也得到了证实[17-18]。已有研究表明，TBL1XR1 通过介导上皮-间质转化促进肝癌、结直肠癌、胃癌和肺癌转移[5-6,19]。与这些研究结果一致，我们的体外研究表明，过表达 TBL1XR1 可诱导头颈鳞癌细胞发生上皮-间质转化改变。

肿瘤干细胞具有自我更新和多能性，是肿瘤的前体细胞，已在乳腺癌、前列腺癌、胰腺癌等多种实体肿瘤中发现。在头颈鳞癌中，通过检测 CD133、CD44 和 ALDH 等标志物也发现了这种细胞亚群[20-21]。肿瘤干细胞和普通干细胞有许多共同的特性，包括自我更新能力、分化能力和抗凋亡能力。TBL1XR1 在造血干细胞、神经干细胞、小鼠胚胎干细胞中均高表达，提示 TBL1XR1 可能在干细胞的维持中起关键作用。在本研究中，我们初步证明过表达 TBL1XR1 的头颈鳞癌细胞肿瘤球形成能力增强、肿瘤干细胞标志物表达增高。

上皮-间质转化和干细胞特性获得之间的直接联系已经在大量恶性肿瘤中得到证实，包括乳腺癌、前列腺癌、卵巢癌、结直肠癌、胰腺癌和头颈鳞癌。以往的报道表明上皮-间质转化过程可以产生具有干细胞性质的细胞[22]。研究发现，肿瘤干细胞除了表达干细胞相关基因外，还具有间充质细胞特征[23]。上皮-间质转化和肿瘤干细胞都与侵袭性细胞的生成和远处转移的形成有关[24]。结合本研究结果，我们认为 TBL1XR1 促进头颈鳞癌细胞的上皮-间质转化和干细胞特性，进而调控头颈鳞癌细胞迁移和侵袭。然而，TBL1XR1



Notes: A~B: Tumorsphere formation assays were used to determine cancer stem cell-like properties after TBL1XR1 overexpression (magnification ×100). C: qRT-PCR was performed to check the expression of cancer stem cell markers including ALDH1, CD44 and CD133. (*: $P<0.05$ *vs* Control. Control: Tu686 cells transfected with empty lentivirus. cDNA: Tu686 cells transfected by lentivirus mediated TBL1XR1 cDNA)

Figure 5 TBL1XR1 enhanced stem cell-like properties of Tu686 cell

调控头颈鳞癌功能的具体机制仍有待进一步探索。

TBL1XR1 可通过调节多种信号转导通路，包括 NF-κB、ERK 和 Wnt/β-catenin 通路，促进肿瘤的发生和进展。在这些复杂的信号通路中，TBL1XR1 和 β-catenin 可以相互招募到 Wnt 靶基因启动子中，从而进行转录激活和肿瘤发生[25]。此外，Wnt/β-catenin 信号通路在包括头颈鳞癌在内的多种人类癌症中促进肿瘤细胞上皮-间质转化发生。我们进一步验证 Wnt/β-catenin 信号通路在 TBL1XR1 介导的头颈鳞癌迁移和侵袭中的作用，结果表明，抑制 Wnt/β-catenin 信号通路可以部分抵消 TBL1XR1 过表达诱导的上皮-间质转化，并逆转 TBL1XR1 介导的头颈鳞癌细胞体外迁移侵袭能力。研究结果表明 Wnt/β-catenin 信号通路参与 TBL1XR1 介导的头颈鳞癌细胞的上皮-间质转化和转移。考虑到 Wnt/β-catenin 信号通路在决定干细胞命运和增殖方面的重要作用。当 Wnt/β-catenin 信号通路在过表达 TBL1XR1 的头颈鳞癌细胞中被抑制时，肿瘤干细胞标志物水平降低，结果表明，TBL1XR1 可能通过 Wnt/β-catenin 信号通路促进上皮-间质转化和干细胞特性，从而调控头颈鳞癌细胞的迁移、侵袭。

本研究在体外水平阐明 TBL1XR1 蛋白可通介导上皮-间质转化和干细胞特性，从而促进头颈鳞癌细胞的迁移、侵袭，其调控机制与 Wnt/β-catenin 信号通路密切相关。该结果仍需进一步进行临床数据及体内实验验证。

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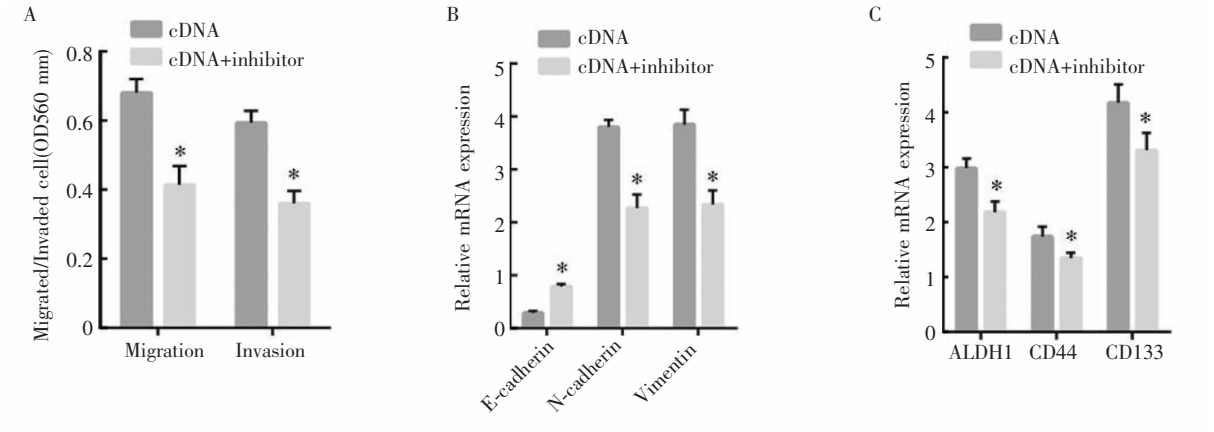

Notes: Specific inhibitor iCRT14(20 μmol/L) was used to impede Wnt/β-catenin signaling pathway in Tu686 cells infected with TBL1XR1 cDNA and empty vector. A: Transwell migration and invasion assays were used to examine the changes of migration and invasion. B~C: qRT-PCR assays were performed to check the expression of epithelial-mesenchymal transition markers and cancer stem cell markers (*: $P<0.05$ *vs* cDNA. cDNA: Tu686 cells transfected by lentivirus mediated TBL1XR1 cDNA, cDNA+inhibitor: Tu686 cells transfected by lentivirus mediated TBL1XR1 cDNA, and iCRT14 was used to impede Wnt/β-catenin signaling pathway)

Figure 6 Wnt/β-catenin signaling pathway was involved in TBL1XR1-mediated Tu686 cell migration and invasion

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