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· 基础研究 ·

过氧化物还原酶-4在口腔鳞状细胞癌中的表达及对癌细胞增殖、迁移和侵袭的影响

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【摘要】　目的　探讨过氧化物还原酶-4(peroxiredoxin-4，PRDX4)在口腔鳞状细胞癌(oral squamous cell carcinoma，OSCC)中的表达及对OSCC细胞增殖、迁移和侵袭的影响。**方法**　通过癌症基因图谱数据库(The Cancer Genome Atlas，TCGA)分析PRDX4在OSCC中的表达，实时荧光定量聚合酶链式反应(real-time fluorescent quantitative polymerase chain reaction，qRT-PCR)和蛋白质印迹实验(Western Blot，WB)分别检测OSCC细胞系中PRDX4的基因与蛋白质表达。将CAL-27细胞中的PRDX4敲低，分为si-PRDX4组和si-NC组；将SCC-9细胞中的PRDX4过表达，分为过表达PRDX4组(转染pcDNA3.1-PRDX4质粒)和Vector组(对照组，转染pcDNA3.1-NC质粒)。采用细胞计数试剂盒(cell counting kit-8，CCK-8)和平板克隆形成实验检测各组细胞增殖能力；细胞划痕和Transwell侵袭实验检测细胞迁移和侵袭能力；WB实验检测敲低和过表达PRDX4及加入p38MAPK激动剂和抑制剂后对OSCC细胞中与p38MAPK相关信号通路蛋白及上皮间充质转化(epithelial-mesenchymal transition，EMT)蛋白表达影响。**结果**　PRDX4在OSCC组织及细胞系中呈高表达。CCK-8实验结果显示，si-PRDX4组较si-NC组在24、48和72 h时OD值低($P<0.05$)；过表达PRDX4组较Verctor组在24、48和72 h时OD值高($P<0.05$)。平板克隆形成实验结果显示，si-PRDX4组较si-NC组集落形成数量少($P<0.05$)；过表达PRDX4组较Vector组集落形成数量多($P<0.05$)。细胞划痕实验结果显示，si-PRDX4组较si-NC组划痕愈合面积少($P<0.05$)；过表达PRDX4组较Vector组划痕愈合面积增多($P<0.05$)。Transwell侵袭实验结果显示，si-PRDX4组较si-NC组穿膜细胞数量减少($P<0.05$)；过表达PRDX4组较Verctor组穿膜细胞数量多($P<0.05$)。WB实验结果显示，敲低和过表达PRDX4可分别下调和上调p38MAPK信号通路及上皮间充质转化相关蛋白表达，而分别加入p38MAPK激动剂和抑制剂后，可显著逆转相关蛋白的表达。**结论**　PRDX4在OSCC中处于高表达状态，敲低OSCC细胞中PRDX4的表达，可下调p38 MAPK信号轴及EMT相关信号蛋白的表达，进而使细胞的增殖、迁移、侵袭能力和上皮间充质转化受到抑制。

【关键词】　氧化物还原酶-4；　促癌因子；　口腔鳞状细胞癌；　p38MAPK信号通路；　上皮间充质转化；　增殖；　侵袭；　迁移
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Expression of peroxiredoxin 4 in oral squamous cell carcinoma and its effects on cancer cell proliferation, migration, and invasion

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【Abstract】 **Objective**　To investigate the expression of peroxiredoxin 4 (PRDX4) in oral squamous cell carcinoma (OSCC) and its effect on the proliferation, migration, and invasion of OSCC cells. **Methods**　The Cancer Genome Atlas (TCGA) database was used to analyze the expression of PRDX4 in OSCC. Quantitative reverse transcription polymerase chain reaction (qRT-PCR) and Western Blot (WB) were used to detect the mRNA and protein expression of PRDX4 in OSCC cell lines and normal oral mucosal epithelial cells. PRDX4 was knocked down in CAL-27 cells and divided into two groups: the si-PRDX4 group and si-NC group. SCC-9 cells overexpressing PRDX4 were divided into two groups: the PRDX4 overexpression group (transfected with pcDNA3.1-PRDX4 plasmid) and the vector group (the control group; transfected with pcDNA3.1-NC plasmid). A cell counting kit-8 (CCK-8) and plate colony formation assay were used to detect cell proliferation. Transwell assay and cell scratch test were used to detect cell invasion and migration ability. WB was used to detect the effects of knockdown or overexpression of PRDX4, p38MAPK agonist or inhibitor on the expression of p38MAPK-related signaling pathway proteins, and epithelial mesenchymal transition proteins in OSCC cells. **Results**　PRDX4 was highly expressed in OSCC tissues and cell lines. The results of qRT-PCR and WB showed that PRDX4 was highly expressed in OSCC cell lines compared with normal oral mucosal epithelial cells. The CCK-8 assay showed that the si-PRDX4 group had significantly lower OD values than the si-NC group at 24, 48, and 72 h ($P < 0.05$). The PRDX4 overexpression group had a significantly higher OD value than the vector group at 24, 48, and 72 h ($P < 0.05$). The plate colony formation assay showed that the si-PRDX4 group had a significantly lower number of colonies than the si-NC group ($P < 0.05$). The number of colonies formed in the PRDX4 overexpression group was significantly higher than that in the vector group ($P < 0.05$). The cell scratch test showed that the wound healing area of the si-PRDX4 group was less than that of the si-NC group ($P < 0.05$). The scratch healing area of the PRDX4 overexpression group was significantly higher than that of the vector group ($P < 0.05$). The Transwell invasion assay showed that the number of transmembrane cells in the si-PRDX4 group was lower than that in the si-NC group ($P < 0.05$). The number of transmembrane cells in the PRDX4 overexpression group was significantly higher than that in the vector group ($P < 0.05$). The WB results showed that knockdown and overexpression of PRDX4 could downregulate and upregulate the expression of the p38MAPK signaling pathway and epithelial-mesenchymal transition related proteins, respectively, and the addition of p38MAPK agonist and inhibitor could significantly reverse the expression of related proteins. **Conclusion**　PRDX4 is highly expressed in OSCC. Knocking down the expression of PRDX4 in OSCC cells can downregulate the expression of p38 MAPK signal axis and EMT-related signal proteins, thereby inhibiting the proliferation, migration, invasion, and epithelial-mesenchymal transition of cells.

【Key words】 peroxiredoxin-4;　cancer-promoting factors;　oral squamous cell carcinoma;　p38 mitogen-activated protein kinase signaling pathway;　epithelial-mesenchymal transition;　proliferation;　invasion;　migration
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口腔鳞状细胞癌(oral squamous cell carcinoma, OSCC)是一种侵袭性较强的上皮恶性肿瘤,位于全球上皮恶性肿瘤中的第六位[1-2]。其具有转移率和复发率高且对于传统的治疗方式易产生抵抗作用的特点[3]。目前,针对OSCC的治疗方式较之前虽有进展,但对于患者预后不良和生存率低的问题仍并未得到很好的解决[4]。

过氧化物还原酶(peroxiredoxin, PRDX)由6个小的抗氧化同工酶组成,属于氧化还原家族蛋白,其广泛分布在人体各组织中[5-6],其中,过氧化物还原酶-4(peroxiredoxin-4, PRDX4)作为其中的家族成员,可在肿瘤的发生发展中发挥多种作用[7-8]。研究表明,PRDX4在子宫内膜癌[9]、肺癌[10]、前列腺癌[11]等多种组织中均异常表达,与恶性肿瘤细胞的多种生物学行为及信号转导存在联系。此外,已有研究证实,PRDX4在口腔鳞状细胞癌组织中高表达,并与患者的不良的预后存在密切关系[12]。本研究前期通过京都基因与基因组百科全书(Kyo-

to Encyclopedia of Genes and Genomes，KEGG）富集分析表明，PRDX4在OSCC中可富集于p38丝裂原活化蛋白激酶（p38 mitogen-activated protein kinase，p38MAPK）信号通路，提示PRDX4可能通过p38MAPK参与调控OSCC发生发展。p38MAPK信号通路在包括癌症在内的各种疾病中发生改变，可通过调节癌症细胞的增殖、侵袭和转移等能力，在癌症进展过程发挥作用。现已有研究证实，PRDX1[13]、PRDX2[14]、PRDX4[15-16]及PRDX6[17]均可通过介导p38MAPK信号通路参与肿瘤细胞生物行为的调控，从而直接或间接参与癌症的形成。但目前PRDX4对OSCC的作用和潜在的分子机理尚未明确。本研究以PRDX4作为分子靶点，通过探讨其在OSCC中的作用并探讨其与p38MAPK信号的潜在机制，为OSCC的分子靶向药物治疗提供实验依据。

1　材料与方法

1.1　主要材料与仪器

OSCC细胞系（CAL-27、SCC-15、SCC-9、HN6和HN30）和正常对照细胞（人口腔黏膜上皮细胞）为山东第二医科大学口腔医学院馈赠。DMEM细胞培养基（SH30022.01B，上海惠颖生物科技有限公司，中国）；角质细胞无血清培养基（MED-1021，武汉赛奥斯生物科技有限公司，中国）；青霉素-链霉素混合液（GA3502，北京鼎国昌盛生物，中国）；胎牛血清（10437028，Gibco，美国）；CCK-8试剂盒（CK04-2，上海经科化学科技有限公司，中国）；基质胶（JK-R5450，上海晶抗生物工程有限公司，中国）；PRDX4抗体（ab184167，Abcam，美国）、p38丝裂原活化蛋白激酶（p38MAPK）抗体（ab308333，Abcam，美国）、E-钙黏附蛋白（E-cadherin）（ab231303，Abcam，美国）、N-钙黏附蛋白（N-cadherin）（ab245117，Abcam，美国）、波形蛋白（Vimentin）（ab92547，Abcam，美国）；Lipofectamine 3000转染试剂盒（L3000-015，上海恒斐生物科技有限公司，中国）；10×封闭-洗涤缓冲液（10×blocking/washing buffer，TBST）（B1009-TBST，北京普利莱基因技术有限公司，中国）、ECL发光液（P1000-25，北京普利莱基因技术有限公司，中国）；p38MAPK抑制剂PD169316（HY-10578，MCE，美国）和p38MAPK激动剂AL-8810（HY-100449，MCE，美国）；小干扰RNA及过表达质粒购自上海英拜生物科技有限公司。酶标仪（HBS-1096A，上海研卉生物科技有限公司，中国）；实时荧光定量聚合酶链反应（quantitative reverse transcription-PCR，qRT-PCR）仪（L988，西安天隆科技有限公司，中国）；光学显微镜（E100，尼康，日本）；离心机（Sorvall ST8，Thermo Fisher，美国）。

1.2　实验方法

1.2.1　在线预测网站　从癌症基因图谱数据库（The Cancer Genome Atlas，TCGA）中下载并整理OSCC的相关数据，进一步明确OSCC中PRDX4的表达水平。同时，以“PRDX4”为检索词，选择OSCC选项，在Kaplan-Meier plotter数据库中分析PRDX4表达与癌症患者预后的相关性。

1.2.2　实验分组与细胞培养、转染　将HN6、HN30、CAL-27、SCC-15和SCC-9细胞于T25培养瓶中进行培养，细胞培养基为含100 μg/mL青霉素、100 μg/mL链霉素和含10%胎牛血清的DMEM完全培养基[18]；口腔黏膜上皮细胞用含0.2 ng/ml重组表皮生长因子（recombinant epidermal growth factor，rEGF）的角质细胞无血清培养基进行培养[19]。将细胞孵育箱的培养条件设置为5% CO_2、37 ℃，定期换液，待细胞铺满瓶底80%～90%时，按比例接种至新的培养瓶中传代及后续实验。在OSCC细胞株中分别以qRT-PCR、Western Blot检测PRDX4的mRNA和蛋白表达。通过RNA提取试剂盒提取各转染组细胞总RNA，随即完成RNA浓度的测定（仪器为超微量分光光度计），最后合成cDNA（试剂为反转录试剂盒）。完成PCR反应体系的配制后，以GAPDH为内参对每一组中PRDX4的mRNA表达进行检测。PRDX4引物分别是：5′-TAGGATCCATGGAGGCGCTGCCGCTGCTAG-3′（上游），5′-TACTCGAGTCAATTCAGTTTATCGAAATAC-3′（下游）。反应结束后，求出各组PRDX4 mRNA的相对表达量，计算公式为$2^{-\triangle\triangle Ct}$。

将CAL-27细胞中的PRDX4敲低，分为si-PRDX4组和si-NC组；将SCC-9细胞中的PRDX4过表达，分为PRDX4组（转染pcDNA3.1-PRDX4质粒）和Vector组（转染pcDNA3.1-NC质粒），siRNA序列见表1。将CAL-27细胞于6孔板内铺板，待细胞继续生长到融合度为70%～90%时开始进行转染。按照操作步骤说明书，用125 μL DMEM稀释5 μL lipo3000，125 μL DMEM稀释2.5 μg siRNA，把稀释的lipo3000和siRNA轻轻混匀，轻轻混匀后静置15 min加入待转染细胞的培养基中；SCC-9细胞转染前准备操作同前，之后用125 μL DMEM稀释5 μL lipo3000，125 μL DMEM稀释5 μL p3000和2.5 μg

pcDNA3.1-PRDX4和pcDNA3.1-NC质粒(由上海英拜生物科技有限公司构建,分别为PRDX4过表达组和过表达对照组),轻轻混匀后静置15 min加入待转染细胞的培养基中。

表1　siRNA序列
Table 1　siRNA sequences

siRNA	Target sequence
si-PRDX4-1	GGCUGCUUCUGCUGCCGCUTT
si-PRDX4-2	GGAUUCCACUUCUUUCAGATT
si-NC	UUCUCCGAACGUGUCACGUTT

PRDX4: peroxiredoxin 4

1.2.3　CCK-8法检测OSCC细胞的增殖能力　收集分别经过敲低和过表达处理的CAL-27和SCC-9细胞,以2×10^{5}个/mL的密度接种于96孔板(每孔中加入100 μL细胞悬液),每组设置3个复孔,同时在周围环绕一圈PBS,以减少蒸发带来的影响。然后在37 ℃含5%CO_2培养箱中培养,于0、24、48、72 h时向每孔加入10 μL稀释后的CCK-8溶液,放入培养箱中继续培养2 h。最后,用酶标仪在450nm处测定吸光度值。最后利用Graphpad Prism 10.1.2软件进行分析。

1.2.4　平板克隆实验检测细胞的克隆形成能力　将转染完成后的各组细胞进行胰蛋白酶消化,消化完成后进行重悬计数。在6孔板上依次将si-PRDX4组、si-NC组、PRDX4组和Vector组细胞进行接种,经过实验前期培养观察,每间隔3 d进行换液,并同时观察细胞的生长状态及培养基的颜色。待孵育14 d后可直接观察到细胞集落形成时,将6孔板中液体吸出,缓冲液冲洗2次后分别用4%多聚甲醛溶液固定、结晶紫染色处理各15 min,晾干后拍照记录(选定背景为白色),计算克隆形成的数量。

1.2.5　Transwell实验检测细胞侵袭能力　将各转染组细胞重悬于不含FBS的DMEM培养基(密度:2×10^{5}个/mL),向Matrigel基质胶包被的Transwell上室中加入细胞悬浮液100 μL。将FBS含量为15%的650 μL DMEM加入下室,转移到5%CO_2以及37 ℃的培养箱内培养2 d。用棉签将Transwell膜上室细胞轻轻拂去,固定、染色后,在光学显微镜下随机挑出5个视野统计穿膜的细胞数,并求出平均值。

1.2.6　抑制或激动p38MAPK信号通路　p38MAPK信号通路激动剂:根据说明书,使用0.2485 mL DMSO作为溶剂将0.1 mg AL-8810制备成浓度为1 mmol/L储备液,等分并在-20 ℃下备用。取需要量的储备液,使用ddH_2O将其稀释为10 μmol/L的AL-8810工作液。然后根据1.2.2方法将完成转染24 h后的CAL-27细胞的悬液(si-NC和si-PRDX4组细胞)加入到六孔板中,24 h后弃掉上清液,并用预热的PBS洗涤3次,然后加入2 mL的AL-8810工作液。将六孔板置于培养箱中,经过2 h后取出,更换为DMEM完全培养基继续培养,并将实验分为si-NC+PD169316组、si-PRDX4+PD169316组、si-NC+DMSO组和si-PRDX4+DMSO组(加入相应浓度的DMSO为对照)进行后续实验;p38MAPK信号通路抑制剂:使用0.2775 mL DMSO作为溶剂将0.1 mg PD169316制备成浓度为1 mmol/L储备液,等分并在-20 ℃下备用。取需要量的储备液,使用ddH_2O将其稀释为10 μmol/L的PD169316工作液。后续步骤同上,将实验分为Vector+PD169316组、PRDX4+PD169316组、Vector+DMSO组和PRDX4+DMSO组(加入等浓度的DMSO)进行后续实验。

1.2.7　Western blot检测PRDX4、p38MAPK信号通路及上皮间充质转化相关蛋白表达　提取并检测各转染组细胞总蛋白和蛋白浓度,凝胶电泳分离蛋白样品并转膜,脱脂奶粉封闭4 h后,4 ℃下分别各一抗工作液[PRDX4(1∶1 000);p38MAPK(1∶1 000);p-p38MAPK(1∶1 000);E-cadherin(1∶1 000);N-cadherin(1∶5 000);Vinmentin(1∶1 000);GAPDH(1∶2 000)]孵育过夜,加二抗(1∶50 000)于室温下孵育3 h。显影,用化学发光成像分析系统拍照并分析灰度值。

1.3　统计学分析

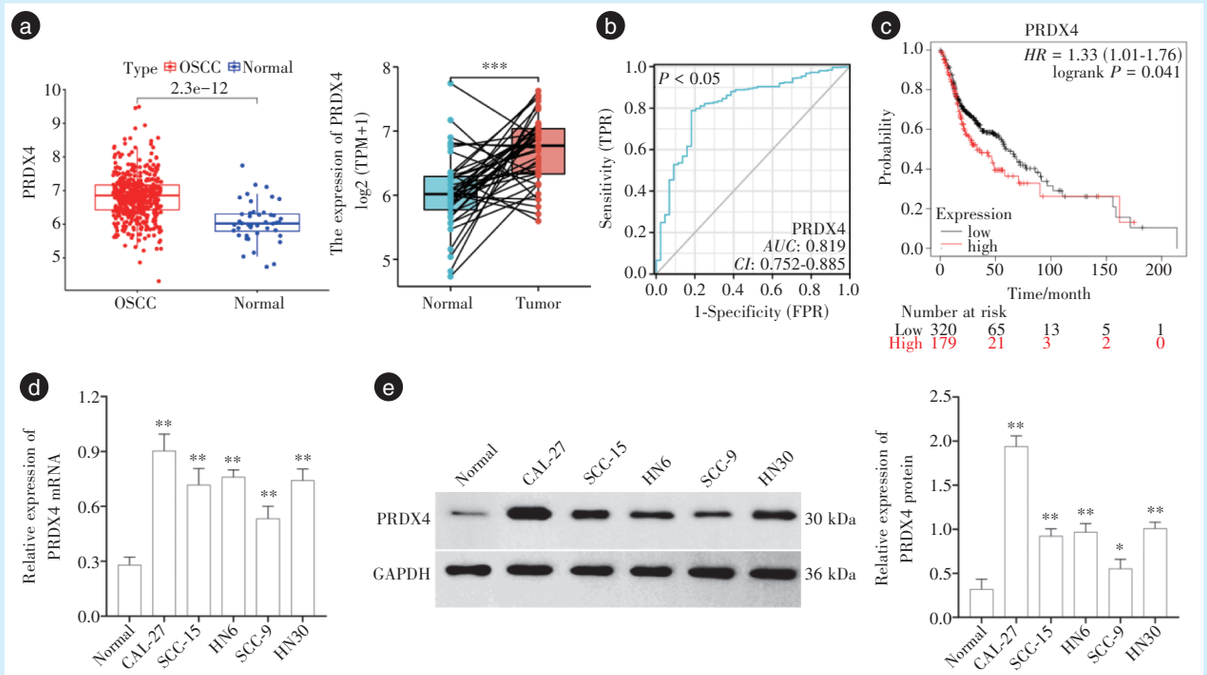
实验的统计学分析使用Graphpad Prism 10.1.2及SPSS16.0软件,数据以$\bar{x} \pm s$表示,采用单因素方差分析行多组间比较,用Student's t检验进行两组间比较,$P<0.05$表示对比结果具有统计学差异。各组实验的次数不少于3次。

2　结　果

2.1　PRDX4在OSCC组织和细胞系中的表达

根据TCGA数据分析可知,OSCC组织中PRDX4的mRNA表达水平明显高于正常组织($P<0.01$)(图1a)。基于ROC曲线对其在OSCC诊断中的价值进行分析可知,ROC曲线下面积为0.819,敏感度和特异度分别是78.0%、81.8%(图1b)。通Kaplan-Meier plotter数据库结果表明,PRDX4高表

达与患者预后呈负性相关(图1c)。qRT-PCR和Western Blot实验检测结果显示,PRDX4在癌细胞系中的mRNA和蛋白表达水平高于正常对照细胞($P<0.05$)(图1d & 1e),其中CAL-27表达较高,进行PRDX4表达敲低处理($P<0.01$),SCC-9表达较低,进行PRDX4过表达处理。



The mRNA and protein expression levels of PRDX4 in cancer cell lines are higher than those in normal control cells, with higher expression in CAL-27. PRDX4 expression knockdown treatment was performed in subsequent experiments; however, PRDX4 expression was low in SCC-9, and additional experiments were conducted to treat PRDX4 overexpression. a: The Cancer Genome Atlas database was used to analyze the mRNA expression of PRDX4 in OSCC; b: ROC curve was used to analyze the value of PRDX4 in the diagnosis of OSCC; c: the relationship between PRDX4 and the survival rate of OSCC patients was analyzed by using the Kaplan-Meier Plotter online analysis tool; d: PRDX4 mRNA expression in OSCC cell lines and normal oral cells; e: protein expression of PRDX4 in OSCC cell lines and normal oral cells. OSCC: oral squamous cell carcinoma; PRDX4: peroxiredoxin 4. $n=3$, $^{*}P<0.05$, $^{**}P<0.01$, $^{***}P<0.001$

Figure 1　Expression of PRDX4 mRNA and protein in OSCC tissues and cell lines

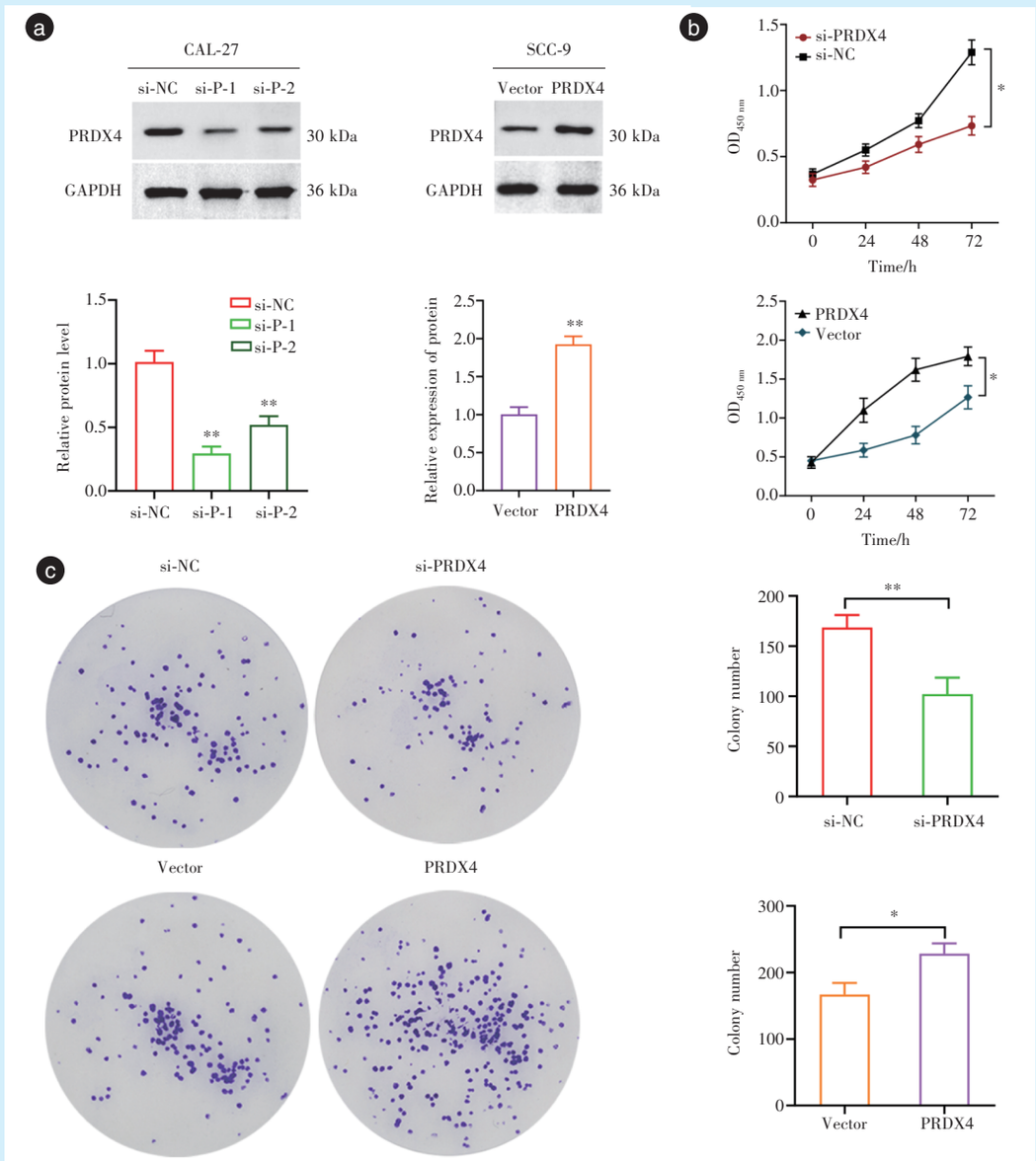
图1　PRDX4在OSCC组织和细胞系中的mRNA及蛋白表达

2.2　敲低或过表达PRDX4可抑制或促进OSCC细胞增殖能力

通过小干扰RNA si-PRDX4-1和si-PRDX4-2对CAL-27中PRDX4进行敲低。结果表明,si-PRDX4-1的敲低效率较si-PRDX4-2效果更为理想,选取si-PRDX4-1进行功能和分子实验($P<0.01$)。通过过表达质粒pcDNA3.1-PRDX4对SCC-9中PRDX4进行过表达,结果表明,相较于Vector组,pcDNA3.1-PRDX4组中PRDX4的表达明显增高($P<0.01$)(图2a)。CCK-8实验检测在体外敲低PRDX4对OSCC细胞增殖的作用,结果发现,敲低PRDX4表达后,si-PRDX4组细胞在450nm处的OD值明显低于si-NC组($P<0.05$);过表达PRDX4后,PRDX4组细胞在450nm处的OD值明显高于Vector组OD值($P<0.05$)(图2b)。平板克隆实验检测结果表明,敲低PRDX4表达后,si-PRDX4组细胞集落形成的数量明显低于si-NC组($P<0.05$);过表达PRDX4后,PRDX4组细胞集落形成的数量明显高于Vector组($P<0.05$)。提示PRDX4可调控OSCC细胞增殖能力(图2c)。

2.3　敲低和过表达PRDX4可抑制和促进OSCC细胞迁移能力

根据划痕实验数据显示,PRDX4表达被敲低后,si-PRDX4组相对愈合面积较si-NC组少($P<0.05$);PRDX4过表达时,PRDX4组比Vector组相对愈合面积多(图3)。说明OSCC细胞迁移能力受PRDX4的调控。



a: the transfection efficiency of si-PRDX4 and pcDNA3.1-PRDX4 was detected by western blotting. Knockdown of PRDX4 in CAL-27 was performed using the small interfering RNAs si-PRDX4-1 and si-PRDX4-2. The results showed that the knockdown efficiency of si-PRDX4-1 was more ideal than that of si-PRDX4-2, and si-PRDX4-1 was selected for subsequent experiments. si-P-1: si-PDX4-1; si-P-2: si-PDX4-2; b: PRDX4 knockdown inhibited the proliferation of CAL-27 cells; overexpression of PRDX4 promoted the proliferation of SCC-9 cells. c: PRDX4 knockdown inhibited the colony formation ability of CAL-27 cells; overexpression of PRDX4 promoted the colony formation ability of SCC-9 cells. OSCC: oral squamous cell carcinoma; PRDX4: peroxiredoxin 4. $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$. Si-NC group: the negative control group for the si-PRDX4 group, using interfering RNA si-NC; si-PRDX4 group: knockdown of PRDX4 expression in CAL-27 cells using interfering RNA si-PRDX4; vector group: the negative control group for the PRDX4 group, transfected with pcDNA3.1-NC plasmid; PRDX4 group: PRDX4 overexpression group, transfected with pcDNA3.1-NC plasmid

Figure 2　Knockdown and overexpression of PRDX4 could inhibit and promote the proliferation of OSCC cells

图2　敲低和过表达PRDX4可抑制和促进OSCC细胞增殖能力

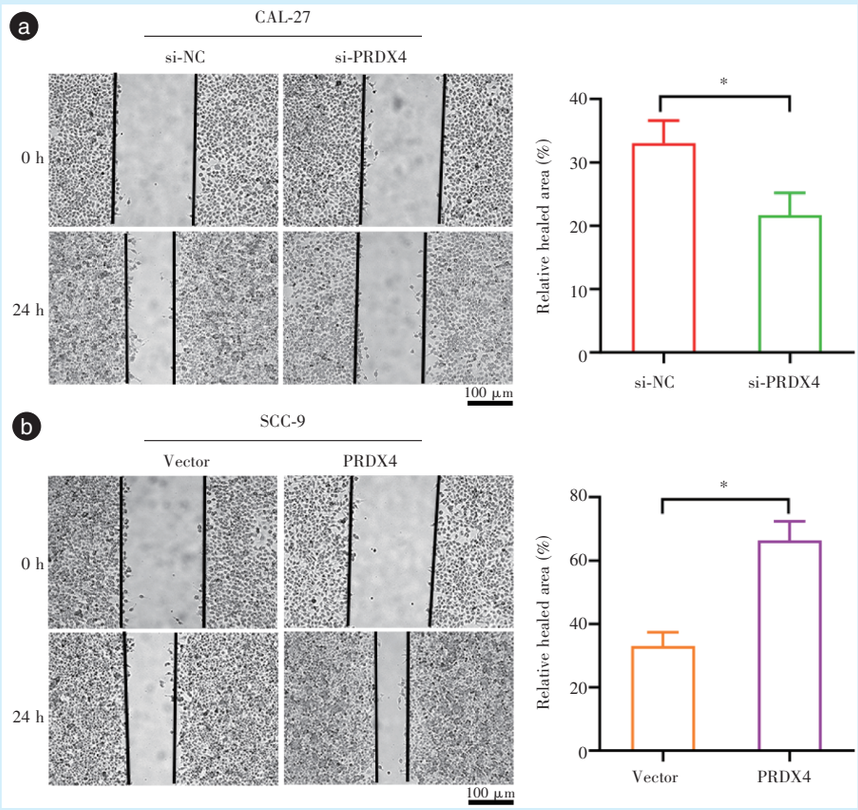
2.4　敲低和过表达PRDX4可抑制和促进OSCC细胞侵袭能力

Transwell侵袭实验数据表明，敲低PRDX4表达后，si-PRDX4组细胞的穿膜细胞数明显低于si-NC组；过表达PRDX4后，PRDX4组细胞穿膜细胞数明显高于Vector组（$P < 0.01$）。提示PRDX4可增强

OSCC细胞侵袭能力($P < 0.05$)(图4)。

2.5　敲低和过表达PRDX4可抑制和促进p38MAPK信号通路及上皮间充质转化蛋白表达

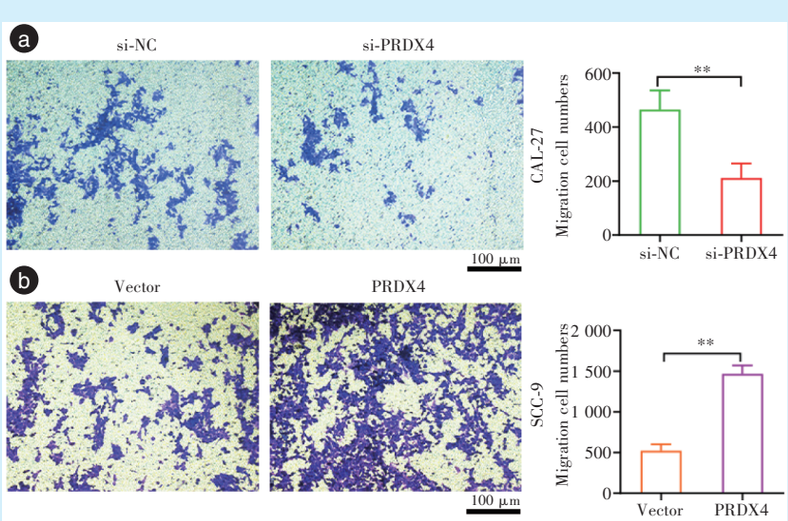
对PRDX4可能的信号通路进行富集分析显示:PRDX4在OSCC中可富集于p38MAPK信号通路(图5a)。Western Blot结果显示,敲低PRDX4表达后,p38MAPK蛋白表达不变、E-cadherin蛋白表达升高,其他蛋白均表达下降($P < 0.05$);而过表



a: effect of PRDX4 knockdown on migration ability of CAL-27 cells. b: effect of PRDX4 overexpression on the migration ability of SCC-9 cells. OSCC: oral squamous cell carcinoma; PRDX4: peroxiredoxin 4. $n = 3$, $^{*}P < 0.05$. Si-NC group: the negative control group for the si-PRDX4 group, using interfering RNA si-NC; Si-PRDX4 group: knockdown of PRDX4 expression in CAL-27 cells using interfering RNA si-PRDX4; vector group: the negative control group for the PRDX4 group, transfected with pcDNA3.1-NC plasmid; PRDX4 group: PRDX4 overexpression group, transfected with pcDNA3.1-NC plasmid

Figure 3　Knockdown and overexpression of PRDX4 inhibited and promoted the migration ability of OSCC cells

图3　敲低和过表达PRDX4可抑制和促进OSCC细胞迁移能力

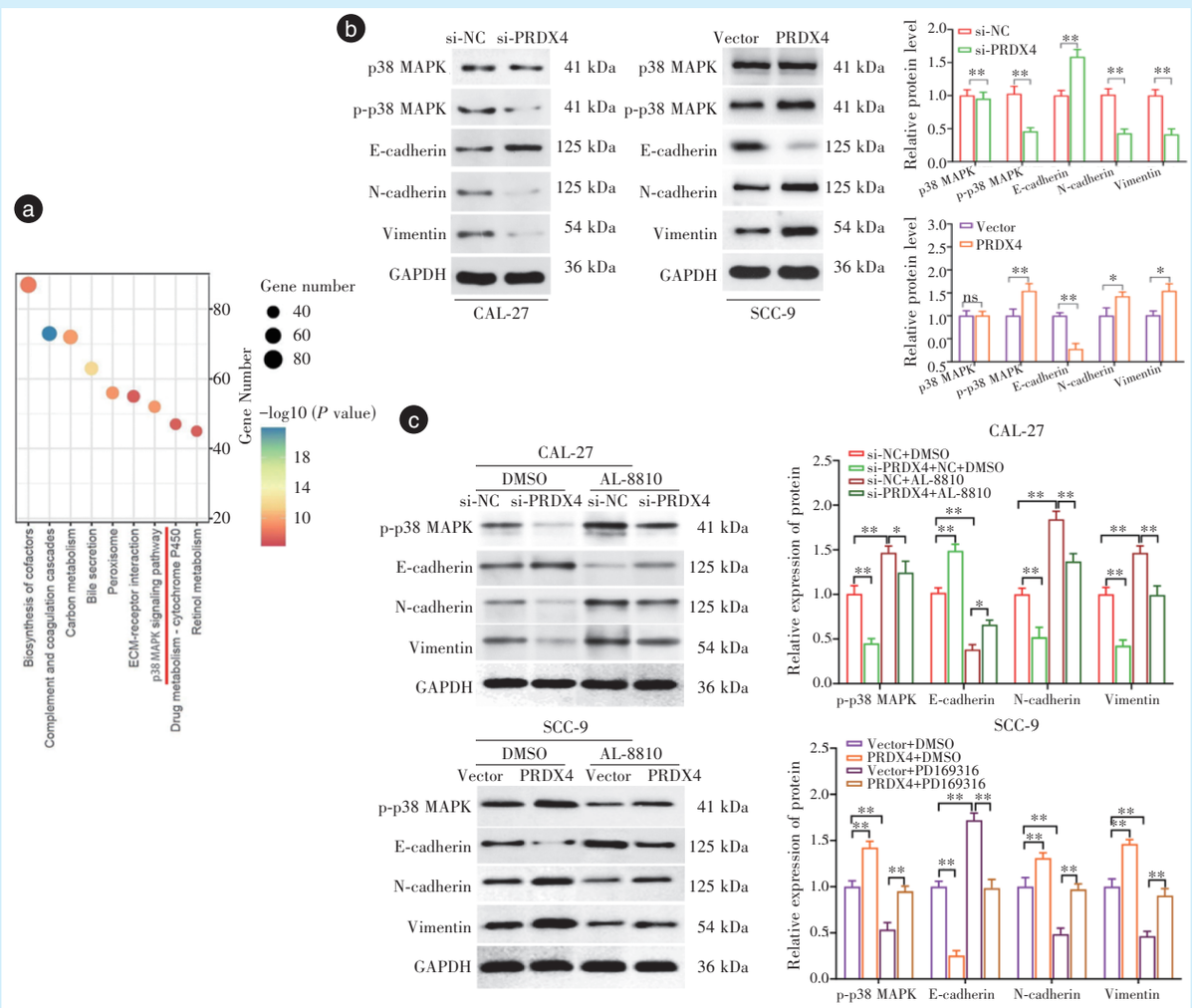


a: knockdown of PRDX4 inhibited CAL-27 cell invasion(x40). b: PRDX4 overexpression promoted SCC-9 cell invasion (x40). OSCC: oral squamous cell carcinoma; PRDX4: peroxiredoxin 4. $n = 3$, $^{**}P < 0.01$. Si-NC group: the negative control group for the si-PRDX4 group, using interfering RNA si-NC; Si-PRDX4 group: knockdown of PRDX4 expression in CAL-27 cells using interfering RNA si-PRDX4; vector group: the negative control group for the PRDX4 group, transfected with pcDNA3.1-NC plasmid; PRDX4 group: PRDX4 overexpression group, transfected with pcDNA3.1-NC plasmid

Figure 4　Knockdown and overexpression of PRDX4 could inhibit and promote the invasion ability of OSCC cells

图4　敲低和过表达PRDX4可抑制和促进OSCC细胞侵袭能力

达PRDX4后，各蛋白表达结果与敲低结果相反（图5b）。此外，分别对CAL-27及SCC-9细胞加入p-p38MAPK激动剂或抑制剂后，对N-cadherin、Vimentin及E-cadherin的影响明显逆转（图5c），表明PRDX4在OSCC中可参与调控p38MAPK信号通路，同时间接调控上皮间充质转化。



a: KEGG analysis showed that PRDX4 could be enriched in the p38MAPK signaling pathway in OSCC. b: PRDX4 knockdown restrained the expression of p38MAPK signal axis and EMT-related proteins in CAL-27; overexpression of PRDX4 promoted the expression of the p38MAPK signaling pathway and EMT-related proteins in SCC-9. c: treatment with p38MAPK agonist (AL-8810, 2 mL, 10 μmol/L) reversed the inhibitory effect of PRDX4 knockdown on the expression of the p38MAPK signaling pathway and EMT-related proteins in CAL-27; p38MAPK inhibitor (PD169316, 2 mL, 10 μmol/L) could reverse the promoting effect of PRDX4 overexpression on the expression of the p38MAPK signaling pathway and EMT-related proteins in SCC-9. DMSO: dimethyl sulfoxide. OSCC: oral squamous cell carcinoma; PRDX4: peroxiredoxin 4. $n = 3$, $^{*}P < 0.05$, $^{**}P < 0.01$. Si-NC group: the negative control group for the si-PRDX4 group, using interfering RNA si-NC; Si-PRDX4 group: knockdown of PRDX4 expression in CAL-27 cells using interfering RNA si-PRDX4; vector group: the negative control group for the PRDX4 group, transfected with pcDNA3.1-NC plasmid; PRDX4 group: PRDX4 overexpression group, transfected with pcDNA3.1-NC plasmid

Figure 5　Knockdown or overexpression of PRDX4 inhibited or promoted the p38MAPK signaling pathway and EMT protein expression

图5　敲低或过表达PRDX4可抑制或促进p38MAPK信号通路及上皮间充质转化蛋白表达

3　讨　论

由于口腔解剖结构相对特殊，颌面部和颈部淋巴结组织众多而且血供丰富，一定程度上影响了OSCC常规手术和放疗的效果，导致患者的5年生存率不足50%，预后不甚理想[20]。不仅如此，如果OSCC晚期患者出现远处转移或复发的现象，5

年生存率普遍不超过50%[21-22]。目前，关于分子靶向治疗肿瘤的研究已成为当前热点，积极探寻新的有效靶点具有重要意义。

PRDX4归属于过氧化物氧化还原酶家族，该蛋白可发挥过氧化氢酶（CAT）活性而具有抗氧化作用，在靠近氮尾端的低活跃区域遍布活性半胱氨酸（cysteine，Cys）[23]。其在肿瘤细胞中，可通过多种信号机制直接或间接与肿瘤的发生发展密切相关。目前实验已经证实，其表达异常可诱导多种癌症的发生发展[24-25]。Park等[26]认为，胃癌组织中PRDX4表达升高，且高表达的PRDX4与患者的不良预后相关。Chen等[27]研究表明，PRDX4在肝癌中表达上调，且高表达的PRDX4与肝癌的远处转移相关。本研究中，结合TCGA和Kaplan-Meier plotter数据库分析发现，其于OSCC组织内处于高表达，且PRDX4在OSCC组织中表达水平与患者生存时间呈负相关性。此外，通过Western Blot实验结果表明，相较于口腔黏膜上皮细胞，PRDX4在OSCC细胞中同样高表达，提示PRDX4在OSCC的发生发展过程中起重要作用，并可能与肿瘤细胞的恶性行为存在紧密联系。因此，笔者推测通过下调PRDX4可以抑制OSCC的恶性进展，有望成为新的分子治疗靶点。

为探究PRDX4对OSCC细胞恶性生物学行为的影响。本研究通过CCK-8实验、平板克隆实验、细胞划痕实验及Transwell侵袭实验分析发现，PRDX4敲低后会抑制细胞增殖、迁移和侵袭能力，而其过表达后则起到促进作用。提示PRDX4参与调控OSCC细胞的增殖、迁移和侵袭能力。此外，通过对TCGA数据库进行数据挖掘，结果表明PRDX4在OSCC可能富集的信号通路中，以p38MAPK信号通路最为显著。因此，基于生信分析的预测结果，本实验对细胞p38MAPK信号通路蛋白的表达水平进行分析。将CAL-27和SCC-9细胞的PRDX4表达进行敲低和过表达后，通过Western Blot实验结果分析，p-p38MAPK表达水平分别出现下降和升高，而p38MAPK的蛋白表达未发生显著变化，即p-p38MAPK/p38MAPK分别降低和增高，表明敲低和过表达PRDX4的表达后，可使p38MAPK信号通路处于抑制/激活状态。提示在OSCC细胞中，PRDX4可激活p38 MAPK信号通路进而促进细胞的增殖、迁移和侵袭能力。p38MAPK是介导肿瘤细胞信号转导的典型应激激活激酶，其作为MAPK通路中的重要分支，在肿瘤发生发展中发挥重要作用[28]。研究表明，p38MAPK通过调控下游的交联信号轴可间接影响癌细胞增殖、迁移和侵袭的进程[29-30]。此外，目前越来越多的研究表明，癌细胞增殖、迁移和侵袭等恶性行为发生发展，可经由p38MAPK信号通路介导下游的关键蛋白进而实现对肿瘤细胞恶性行为的间接调节[31]。有研究表明，该信号可影响耐药相关基因及蛋白的表达，调控癌细胞的增殖、侵袭和转移能力，使癌细胞对抗癌药物的敏感性降低。此外，在多种恶性肿瘤中，抑制p38MAPK信号通路的活化，可降低肿瘤细胞增殖、迁移和侵袭能力，从而使肿瘤细胞的恶性生物学活性受抑[32-33]。

EMT是指上皮细胞失去黏附能力而获得侵袭能力，成为间充质细胞的过程[34-35]。目前已有多项研究指出EMT为肿瘤侵袭、转移的关键环节，同时可促进肿瘤对化疗药物的抵抗性，与药物抵抗密切相关[36-37]。当细胞发生上皮间充质转化时，可有相关表型存在变化，如E-cadherin的表达下降以及N-cadherin、Vimentin的表达增加[38-39]。目前，已有数据证实，p38MAPK信号可调控EMT过程，下调p38MAPK信号通路后，EMT关键蛋白N-cadherin、Vimentin的表达减少，E-cadherin的表达增加[40]。本研究中，敲低OSCC中PRDX4蛋白表达后，p-p38MAPK蛋白表达降低，N-cadherin、Vimentin的表达减少，E-cadherin的表达增加。而过表达PRDX4蛋白表达后，p-p38MAPK蛋白表达增高，N-cadherin、Vimentin的表达增高，E-cadherin的表达降低。而分别对CAL-27及SCC-9细胞加入p-p38MAPK激动剂和抑制剂后，对N-cadherin、Vimentin及E-cadherin的影响明显逆转。表明PRDX4在OSCC中可通过介导p38MAPK信号进而调控E-cadherin、N-cadherin及Vimentin的表达，使肿瘤细胞的极性和黏附能力发生改变，影响EMT进程，最终调控肿瘤细胞侵袭性、转移性细胞表型的转变。提示在OSCC中PRDX4可激活p38 MAPK信号通路和EMT进程而促进细胞的迁移和侵袭能力。

基于目前的研究，未来将在此基础上开展关于PRDX4的生物抑制剂的研制，通过大量的体外实验、临床前动物实验及临床实验对PRDX4调控OSCC发生发展的具体机制进行详细的探讨及验证，进而对OSCC中PRDX4高表达的患者群体进行靶向治疗。通过结合目前的纳米技术，使生物抑癌制剂在靶向输送、缓释给药、改变体内分布以及提高生物利用度等方面具有新的应用前景。尽管

关于PRDX4的生物抑制剂在OSCC的治疗中显示出了良好的治疗前景，但也有很多挑战困扰着其进一步的推广。PRDX4抑制剂在肿瘤治疗中产生的耐药和不良反应问题需亟待解决；在治疗中的用药剂量和频次还有待讨论；当前关于PRDX4抑制剂治疗OSCC的研究临床数据并不完整，PRDX4抑制剂的真实效果需要更大的样本量进行分析。

综上所述，在OSCC中PRDX4处于高表达状态，敲低OSCC细胞中PRDX4的表达，可下调p38 MAPK信号轴及EMT相关信号蛋白的表达，进而使细胞的增殖、迁移、侵袭能力和上皮间充质转化受抑。

【Author contributions】 Geng H performed the experiments, analyzed the data and wrote the article. Li L performed the experiments, analyzed the data and revised the article. Yang J analyzed the data and revised the article. Liu YX analyzed the data and revised the article. Chen XD designed the study, conceptualized and reviewed the article. All the authors read and approved the final manuscript as submitted.

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