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# 靶向灭活肺腺癌干细胞OCT4多潜能转录因子后的差异表达基因分析

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**摘要** 应用全基因组cDNA表达芯片杂交技术, 筛查了OCT4多潜能转录因子阳性(OCT4$^+$)的肺腺癌干细胞及其*OCT4*表达沉默(OCT4-KD)细胞中的差异表达基因。发现与OCT4$^+$细胞比较, OCT4-KD细胞有2 138个基因显示差异表达, 其中1 554个基因表达上调, 584个基因表达下调。这些差异表达基因涉及细胞周期、细胞增殖、细胞凋亡以及细胞迁移等功能, 参与肿瘤通路、EGFR信号通路和低氧/p53信号通路等多个信号转导通路。选择差异表达最显著的基因进行分析, 显示其中20个基因特异性表达在OCT4$^+$细胞, 而47个基因仅在OCT4-KD细胞中表达, 该组基因可以作为肺腺癌中OCT4$^+$细胞可资鉴别的特征性基因。此外, 研究还发现在肺腺癌干细胞中, OCT4表达受HIF1α调控, 应用RNA干扰技术选择性灭活*HIF1α*基因引起OCT4表达转阴和细胞表型分化, 而采用棘霉素干预HIF1α的转录活性也明显下调了OCT4表达。实验结果揭示, 肺腺癌干细胞中存在HIF1-OCT4调控轴, 此发现为发展OCT4靶向治疗新技术提供了研究思路。

**关键词** 肺腺癌; 肿瘤干细胞; OCT4

# Profiling of Genes Differentially Expressed After Targeted Inactivation of OCT4 Pluripotent Transcription Factor in Human Lung Adenocarcinoma Stem Cells

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**Abstract** An analysis of cDNA microarray database was performed to search for the differentially expressed genes between the OCT4 pluripotent transcription factor-positive lung adenocarcinoma stem cells (OCT4$^+$ cells) and their *OCT4* knockdown (OCT4-KD) cells. In comparison with OCT4$^+$ cells, OCT4-KD cells were found to exhibit differential expression in 2 138 genes, among which 1 554 genes were up-regulated and 584 genes down-regulated. These genes were involved in several cell functions such as cell cycle, proliferation, apoptosis and migration. They were also participated in some signaling pathways especially pathways in cancer, EGFR signaling pathway and hypoxia/p53 pathway. Analysis with the top differentially expressed genes revealed that 20 genes were specifically expressed in OCT4$^+$ cells, while 47 genes were exclusively existed in OCT4-KD cells. This signature

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represents a unique type of genes that enable to identify the OCT4$^+$ cells in human lung adenocarcinoma. Moreover, our studies demonstrated that OCT4 expression in lung adenocarcinoma stem cells was regulated by HIF1α. With RNA interference technique, we showed that targeted inactivation of HIF1α in OCT4$^+$ cells was able to shut down OCT4 expression and thereby, to induce their phenotypic differentiation. Moreover, interference of HIF1 transcriptional activity with echinomycin also significantly reduced the expression of OCT4. Collectively these data pointed out the presence of HIF1α-OCT4 axis in lung adenocarcinoma stem cells. This finding provided a new avenue for development of OCT4-based targeting approaches to treat this tumor.

**Key words** lung adenocarcinoma; cancer stem cell; OCT4

八聚体结合转录因子4(octamer-binding transcription factor 4, OCT4)是胚胎发育早期原始未分化细胞如胚胎干细胞的特征性标志, 其主要功能是维持干细胞的自我更新(self-renewal)及多潜能(pluripotent)分化[1-2]。但研究揭示, 肿瘤中也存在干细胞的层级(hierarchy)结构, 其中的恶性干细胞称为肿瘤干细胞(cancer stem cells, CSC)[3]。CSC能够自我更新及分化形成肿瘤内各种类型癌细胞, 并具有显著的致瘤、转移和耐药特性, 因而是影响疾病转归和患者生存的主要细胞亚群[4-5]。近年来的文献报道, 在肺腺癌、肝癌和食管癌等常见肿瘤中, CSC不仅表达OCT4, 而且原发肿瘤中存在OCT4$^+$细胞者预后差[6-14]。

肺腺癌是肺癌最常见的组织类型之一, 其临床表现为易早期发生转移且对细胞毒化疗药物不甚敏感, 因而预后较差[15]。但OCT4如何调控肺腺癌CSC的生物学行为从而威胁患者生存, 目前还知之甚少。为了探讨此问题, 我们曾采用小RNA(microRNA)介导的基因沉默技术选择性地灭活了此类CSC中的*OCT4*基因, 并借助基因组学检测技术(基因芯片)筛查了具有OCT4结合位点(八聚体基序, octamer mortif)的特异性靶基因, 研究结果揭示, OCT4在此类细胞中具有转录调控功能、能够影响271个靶基因表达[16]。本文则利用上述基因芯片数据库进一步分析了*OCT4*沉默后的其他差异表达基因, 目的是寻找其特征性的基因群并为研发基于*OCT4*多潜能基因的CSC靶向治疗新技术提供研究思路。

## 1 材料与方法

### 1.1 材料

胎牛血清、DMEM及DMEM/F12培养基购自HyClone公司。免疫荧光检测抗体: 兔抗人OCT4多克隆抗体、兔抗人SP-C多克隆抗体、荧光素Rhodamine标记的驴抗兔IgG抗体均购自Santa Cruz公司。磁性细胞分选试剂: 鼠抗人CD221抗体购自BD公司, 鼠抗人MUC1抗体购自Santa Cruz公司, 羊抗鼠IgG免疫磁珠购自Miltenyi公司。生长因子及酪氨酸激酶抑制剂: IGF-1(insulin-like growth factor-1)和EGF(epidermal growth factor)购自Serotec公司, p38 MAPK抑制剂SB239063和糖原合成酶激酶-3(GSK-3)抑制剂BIO购自Sigma公司。*HIF1α*特异性shRNA慢病毒购自Santa Cruz公司。HIF1抑制剂棘霉素(Echinomycin, ECH)购自BioViotica公司。

### 1.2 方法

1.2.1 *细胞培养* 肺腺癌SPC-A1细胞购自中国科学院上海生命科学院生物化学与细胞生物学研究所细胞库。细胞培养条件为含10%胎牛血清、100 U/mL青霉素和100 g/mL链霉素的DMEM或DMEM/F12培养基、37 °C、5% $CO_2$的培养箱中培养。实验选择对数增殖期细胞, 经0.25%胰酶消化后收集。

磁性细胞分选(MACS)后的OCT4$^+$细胞(采用CD221抗体分选)在SMC(stemness-maintaining combinations)特种培养基中传代培养。SMC配方: DMEM培养基、10%胎牛血清、IGF-1(20 ng/mL)、EGF(20 ng/mL)、SB239063(5 μmol/L)和BIO(1 μmol/L)。

1.2.2 *磁性细胞分选* 分别采用CD221和MUC1抗体进行磁性细胞分选(MACS), 实验方法见文献[16-17]。简述之, 按2 μg抗体/$10^6$细胞比例加入鼠抗人CD221或MUC1抗体, 4 °C避光温育30 min后用PBS离心洗涤2次去除未结合的抗体, 按10个磁珠/细胞比例加入羊抗鼠IgG磁珠, 4 °C避光孵育30 min, PBS 离心洗涤后用miniMACS磁性分选柱分离获得阳性细胞。

1.2.3 *基因表达沉默* *OCT4*表达沉默细胞(*OCT4*-knockdown, 简称OCT4-KD)的制备见文献[16]。*HIF1α*表达沉默采用shRNA介导的慢病毒转染技术, 按生产商提供的配套试剂及实验指南操作: 6孔板

(Costa公司)内每孔种植3.0×10$^5$ OCT4$^+$细胞(1 mL培养基), 培养24 h后每孔加入2 μL聚凝胺(Polybrene, 终浓度为10 μg/mL), 作用30 min后加入30 μL慢病毒。感染后次日更换培养基, 第2日开始用10 μg/mL嘌呤霉素(Puromycin)筛选, 2周后获得抗性细胞克隆。

1.2.4 免疫荧光检测 将细胞(1×10$^5$细胞, 500 μL培养基)接种于置有无菌盖玻片的24孔板(Costa公司)中, 培养过夜后按实验要求分为二类: 表型检测实验直接经4%多聚甲醛固定; 药物抑制实验则加入500 μL含ECH培养基(终浓度为10 nmol/L)并继续培养24 h后固定。多聚甲醛固定30 min后, PBS洗3次(每次洗3 min, 下同), 0.25% Triton X-100通透15 min, 1% BSA封闭非特异性结合3 h, PBS洗3次后加入一抗(1:100稀释), 设阴性对照(用PBS代替一抗), 4 °C过夜。PBS洗3次去除非结合抗体后加二抗(1:100稀释), 室温避光1 h, PBS洗3次, 滴加Hoechest 33342(1:50稀释)染核5 min, PBS洗2次, 加封片剂于Olympus IX51型荧光显微镜下观察。

1.2.5 基因表达芯片检测及生物信息学分析 以体外培养的OCT4$^+$细胞为对照组, OCT4-KD细胞为实验组, 提取总RNA后采用Human-12T Illumina Beadchip进行全基因组表达筛查, 详见文献[16]。差异表达基因按照差异分值(DiffScore)遴选。差异分值体现一个探针在两个样本中的表达差异程度, 该数值 ≥13时显示两个样本间探针检测信号的差异具有统计学意义($P$<0.05)。然后依据GenBank注译基因从差异表达基因中获得功能已知基因群, 并将结果上传至DAVID数据库(http://david.abcc.ncbicrf.gov/home.jsp)和KEGG(Kyoto Encyclopedia of Genes and Genomes)数据库, 进行基因类型(Gene ontology, GO)分类和信号转导通路分类。

## 2 结果

### 2.1 *OCT4*灭活后的基因表达芯片筛查结果

依据前期研究获得的Human-12T Illumina Beadchip基因芯片数据库[16], 共筛查获得了2 138个差异表达基因, 其中1 554个基因在OCT4-KD细胞中表达上调, 584个基因表达下调。

我们曾报道, OCT4$^+$细胞具有显著的致瘤、转移能力并对顺铂和卡铂高度耐药[18-21], 因而通过GO分析我们筛查了与细胞迁移、增殖和凋亡等功能相关的差异表达基因。图1结果显示, 涉及细胞迁移、细胞周期和细胞凋亡的基因分别为171、99和82个, 而与细胞增殖及DNA损伤应答的基因各为48和53个。KEGG分析揭示, 差异表达基因也参与了多个信号通路, 主要集中在肿瘤通路、表皮生长因子受体(epidermal growth factor receptor, EGFR)信号通路和低氧(hypoxia)/p53抑癌基因信号通路等。

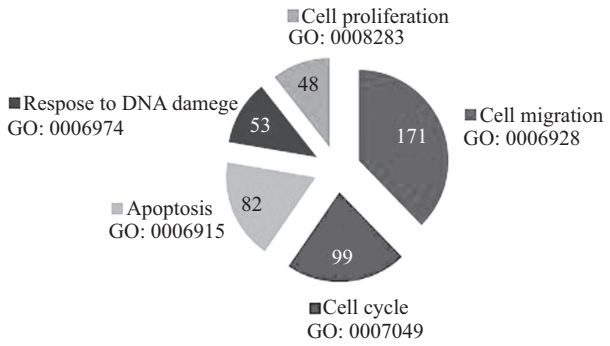



**图1 差异表达基因的基因类型**

**Fig.1 Gene ontology of differentially expressed genes**

### 2.2 OCT4$^+$肺腺癌细胞的特征性基因分析

采用以下2项指标, 我们从基因芯片数据库中寻找OCT4$^+$细胞的特征性基因: (1)在OCT4$^+$细胞中不表达(探针信号值与背景信号值之间的统计学差异无显著意义, $P$>0.05), 但在OCT4-KD细胞表达($P$<0.05)并且二组间差异分值≥20。(2)在OCT4$^+$细胞中表达, 但在OCT4-KD细胞中不表达并且二组间差异分值≥20。差异分值≥20表示所测基因在二组样本间的表达差异具有统计学极显著意义($P$<0.01)。

按上述第(1)项遴选指标共发现47个基因的表达显著上调(表1)。在该组基因中, 黏蛋白MUC1是呼吸上皮中II型肺泡细胞(alveolar type 2 cells, AT2)的特征性标志之一[22], 该标志在*OCT4*沉默后阳性表达提示肺腺癌CSC失去*OCT4*多潜能基因后主要向肺泡上皮分化, 此结论与我们的前期研究结果一致[17,23]。为了进一步验证MUC1与OCT4之间的关系, 我们采用MACS技术从SPC-A1细胞株中分离获得了MUC1$^+$细胞亚群, 然后采用免疫荧光检测技术分别检测了OCT4和AT2细胞标志C型活性蛋白(surfactant protein C, SP-C)的表达, 结果证实此类细胞具有OCT4$^-$和SP-C$^+$表型(图2)。

按上述第(2)项遴选指标也发现20个基因的表达显著下调(表2), 其中IX型碳酸酐酶(carbonic anhydrase IX, *CA9*)位居第三。我们曾选择该标志进行过分析,

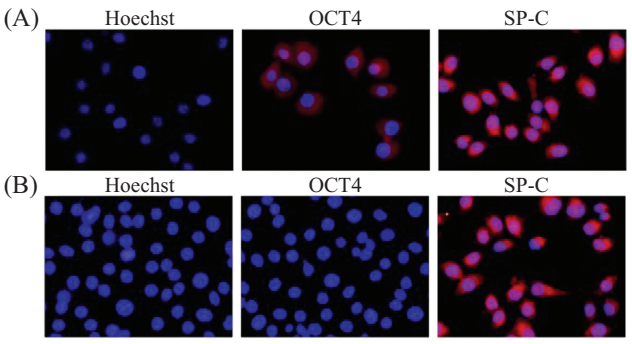
**表1 *OCT4*沉默后的表达上调基因**

**Table 1 Genes upregulated after *OCT4* knockdown**

| 基因<br>Genes | 基因全称<br>Gene name | $OCT4^+$细胞*<br>$OCT4^+$ cell* | OCT4-KD*<br>OCT4-KD* | 差异分值<br>Diffscore |
|---|---|---|---|---|
| *RETNLB* | Resistin like beta | 27.449 | 11 369.050 | 327.593 |
| *OLR1* | Oxidized low density lipoprotein (lectin-like) receptor 1 | –22.916 | 513.610 | 96.632 |
| *NNMT* | Nicotinamide N-methyltransferase | 27.884 | 606.484 | 77.396 |
| *NRG1* | Neuregulin 1, transcript variant GGF2 | 16.604 | 453.468 | 69.880 |
| *ANKRD1* | Ankyrin repeat domain 1 (cardiac muscle) | 19.503 | 498.482 | 65.486 |
| *C8orf4* | Chromosome 8 open reading frame 4 | 33.523 | 410.962 | 60.028 |
| *LOC100130009* | PREDICTED: similar to high mobility group protein | 17.458 | 363.357 | 59.762 |
| *MAZ* | MYC-associated zinc finger protein, transcript variant 2 | 16.472 | 366.099 | 58.979 |
| *EFEMP1* | EGF-containing fibulin-like extracellular matrix protein 1 | 31.320 | 448.312 | 52.390 |
| *LEPREL1* | Leprecan-like 1 | 23.889 | 361.677 | 52.296 |
| *MYLK* | Myosin light chain kinase, transcript variant 8 | 35.151 | 487.704 | 51.870 |
| *IER5L* | Immediate early response 5-like | –4.458 | 276.281 | 50.966 |
| *PSG4* | Pregnancy specific beta-1-glycoprotein 4, transcript variant 1 | 21.063 | 333.370 | 48.830 |
| *SPHK1* | Sphingosine kinase 1, transcript variant 1 | 40.300 | 345.238 | 48.253 |
| *ABCA13* | ATP-binding cassette, sub-family A(ABC1), member 13 | –12.100 | 287.056 | 46.349 |
| *NEXN* | Nexilin (F actin binding protein) | 14.973 | 337.873 | 43.350 |
| *CHAC2* | ChaC, cation transport regulator homolog 2(*E.coli*) | 41.931 | 372.848 | 43.059 |
| *MUC1* | Mucin 1, cell surface associated, transcript variant 1 | 19.881 | 274.001 | 38.889 |
| *RCAN1* | Regulator of calcineurin 1, transcript variant 2 | 31.853 | 290.433 | 35.499 |
| *DIAPH3* | Diaphanous homolog 3 (*Drosophila*), transcript variant 2 | 27.449 | 11 369.050 | 327.593 |
| *IFI27* | Interferon, alpha-inducible protein 27, transcript variant 2 | –7.211 | 235.390 | 34.881 |
| *NFKB2* | Nuclear factor of kappa light polypeptide gene enhancer | 16.910 | 286.444 | 34.430 |
| *SUPT6H* | Suppressor of Ty 6 homolog (*S.cerevisiae*) | 32.288 | 281.455 | 32.591 |
| *NKAP* | NF-kappaB activating protein | 36.805 | 263.790 | 31.175 |
| *SLC3A2* | Solute carrier family 3, member 2, transcript variant 6 | 19.137 | 297.211 | 31.173 |
| *XK* | X-linked Kx blood group (McLeod syndrome) | 7.864 | 244.403 | 30.268 |
| *MTF1* | Metal-regulatory transcription factor 1 | 29.037 | 231.789 | 29.315 |
| *RPS19BP1* | Ribosomal protein S19 binding protein 1 | 40.297 | 268.356 | 29.230 |
| *DOLK* | Dolichol kinase | 37.498 | 245.803 | 29.116 |
| *KIAA0460* | KIAA0460 | 24.718 | 250.371 | 27.924 |
| *SNX25* | Sorting nexin 25 | 10.287 | 200.283 | 25.720 |
| *JARID1C* | Jumonji, AT rich interactive domain 1C | 14.556 | 209.902 | 25.717 |
| *OGFRL1* | Opioid growth factor receptor-like 1 | –15.128 | 175.086 | 25.406 |
| *SRFBP1* | Serum response factor binding protein 1 | 31.321 | 225.118 | 24.912 |
| *FAM100A* | Family with sequence similarity 100, member A | 24.643 | 311.667 | 24.854 |
| *SFTA1P* | Surfactant associated 1 (pseudogene), non-coding RNA | 36.376 | 254.012 | 24.831 |
| *PUSL1* | Pseudouridylate synthase-like 1 | 35.521 | 243.657 | 24.380 |
| *HYI* | Hydroxypyruvate isomerase homolog (*E.coli*) | 38.385 | 234.384 | 24.255 |
| *CNOT3* | CCR4-NOT transcription complex, subunit 3 | 34.268 | 261.149 | 22.712 |
| *GOLGA4* | Golgi autoantigen, golgin subfamily a, 4 | 41.787 | 245.138 | 22.530 |
| *RHBDD3* | Rhomboid domain containing 3 | 0.466 | 160.015 | 22.059 |
| *TERF1* | Telomeric repeat binding factor (NIMA-interacting) 1 | –0.228 | 168.805 | 21.806 |
| *ZFP91* | Zinc finger protein 91 homolog (mouse) | 38.590 | 213.104 | 21.110 |
| *ECHDC1* | Enoyl Coenzyme A hydratase domain containing 1 | 38.716 | 209.238 | 21.029 |
| *SFMBT1* | Scm-like with four mbt domains 1, transcript variant 2 | 37.758 | 222.368 | 20.766 |
| *SMARCC2* | SWI/SNF related, matrix associated, actin dependent regulator of chromatin, subfamily c, member 2, transcript variant 1 | 15.933 | 206.851 | 20.479 |
| *GBF1* | Golgi-specific brefeldin A resistant guanine nucleotide exchange factor 1 | 41.760 | 229.762 | 20.398 |

*探针的信号检测值。

*Signaling value detected by probes.




A: SPC-A1细胞中OCT4$^+$细胞亚群均为OCT4和SP-C染色阳性; B: MACS分选MUC1$^+$亚群后染色显示SP-C阳性, 但OCT4阴性。
A: the OCT4$^+$ subtype in SPC-A1 cell line was stained positive for OCT4 and SP-C; B: the MUC1$^+$ subtype isolated by MACS showed positive staining for SP-C but negative for OCT4.

**图2 OCT4与MUC1表达的关系**

**Fig.2 The correlation between OCT4 and MUC1**

证明CA9$^+$细胞属于OCT4阳性细胞亚群[17]。

### 2.4 HIF1α转录调控OCT4表达

CA9是一种公认的内源性低氧表面标志, 其表达受低氧诱导因子1(hypoxia inducible factor 1, HIF1)转录调控[24]。*OCT4*沉默引起*CA9*表达转阴提示在肺腺癌CSC中OCT4与HIF1之间存在内在联系, 于是我们从基因芯片数据库中寻找相关线索, 发现OCT4$^+$细胞主要表达*HIF1α*、*HIF2α*和*HIF1β*, 而*OCT4*基因沉默引起上述3个HIF家族成员的表达明显上调(图3A)。免疫荧光检测也证实在*OCT4*沉默前后, HIF1α和HIF2α蛋白均呈阳性表达(图3B和图3C)。进一步筛查发现, *OCT4*沉默还引起*CITED2*和*CITED4*的表达明显上调(图3A), 此2个蛋白能够与HIF1结合形成复合物, 从而干扰HIF1与低氧反

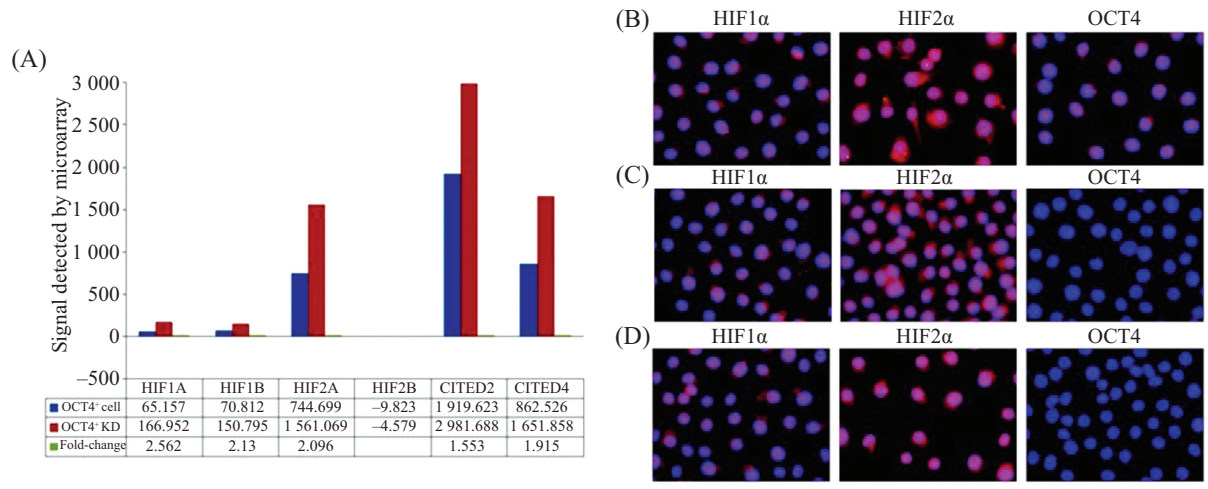
**表2 *OCT4*沉默后的表达下调基因**

**Table 2 Genes down-regulation after *OCT4* knockdown**

| 基因<br>Genes | 基因全称<br>Gene name | OCT4$^+$细胞*<br>OCT4$^+$ cell* | OCT4-KD*<br>OCT4-KD* | 差异分值<br>Diffscore |
|---|---|---|---|---|
| *GBP2* | Guanylate binding protein 2, interferon-inducible | 500.361 | 1.223 | –104.636 |
| *RN7SK* | RNA, 7SK small nuclear, non-coding RNA | 629.065 | 8.0292 | –71.053 |
| *CA9* | Carbonic anhydrase IX | 353.364 | –11.651 | –69.114 |
| *EGR2* | Early growth response 2 (Krox-20 homolog, *Drosophila*) | 376.461 | 13.260 | –60.225 |
| *ALOX5* | PREDICTED: arachidonate 5-lipoxygenase | 388.250 | 27.763 | –48.955 |
| *RNU6ATAC* | RNA, U6atac small nuclear (U12-dependent splicing) | 423.693 | 25.666 | –48.885 |
| *CYP1A1* | Cytochrome P450, family 1, subfamily A, polypeptide 1 | 311.511 | –17.478 | –42.775 |
| *SLC29A4* | Solute carrier family 29 (nucleoside transporters), member 4 | 306.857 | 27.547 | –42.637 |
| *MALL* | Mal, T-cell differentiation protein-like | 178.591 | –9.999 | –32.376 |
| *MIR25* | MicroRNA 25 | 225.843 | 10.743 | –28.671 |
| *WFDC3* | WAP four-disulfide core domain 3, transcript variant 2 | 227.638 | 27.200 | –28.360 |
| *ACCN2* | Amiloride-sensitive cation channel 2, transcript variant 2 | 169.916 | –6.790 | –27.982 |
| *GALNT9* | Polypeptide N-acetylgalactosaminyltransferase 9 | 207.018 | 25.047 | –26.474 |
| *TAGLN* | Transgelin, transcript variant 2 | 227.229 | 21.629 | –26.259 |
| *FLJ44342* | PREDICTED: hypothetical LOC645460, miscRNA | 215.287 | 20.760 | –26.047 |
| *LOC100132761* | PREDICTED: hypothetical protein LOC100132761 | 163.913 | –7.533 | –23.449 |
| *ETV5* | Ets variant gene 5 (ets-related molecule) | 242.514 | 31.383 | –22.535 |
| *REEP6* | Receptor accessory protein 6 | 225.116 | 25.840 | –20.980 |
| *NDUFA4L2* | NADH dehydrogenase 1 alpha subcomplex, 4-like 2 | 155.514 | –13.279 | –20.800 |
| *SPRY1* | Sprouty homolog 1, antagonist of FGF signaling (*Drosophila*) | 198.312 | 26.768 | –20.282 |

*探针的信号检测值。

*Signaling value detected by probes.



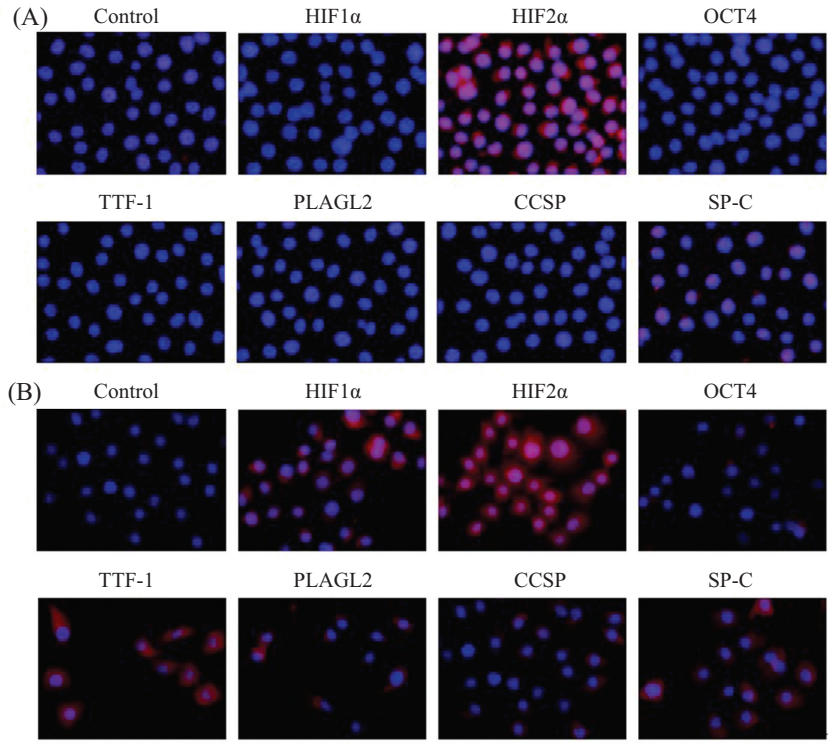

| | HIF1A | HIF1B | HIF2A | HIF2B | CITED2 | CITED4 |
|---|---|---|---|---|---|---|
| OCT4+ cell | 65.157 | 70.812 | 744.699 | −9.823 | 1 919.623 | 862.526 |
| OCT4+ KD | 166.952 | 150.795 | 1 561.069 | −4.579 | 2 981.688 | 1 651.858 |
| Fold-change | 2.562 | 2.13 | 2.096 | | 1.553 | 1.915 |

A: 基因芯片检测结果; B-D: 免疫荧光检测。B: OCT4$^+$细胞; C: OCT4-KD细胞; D: OCT4$^+$细胞经10 nmol/L ECH作用24 h后。

A: microarray analysis; B-D: immunostaining. B: OCT4$^+$ cells; C: OCT4-KD cells; D: OCT4$^+$ cells treated with 10 nmol/L ECH for 24 h.

**图3 HIF1调控OCT4表达**

**Fig.3 HIF1 regulates OCT4 expression**




A: 免疫荧光检测显示OCT4-KD细胞的表型标志表达; B: OCT4$^+$细胞对照组的表型标志表达。

A: immunofluorescent assay revealed the expression of phenotypic markers in OCT4-KD cells; B: the expression of these markers in OCT4$^+$ cells as control.

**图4 靶向灭活HIF1下调OCT4表达并促使OCT4$^+$细胞表型分化**

**Fig.4 Down-regulation of OCT4 expression and induction of phenotypic differentiation by targeted inactivation of HIF1 in OCT4$^+$ cells**

应元件(hypoxia response element, HRE)结合并导致转录活性下降。该作用机制可以解释*OCT4*沉默后的CA9表达下调现象。

人OCT4编码基因*POU5f1*的启动子中也存在HRE基序, 选择性灭活*HIF1α*或*HIF2α*均能够转录抑制OCT4表达[27-28]。本文选用棘霉素(ECH)阻断HIF1-HRE相互作用[29], 评估该药物对OCT4表达的抑制效应。图3D结果显示, 经10 nmol/L ECH作用OCT4$^+$细胞24 h后, HIF1α和HIF2α蛋白仍阳性, 但OCT4表达显著下调, 提示HIF1α可能是诱导OCT4表

达的主要调控因子。

为了验证上述推测, 我们采用*HIF1α*特异性shRNA慢病毒转染OCT4$^+$细胞, 后者经嘌呤霉素筛选后获得了稳转细胞克隆(称为HIF1-KD)。RT-PCR检测显示HIF1-KD细胞阳性表达*HIF2α*特异性mRNA, 而*HIF1α*不表达, 说明实验选用的shRNA具有靶基因选择性(资料未列)。免疫荧光检测证实在HIF1-KD细胞中, HIF1α和OCT4均呈阴性表达, 而HIF2α仍阳性(图4A)。进一步分析发现, HIF1-KD细胞失去了远端呼吸上皮的特征性转录因子及表型标志, 如甲状腺转录因子-1(thyroid transcription factor-1, TTF-1)、多形腺瘤样基因2(pleiomorphic adenoma gene-like 2, PLAGL2)和CCSP, 同时SP-C表达明显下调(图4A)。而OCT4$^+$细胞对照组阳性表达上述转录因子及表型标志(图4B), 与我们的前期报道一致[17,23]。综合以上实验结果可以得出结论, 在肺腺癌细胞中OCT4表达主要受HIF1α的转录调控, 靶向灭活*HIF1α*导致OCT4表达转阴并促使此类细胞分化, 表现为上述4种远端呼吸上皮标志消失或显著下调。

## 3 讨论

肺癌CSC是近年来进展迅速的一个研究领域, 自2007年Ho等[30]首次报道以来, 世界各国对肺癌CSC的研究多聚焦于非小细胞肺癌(NSCLC)尤其是肺腺癌, 现有证据均支持此类肿瘤中存在具有未分化干细胞特性的高致瘤和高度耐药的CSC[31-33]。这些肺癌CSC分属多个表型不同的亚群, 如侧群细胞(side population, SP)、CD133$^+$和ALDH$^{HIGH}$等, 但其共性特征是均表达多潜能干细胞基因尤其是*OCT4*[6]。台湾研究者Chiou等[34]率先报道, 异位(ectopic)表达*OCT4*等多潜能基因可以促使肺腺癌细胞逆向分化成为高度恶性的CSC样细胞, 而Chen等[35]采用RNA干扰技术灭活CSC中的*OCT4*, 证明*OCT4*表达沉默可以显著抑制CSC的体内致瘤能力并提高顺铂等药物的体外抗癌作用。由此说明, *OCT4*是肺腺癌CSC的一个重要调控基因, 靶向抑制该基因具有显著抗癌效应。

低氧是实体肿瘤的一个普遍现象。研究揭示, 低氧既是促使肿瘤转移的主要微环境因子, 低氧区又是维持CSC生物学特性的"巢(niche)"[36-37]。实验证明, 细胞对低氧的反应是转录上调HIF, 后者与OCT4编码基因*POU5f1*启动子中的HRE基序结合后启动OCT4表达[27]。因此, 针对低氧-HIF-OCT4轴是靶向杀灭CSC或诱导其分化的一条有效途径。近年来, 围绕该调控轴我们曾开展了OCT4靶向治疗的探索性研究。前文我们报道, 通过15-脱氧–前列腺素J2(15d-PGJ2)与去铁胺(desferrioxamine, DFO)组合可以有效阻止HIF2α转译从而使得OCT4表达转阴, 并且该药物组合具有显著的体外抗癌效应[16]。最近我们发现, 上述组合不仅下调HIF2α而且也抑制HIF1α表达(未发表资料)。本研究揭示, 在此类癌细胞中不仅靶向灭活*HIF1α*基因也具有相同效应, 而且HIF抑制剂ECH也能够显著下调OCT4表达。据此我们认为, 肺腺癌CSC的生物学特性受HIF1α-OCT4轴调控, 而且该调控轴是可以通过药物进行干预的。因而, 围绕该研究思路继续寻找适合临床应用的有效药物, 将能够为肺癌CSC的靶向治疗提供有效手段。

综上所述, *OCT4*多潜能基因作为肺腺癌CSC的特征性标志和治疗靶标已逐渐得到研究者的认同, 但目前关于此类CSC的表型特征尚未取得共识。除了SP、CD133$^+$和ALDH$^{HIGH}$等OCT4$^+$亚群外, 我们曾从SPC-A1等细胞株中分离鉴定了一个新的OCT4$^+$亚群, 此类细胞表达呼吸道远端祖细胞(progenitor cells)的表型标志, 如甲状腺转录因子-1(thyroid transcription factor-1, TTF-1)、多形腺瘤样基因2(pleomorphic adenomatous gene-like 2, PLAGL2)、SP-C和Clara细胞分泌蛋白(Clara cell secretory proteins, CCSP)等。采用基因沉默技术我们分别灭活了*OCT4*、*TTF-1*和*PLAGL2*, 发现上述基因表达沉默促使癌细胞向肺泡上皮分化[16-17,23]。这些研究结果提示, 肺腺癌可能源自呼吸道远端祖细胞的恶性转化。但此类CSC的属性(identity)仍有待深入研究, 基因组学研究技术有助于阐明此问题。本文通过全基因组表达筛查, 首次报道了OCT4$^+$肺腺癌细胞具有可资鉴别的特征性基因群, 其中20个基因在此类细胞中高表达, 而47个基因在*OCT4*沉默后明显表达。该群基因对深化肺腺癌CSC的研究具有重要价值。

简言之, 随着CSC研究的深入, 其特异性靶向治疗已日益受到关注。本文结果为推进上述研究提供了新的思路。相信随着研究的不断深入, 肺癌CSC的特异性靶向治疗最终将成为一种临床治疗新技术, 而此类治疗无疑将为改善患者生存带来希望。

## 参考文献 (References)


1 Pan GJ, Chang ZY, Scholer HR, Pei D. Stem cell pluripotency and transcription factor Oct4. Cell Res 2002; 12(5/6): 321-9.

2 Boyer LA, Lee TI, Cole MF, Levine SS, Zucker JP, Guenther MG, *et al*. Core transcriptional regulatory circuitry in human embryonic stem cells. Cell 2005; 122(6): 947-56.

3 Reya T, Morrison SJ, Clarke MF, Weissman IL. Stem cells, cancer, and cancer stem cells. Nature 2001; 414(6859): 105-11.

4 Clarke MF, Dick JE, Dirks PB, Eaves CJ, Jamieson CH, Jones DL, *et al*. Cancer stem cells—perspectives on current status and future directions: AACR workshop on cancer stem cells. Cancer Res 2006; 66(19): 9339-44.

5 Frank NY, Schatton T, Frank MH. The therapeutic promise of the cancer stem cell concept. J Clin Invest 2010; 120(1): 41-50.

6 Akunuru S, James Zhai Q, Zheng Y. Non-small cell lung cancer stem/progenitor cells are enriched in multiple distinct phenotypic subpopulations and exhibit plasticity. Cell Death Dis 2012; 3: e352.

7 Zhang XY, Dong QG, Huang JS, Huang AM, Shi CL, Jin B, *et al*. The expression of stem cell-related indicators as a prognostic factor in human lung adenocarcinoma. J Surg Oncol 2010; 102: 856-62.

8 Cortes-Dericks L, Galetta D, Spaggiari L, Schmid RA, Karoubi G. High expression of octamer-binding transcription factor 4A, prominin-1 and aldehyde dehydrogenase strongly indicates involvement in the initiation of lung adenocarcinoma resulting in shorter disease-free intervals. Eur J Cardiothorac Surg 2012; 41: e173-81.

9 Chen ZG, Wang T, Cai L, Su CH, Zhong BL, Lei YY, *et al*. Clinicopathological significance of non-small cell lung cancer with high prevalence of Oct-4 tumor cells. J Exp Clin Cancer Res 2012; 31: 10-9.

10 Li XX, Wang JG, Xu ZY, Ahmad A, Li EC, Wang Y, *et al*. Expression of *Sox2* and *Oct4* and their clinical significance in human non-small-cell lung cancer. Int J Mol Sci 2012; 13: 7663-75.

11 Qian YW, Chen Y, Yang W, Fu J, Cao J, Ren YB, *et al*. p28(GANK) prevents degradation of Oct4 and promotes expansion of tumor-initiating cells in hepatocarcinogenesis. Gastroenterology 2012; 142(7): 1547-58.

12 Dong Z, Zeng Q, Luo H, Zou J, Cao C, Liang J, *et al*. Increased expression of OCT4 is associated with low differentiation and tumor recurrence in human hepatocellular carcinoma. Pathol Res Pract 2012; 208(9): 527-33.

13 Li C, Yan Y, Ji W, Bao L, Qian H, Chen L, *et al*. OCT4 positively regulates survivin expression to promote cancer cell proliferation and leads to poor prognosis in esophageal squamous cell carcinoma. PLoS One 2012; 7(11): e49693.

14 He W, Li K, Wang F, Qin YR, Fan QX. Expression of OCT4 in human esophageal squamous cell carcinoma is significantly associated with poorer prognosis. World J Gastroenterol 2012; 18: 712-9.

15 廖美琳. 肺部肿瘤学. 上海: 上海科学技术出版社(Liao Meilin. Pulmonary Oncology. Shanghai: Shanghai Scientific & Technical Publishers), 2008, 7-61.

16 宁仁利, 黄进肃, 吴丽霞, 李 榕, 徐慧莉, 周 瑾, 等. 靶向肺腺癌干细胞OCT4多潜能基因的体外抗癌效应研究. 中国细胞生物学学报(Ning Renli, Huang Jinsu, Wu Lixia, Li Rong, Xu Huili, Zhou Jin, *et al*. A study on the *in vitro* anti-cancer effects by targeting OCT4 pluripotent gene in lung adenocarcinoma stem cells. Chinese Journal of Cell Biology) 2012; 34(11): 1101-9.

17 薛明明, 宁仁利, 黄进肃, 李 榕, 李 钟, 徐慧莉, 等. 人肺腺癌干细胞分子表型及PLAGL2基因的生物学功能研究. 中国细胞生物学学报(Xue Mingming, Ning Renli, Huang Jinsu, Li Rong, Li Zhong, Xu Huili, *et al*. A study on the molecular phenotype of human lung adenocarcinoma stem cells and biological functions of *PLAGL2* gene. Chinese Journal of Cell Biology) 2012; 34(4): 366-75.

18 董强刚, 姚 明, 耿 沁, 周 瑾, 闫明霞. 人体肺腺癌干细胞的分离及鉴定. 肿瘤(Dong Qianggang, Yao Ming, Geng Qin, Zhou Jin, Yan Mingxia. Isolation and identification of human lung adenocarcinoma stem cell. Tumor) 2008; 28(1):1-7.

19 耿 沁, 董强刚, 姚 明, 郑必强, 胡晶莹, 周 瑾, 等. 肺癌干细胞的球体形成与致瘤性分析. 肿瘤(Geng Qin, Dong Qianggang, Yao Ming, Zheng Biqiang, Hu Jingying, Zhou Jin, *et al*. The sphere formation and tumorigenesis of lung cancer stem cells. Tumor) 2008; 28(9): 751-4.

20 郑必强, 周 瑾, 耿 沁, 董强刚. 人肺腺癌干细胞源自肺脏细支气管肺泡干细胞的初步研究. 中国肺癌杂志(Zheng Biqiang, Zhou Jin, Geng Qin, Dong Qianggang. A preliminary study on the origin of human lung adenocarcinoma stem cells from lung bonchioalveolar stem cells. Chinese Journal of Lung Cancer) 2008; 11(6): 759-64.

21 黄进肃, 亓雪莲, 张雪颜, 李 焱, 韩宝惠, 耿 沁, 等. 人肺腺癌干细胞对顺铂和卡铂的药物敏感性分析. 肿瘤(Huang Jinsu, Qi Xuelian, Zhang Xueyan, Li Yan, Han Baohui, Geng Qin, *et al*. The drug sensitivity of human lung adenocarcinoma stem cells to cisplatin and carboplatin. Tumor) 2010; 30(2): 95-9.

22 Yoo SH, Jung KC, Kim JH, Sung SW, Chung JH, Shim YS, *et al*. Expression patterns of markers for type II pneumocytes in pulmonary sclerosing hemangiomas and fetal lung tissues. Arch Pathol Lab Med 2005; 129: 915-9.

23 李 焱, 薛明明, 亓雪莲, 耿 沁, 徐慧莉, 董强刚. 靶向TTF-1转录因子诱导人肺腺癌干细胞表型分化. 中国细胞生物学学报(Li Yan, Xue Mingming, Qi Xuelian, Geng Qin, Xu Huili, Dong Qianggang. Targeting the thyroid transcription factor-1 induces phenotypic differentiation of human lung adenocarcinoma stem cells. Chinese Journal of Cell Biology) 2011; 33(2): 119-27.

24 Stefan K, Milota K, Liao SY, Michael L, Eric JS. Transcriptional control of the tumor- and hypoxia-marker carbonic anhydrase 9: a one transcription factor (HIF-1) show? Biochim Biophys Acta 2009; 1795: 162-72.

25 Fox SB, Bragança J, Turley H, Campo L, Han C, Gatter KC, *et al*. CITED4 inhibits hypoxia-activated transcription in cancer cells, and its cytoplasmic location in breast cancer is associated with elevated expression of tumor cell hypoxia-inducible factor a. Cancer Res 2004; 64: 6075-81.

26 Yoon H, Lim JH, Cho CH, Huang LE, Park JW. CITED2 controls the hypoxic signaling by snatching p300 from the two distinct activation domains of HIF-1a. Biochim Biophys Acta 2011; 1813: 2008-16.

27 Covello KL, Kehler J, Yu H, Gordan JD, Arsham AM, Hu CJ, *et al*. HIF-2a regulates Oct-4: effects of hypoxia on stem cell function, embryonic development, and tumor growth. Genes Dev 2006; 20: 557-70.

28 Mathieu J, Zhang Z, Zhou W, Wang AJ, Heddleston JM, Pinna CM, *et al*. HIF induces human embryonic stem cell markers in cancer cells. Cancer Res 2011; 71: 4640-52.

29 Kong D, Park EJ, Stephen AG, Calvani M, Cardellina JH, Monks A, *et al*. Echinomycin, a small-molecule inhibitor of hypoxia-inducible factor-1 DNA-binding activity. Cancer Res 2005; 65(19): 9047-55.

30 Ho MM, Ng AV, Lam S, Hung JY. Side population in human lung cancer cell lines and tumors is enriched with stem-like cancer cells. Cancer Res 2007; 67: 4827-33.

31 Eramo A, Lotti F, Sette G, Pilozzi E, Biffoni M, Di Virgilio A, *et al*. Identification and expansion of the tumorigenic lung cancer stem cell population. Cell Death Differ 2007; 15(3): 501-14.

32 Jiang F, Qiu Q, Khanna A, Todd NW, Deepak J, Xing L, *et al*. Aldehyde dehydrogenase 1 is a tumor stem cell-related marker in lung cancer. Mol Cancer Res 2009; 7(3): 330-8.

33 Leung EL, Fiscus RR, Tung JW, Tin VP, Cheng LC, Sihoe AD, *et al*. Non-small cell lung cancer cells expressing CD44 are enriched for stem cell-like properties. PLoS One 2010; 5(11): e14062.

34 Chiou SH, Wang ML, Chou YT, Chen CJ, Hong CF, Hsieh WJ, *et al*. Coexpression of Oct4 and Nanog enhances malignancy in lung adenocarcinoma by inducing cancer stem cell-like properties and epithelial-mesenchymal transdifferentiation. Cancer Res 2010; 70(24): 10433-44.

35 Chen YC, Hsu HS, Chen YW, Tsai TH, How CK, Wang CY, *et al*. Oct-4 expression maintained cancer stem-like properties in lung cancer-derived CD133-positive cells. PLoS One 2008; 3(7): e2637.

36 Lu X, Kang Y. Hypoxia and hypoxia-inducible factors: master regulators of metastasis. Clin Cancer Res 2010; 16(24): 5928-35.

37 Keith B, Simon MC. Hypoxia-inducible factors, stem cells, and cancer. Cell 2007; 129(3): 465-72.