**SIRT2 suppresses non-small cell lung cancer growth by targeting** **JMJD2A**

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**Supplementary materials and methods**

**Quantitative RT-PCR (qRT-PCR)**

Total RNA from tissues and cells was extracted using TRIzol reagent (Invitrogen) and reverse transcribed into cDNA using SuperScriptIII Reverse Transcriptase Kit (Invitrogen), following the manufacturer’s instructions. Quantitative RT-PCR (qRT-PCR) for mRNA level was performed using the SYBR® Premix Dimmer Eraser Kit (TaKaRa) on the ABI7500 system (Applied Biosystems, CA). GAPDH served as an internal standard. The relative fold change of mRNAs was calculated using 2−ΔΔCt method. Primer sequences were referenced from the previous research (Li et al., 2013; Mallette and Richard, 2012).

**Western blotting**

Total protein from NSCLC tissues and cells was extracted and the protein concentration was measured by the Bio-Rad protein assay. Western blot was performed as previously described (Das et al., 2014). The following antibodies were used: JMJD2A (Santa Cruz), SIRT2 (Santa Cruz), β-actin (Santa Cruz) and GAPDH (Santa Cruz). All of the western blot data have been repeated three times independently and only representative images are shown in the Figures.

**Cell** **infection and transient transfection**

For over-expression, A549 cell lines were infected with adenovirus expressing GFP (Ad-NC) or SIRT2 (Ad-SIRT2) as previously described (Li et al., 2013). The human SIRT2 cDNA was inserted into D-TOPO vector (Invitrogen). The D-TOPO- SIRT2 plasmid was cloned into the pAd/CMV/V5-DEST vector (Invitrogen) using LR Clonase (Invitrogen). The plasmid was linearized with PacI and was transfected into A549 cells for production of adenovirus.

For SIRT2 knockdown, retrovirus infection Sh-RNA was produced as Jing et al. described (Han et al., 2013). Briefly, STRT2 shRNAs were designed using the Dhamarcon website. Stable retroviral transduction of A549 cells was achieved by infection for 12-16 h. Selection was initiated with either Puromycin or Zeocin and stopped as soon as the noninfected control cell died off, and the media were replaced with normal-growing media.

**References**

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**Supplementary Tables**

**Supplementary Table 1** The culture condition of the cell lines.

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| **Cell lines** | **Culture condition** |
| HBE | RPMI 1640+10% FBS+1% PS |
| MRC-5 | MEM+10% FBS+1% PS |
| A549 | F12K+10% FBS+1% PS |
| H460 | RPMI 1640+10% FBS+1% PS |
| NCI-H1299 | RPMI 1640+10% FBS+1% PS |
| NCI-H520 | RPMI 1640+10% FBS+1% PS |

**Supplementary Table 2** Primers used for ChIP qPCR

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| --- | --- | --- |
| **Region** | **Forward primer (5’→3’)** | **Reverse primer (5’→3’)** |
| R1(-1,606~-1,450) | TCTACCGCAGGATTTGGGT | GCAGCCACAAGTCTTTCACAAT |
| R2(-790~-600) | CGATTCTCCTGCCTCAAC | GTCCCTCACGCCTGTAAT |
| R3(-232~-35) | TCAACCCTGATTAGTTATGGG | TACACCAGCCAATGAGCA |
| R4(+116~+311) | TTTGGGCTGTAGGTGAGAA | CTTGAAGATTGGAAAGGACC |
| R5(+783~+804) | GGAAGGTGTCATGAAACGGG | TTCCTCACCCTCACCTCAAT |
| R6(+1,486~+1,644) | CTGAACCGCCATACCTTT | CGCAATAGACCACCTCAAC |