

Supplemental Data

Secreted Frizzled Related Protein 1 Modulates Taxane Resistance of Human Lung Adenocarcinoma

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Supplementary Table S1. Primer sequences used for Real-time PCR and shRNA sequence for SFRP1.

mRNA	Primers	Expected size (bp)
<i>SFRP1</i>	Forward: 5'- AGAAGATGGTGCTGCCCAACCTG -3' Reverse: 5'- AGTTCTTGTGAGCAGGGGCACC -3'	93
<i>SFRP4</i>	Forward: 5'- CTCCCGGAGGATGTTAAGTG -3' Reverse: 5'- GGGCTTAGGCGTTACAGTC -3'	89
<i>SFRP1(U)</i>	Forward: 5'- GTAGAGGAAGATTGGGTGTCGGT -3' Reverse: 5'- GCGAACCTACGACTATACCACAACA -3'	150
<i>SFRP1(M)</i>	Forward: 5'- GTAGAGGAAGATTGGGTGTCGGC -3' Reverse: 5'- GCGAACCTACGACTATACCACAACG -3'	150
<i>β-catenin</i>	Forward: 5'- AAGACATCACTGAGCCTGCCAT -3' Reverse: 5'- CGATTGCGGGACAAAGGGCAA -3'	98
<i>c-myc</i>	Forward: 5'- ACCACCAGCAGCGACTCTGA -3' Reverse: 5'- TCCAGCAGAAGGTGATCCAGACT -3'	127
<i>cyclin-D1</i>	Forward: 5'- AATGACCCCGCACGATT -3' Reverse: 5'- GCACAGAGGGCAACGAAGG -3'	108
<i>E-cadherin</i>	Forward: 5'- CATTCCCAACTCCTCTCCTGGC -3' Reverse: 5'- ATGGGCCTTTTTCATTTCTGGG -3'	90
<i>Vimentin</i>	Forward: 5'- AGTTCAAGAACACCCGCACCAAC -3' Reverse: 5'- CAGGAAGCGCACCTTGTCGATGT -3'	89
<i>N-cadherin</i>	Forward: 5'- CTTCAGGCGTCTGTAGAGGCTTC -3' Reverse: 5'- TGCACATCCTTCGATAAAGACTGC -3'	95
<i>GAPDH</i>	Forward: 5'- GCACCGTCAAGGCTGAGAAC -3' Reverse: 5'- TGGTGAAGACGCCAGTGGG -3'	128
shRNA	Forward: 5'- TTTGCCGGAGAGTTATCCTGATAAACTC GAGTTATCAGGATAACTCTCCGGTTTTTG -3' Reverse: 5'- GATCCAAAACCGGAGAGTTATCCTGAT AAACTCGAGTTATCAGGATAACTCTCCGG -3'	—
shcontrol	Forward: 5'- TTTGGCATTGCTTCTGTGTAATAACTCG AGTAATTACACAGAAGCAATGCTTTTTG -3' Reverse: 5'- GATCCAAAAGCATTGCTTCTGTGTAAT TACTCGAGTAATTACACAGAAGCAATGC -3'	—

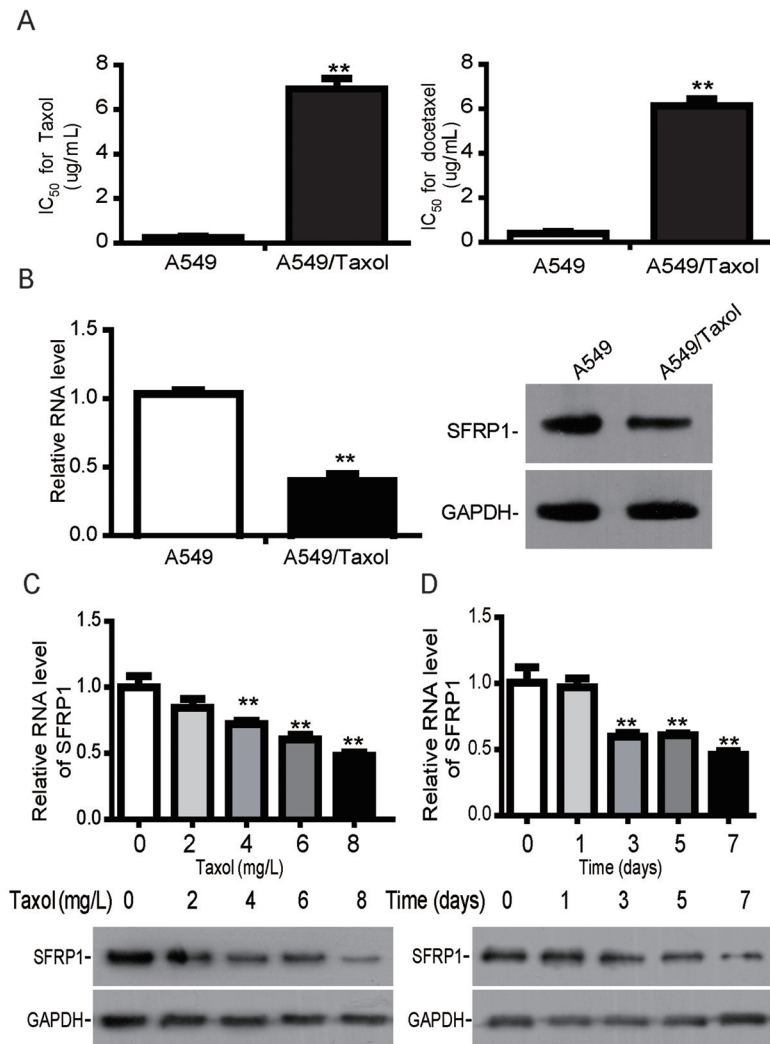
SFRP1 MODULATES TAXANES RESISTANCE

Supplementary Table S2. Differential methylation status in the CpG islands of genes in docetaxel-resistant SPC-A1/DTX cell line compared with parental SPC-A1 cell line.

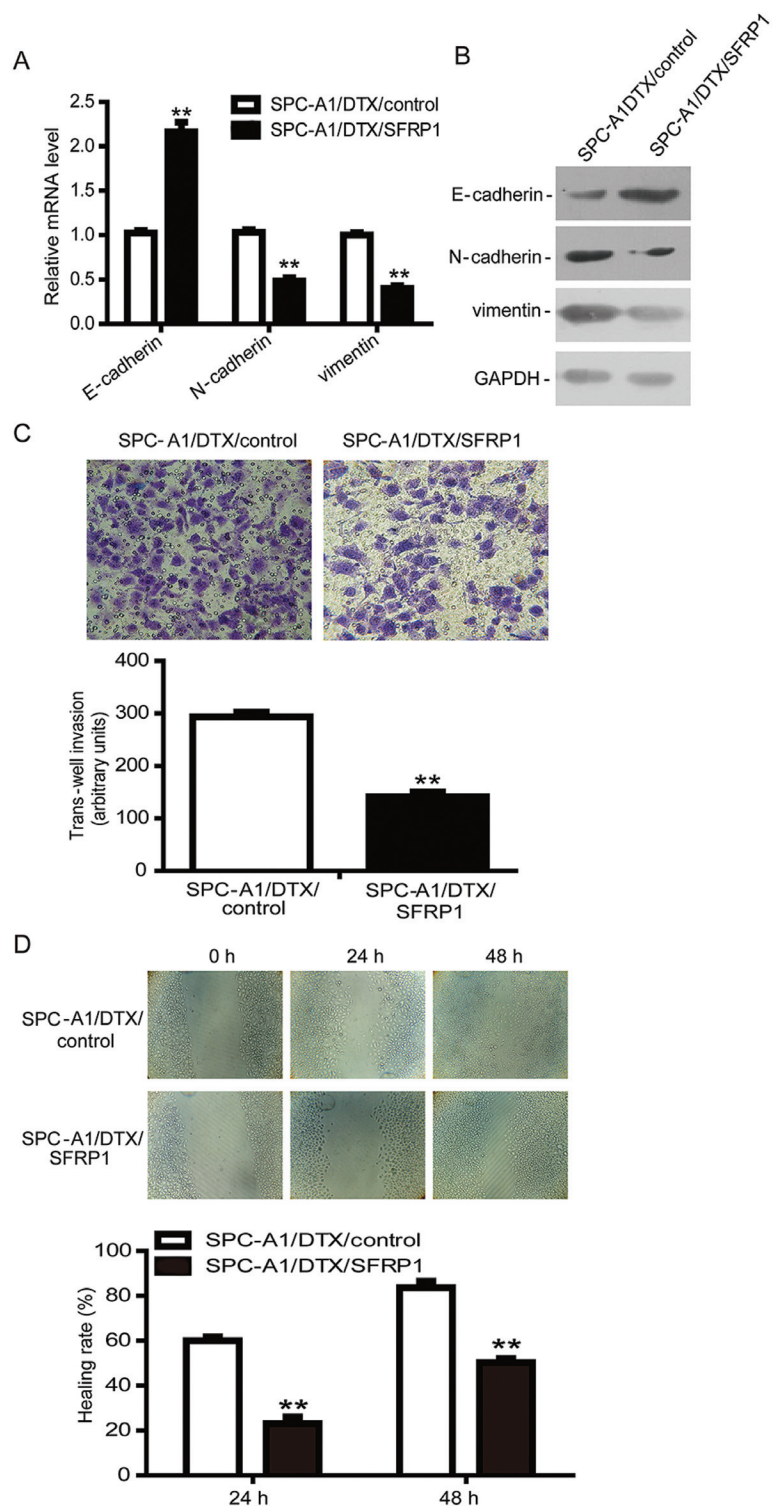
Hypermethylated Clone (rate)	BLAST Homology <i>Homo sapiens</i>	Gene name (Symbol)	CpG island
1 (95.2%)	NM_002015	Forkhead box O1A (<i>FOXO1A</i>)	+
2 (91.5%)	NM_033615	ADAM metallopeptidase domain 33 (<i>ADAM33</i>)	+
3 (89.7%)	NM_004364	CCAAT/enhancer binding protein, alpha (<i>CEBPA</i>)	+
4 (88.2%)	NM_003012	Secreted frizzled related protein 1 (<i>SFRP1</i>)	+
5 (87.3?)	AF252549	Filamin C, gamma (<i>FLNC</i>)	+
6 (86.2%)	NM_004938	Death-associated protein kinase 1 (<i>DAPK1</i>)	+
8 (84.2%)	BC005022	Sorting nexin 9 (<i>SNX9</i>)	+
9 (83.1%)	BC010444	Matrilin 2 (<i>MATN2</i>)	+
10 (81.9%)	BC104883	Nucleosome assembly protein 1-like 5 (<i>NAP1L5</i>)	+
11 (81.6%)	U01184	Signal-transducing adaptor protein-2 (<i>STAP2</i>)	+
13 (80.7%)	AF023476	ADAM metallopeptidase domain 12 (<i>ADAM12</i>)	+
14 (80.6%)	AF319441	Homeobox D11 (<i>HOXD11</i>)	+
15 (80.4%)	NM_001992	Coagulation factor II receptor (<i>F2R</i>)	+
16(80.3%)	NM_018009	TAP binding protein-like (<i>TAPBPL</i>)	+
17 (80.2%)	AF158733	Matrix metalloproteinase 14 (<i>MMP14</i>)	+
18 (80.1%)	AL133414	Runt-related transcription factor 3 (<i>RUNX3</i>)	+

Supplementary Table S3. Differentially expressed genes in docetaxel-resistant SPC-A1/DTX cell line compared with parental SPC-A1 cell line.

GeneBank No.	Gene name (Symbol)	Chromosome	Fold change
NM_152578	Fragile X mental retardation 1 neighbor (<i>FMR1NB</i>)	Xq27	31.52
NM_004938	Death-associated protein kinase 1 (<i>DAPK1</i>)	9q34	29.45
NM_012253	Transketolase-like 1 (<i>TKTL1</i>)	Xq28	28.18
NM_144594	Hypothetical protein FLJ32942 (<i>FLJ32942</i>)	12q13	27.98
AF023476	ADAM metallopeptidase domain 12 (<i>ADAM12</i>)	10q26	25.26
NM_003012	Secreted frizzled related protein 1 (<i>SFRP1</i>)	2q31	24.15
NM_005367	Melanoma antigen family A, 12 (<i>MAGEA12</i>)	Xq28	22.42
AF158733	Matrix metalloproteinase 14 (<i>MMP14</i>)	14q11	20.65
NM_152578	Fragile X mental retardation 1 neighbor (<i>FMR1NB</i>)	Xq27	19.10
NM_006228	Prepronociceptin (<i>PNO</i>)	8p21	18.38
NM_005602	Claudin 11 (<i>CLDN11</i>)	3q26	17.12
NM_004988	Melanoma antigen family A, 1 (<i>MAGEA1</i>)	Xq28	13.66
NM_001002915	Insulin growth factor-like family member 2 (<i>IGFL2</i>)	19q13	12.52
NM_013238	DnaJ homolog, subfamily C-15 (<i>DNAJC15</i>)	13q14	10.33
NM_003480	Microfibrillar associated protein 5 (<i>MFAP5</i>)	12p13	8.28
NM_005213	Cystatin A (<i>CSTA</i>)	3q21	7.10
NM_003289	Tropomyosin 2 (beta) (<i>TPM2</i>)	9p13	5.82
NM_032048	Elastin microfibril interfacer 2 (<i>EMILIN2</i>)	18p11	5.07
NM_001323	Cystatin E/M (<i>CST6</i>)	11q13	5.04
NM_173571	Hypothetical protein LOC255313 (<i>LOC255313</i>)	Xq24	5.02



Supplementary Figure S1. The expression of SFRP1 was decreased in a dose and time dependent manner after exposure to Taxol in A549 cell line. (A) The IC₅₀ values of docetaxel or Taxol for A549 and A549/Taxol cell lines were determined using MTT assay. **: $P < 0.01$. (B) The mRNA and protein levels of SFRP1 in A549 and A549/Taxol cell lines were determined by Real-time PCR and western blotting. **: $P < 0.01$. (C) A549 cells were cultured in the presence of various concentrations of Taxol (0, 2, 4, 6 and 8 $\mu\text{g/L}$) for 24 h. Real-time PCR and western blotting were performed to detect the mRNA and protein levels of SFRP1. **: $P < 0.01$. (D) A549 cells were cultured in the presence of Taxol (4 $\mu\text{g/L}$) for various time points (0, 1, 3, 5 and 7 d). Real-time PCR and western blotting were performed to detect the mRNA and protein levels of SFRP1. **: $P < 0.01$.



Supplementary Figure S2. Ectopic of SFRP1 suppressed EMT and the invasive ability of SPC-A1/DTX cell line. The levels of epithelial and mesenchymal markers were detected by Real-time PCR (A) and western blotting (B) in SPC-A1/DTX/control and SPC-A1/DTX/SFRP1 cell line. **: $P < 0.01$. (C) The invasive ability of SPC-A1/DTX/SFRP1 cells was determined by transwell assay. **: $P < 0.01$. (D) The migratory potential of SPC-A1/DTX/SFRP1 cells was detected by wound healing assay. **: $P < 0.01$.