

**Long noncoding RNA DICER1-AS1 functions in methylation regulation on the multi-drugresistance of osteosarcoma cells via miR-34a-5p and GADD45A**

### Supplementary Fig. 1

The sequence of primers and probes of DICER1-AS1:

hDICER1-AS1 F: 5'-TGGGATTACGGGCGTGAG-3'

hDICER1-AS1 R: 5'-CCTGGGCACTCCTTCAGC-3'

hDICER1-AS1 P: 5'-CY5-CACCGTACCCAGCCTGCTTCCTG-3'

The sequence of primers and probes of GADD45A:

hGADD45A F: 5'-CCCCGATAACGTGGTGTGTGT-3'

hGADD45A R: 5'-GATGTCTGTTCTCGCAGCAAA-3'

hGADD45A P: 5'-FAM-CTCTGCAGATCCACTTCACCCTGATCCA-3'

The sequence of si-DICER1-AS1:

si-DICER1-AS1\_001 GGAATAACTTCCAACAAGT

si-DICER1-AS1\_002 GACGATTGTCTAAGGATGA

si-DICER1-AS1\_003 CACATTTCTTACTCCATA

The sequence of si-GADD45A:

si-GADD45A-1

GCGAGAACGACAUCAACAUTT

AUGUUGAUGUCGUUCUCGCTT

si-GADD45A-2

CCUGCCUUAAGUCAACUUATT

UAAGUUGACUUAAGGCAGGTT

si-GADD45A-3

CCGAAAGGGUUAUCAUAUTT

AUAUGAUUAACCCUUCGCGTT

The sequence of sh-DICER1-AS1:

DICER1-AS1-shRNA(GGAATAACTTCCAACAAGT)

TATGTTTTAAATGGACTATCATATGCTTACCGTAACTTGAAAGTATTTTCGAT

TTCTTGGGTTTATATATCTTGTGGAAAGGACGCGGGATCCCGGAATAACTTC

CAACAAGTTTGATATCCGACTTGTTGGAAGTTATTCCTTTTTTCCAAAAGCT

TAAGTTTAAACCGCTGATCAGCCTCGACTGTGCCTCTAAATAGTATCATTGG

GC

The sequence of hsa-miR-34a-5p inhibitor:

ACAACCAGCTAAGACACTGCCACGATACAACCAGCTAAGACACTGCCATC

ACACAACCAGCTAAGACACTGCCA

The sequence of hsa-miR-34a-5p-O/E:

TGGCAGTGTCTTAGCTGGTTGT

The sequence of hsa-miR-34a-5p NC-O/E:

TTCTCCGAACGTGTCACGT

The sequence of DICER1-AS1-O/E:

ATGTTGGCCATGATGGTCTCGATTTCTGACCTTGTCATCCGTCCGCCTTGG  
CCTCCCAAAGTGCTGGGATTACGGGCGTGAGCCACCGTACCCAGCCTGCT  
TCCTGTTTTAACAGACGAAGAAATGGAATAACTTCCAACAAGTTGGAGCT  
GAAGGAGTGCCCAGGCTGCGGATGGAGGAAGGACGTGCTCGGGAGAAGG  
TGAAGATGGGAGAGAAGCAAGATCTTCTGTGTTTGGACCAAGGACACATT  
TCCTTACTCCATAGCAGCTGTGGTCAGTGGCTTAGCTCGGACAAGGAGAT  
GAGAGCCCATGTGTTGTGAGGGTTCTTCTGGGAACTCTGAGAAGGCAGGA  
GCCGCCCCCGCCCTTCACTGCCTCTCTTCCATTCTGCAGCATGGAATATGG  
ATGTGTGGTCTGGATGTACAGACACCATTTTGGACCATAAAGACAAGGAC  
GATTGTCTAAGGATGACACAGCCAGAGCACTGAGAGTGGCGAGAAGGAC  
AGGGGAGCTGCTGTGCCAAGCAGGACTGTCTCTCTTAAGAGAAAAATAA

The sequence of DICER1-AS1-BSP primers:

DICER1-AS1-F 5' TATTTGGGTTTGTAGTAGT 3'

DICER1-AS1-R 5' AAACCAATAAAATTCTCCA 3'

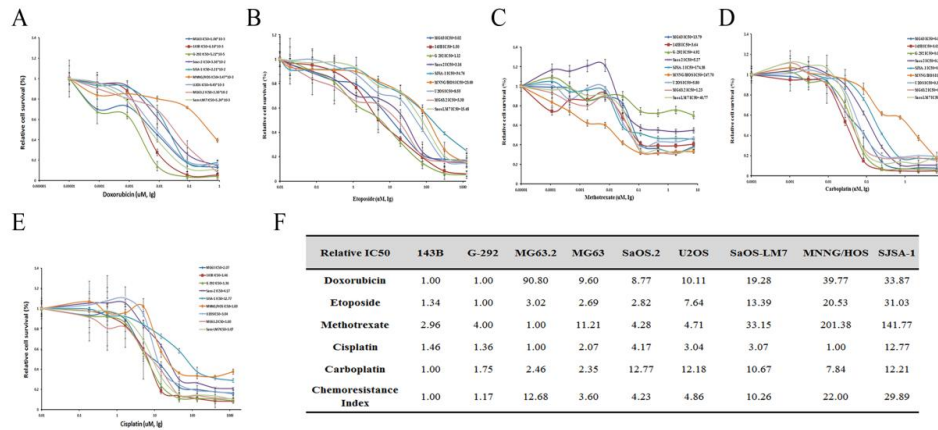
The sequence of DICER1-AS1-wt:

CTCGAGCGGATGGAGGAAGGACGTGCTCGGGAGAAGGTGAAGATGGGAG  
AGAAGCAAGATCTTCTGTGTTTGGACCAAGGACACATTTCTTACTCCATA  
GCAGCTGTGGTCAGTGGCTTAGCTCGGACAAGGAGATGAGAGCCCATGTG  
TTGTGAGGGTTCTTCTGGGAACTCTGAGAAGGCAGGAGCCGCCCCCGCCC  
TTCACTGCCCTCTCTTCCATTCTGCAGCATGGAATATGGATGTGTGGTCTGGA  
TGTACAGACACCATTTTGGACCATAAAGACAAGGACGATTGTCTAAGGATG  
ACACAGCCAGAGCACTGAGAGTGGCGAGAAGGACAGGGGAGCTGCTGTG  
CCAAGCAGGACTGTCTCTCTTAAGAGGCGGCCGC

The sequence of DICER1-AS1-mut:

CTCGAGCGGATGGAGGAAGGACGTGCTCGGGAGAAGGTGAAGATGGGAG  
AGAAGCAAGATCTTCTGTGTTTGGACCAAGGACACATTTCTTACTCCATA  
GCAGCTGTGGTCAGTGGCTTAGCTCGGACAAGGAGATGAGAGCCCATGTG  
TTGTGAGGGTTCTTCTGGGAACTCTGAGAAGGCAGGAGCCGCCCCCGCCC  
TTGAGTTCACTCTTCCATTCTGCAGCATGGAATATGGATGTGTGGTCTGGA  
TGTACAGACACCATTTTGGACCATAAAGACAAGGACGATTGTCTAAGGATG  
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CCAAGCAGGACTGTCTCTCTTAAGAGGCGGCCGC

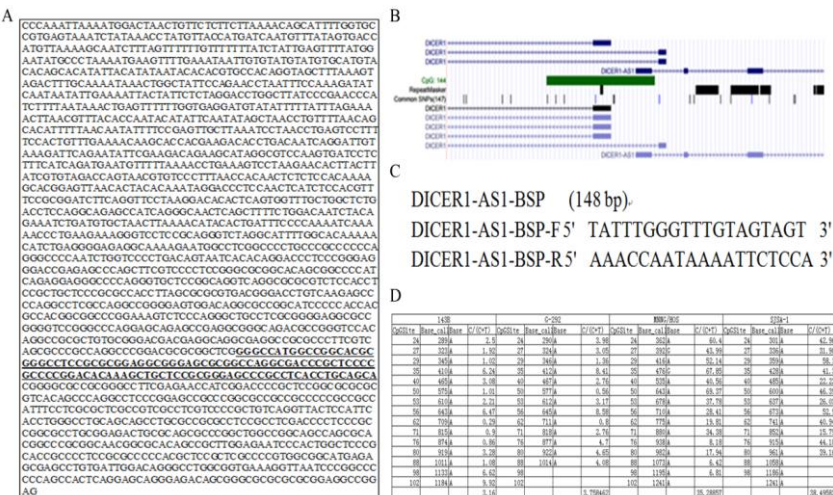
Supplementary Fig. 2



Drug resistance profiling of nine osteosarcoma cell lines.

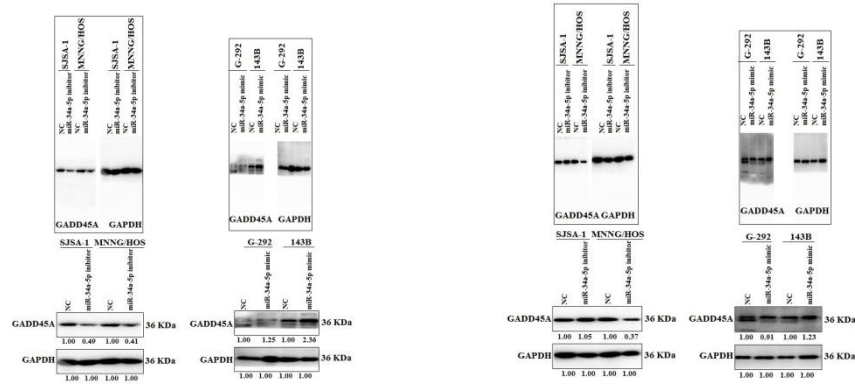
(A-E).IC<sub>50</sub> values of the five indicated chemotherapeutics for nine osteosarcoma cell lines. The cell survival rates were calculated as percentages relative to the mock treatment and plotted against lg μM of drug. (F).The IC<sub>50</sub> (-fold) values relative to those of the most sensitive cell line (143B) are presented in the table. Judged by the fold difference over the lowest IC<sub>50</sub>, in this manuscript, G-292 and 143B regard as the multi-drugsensitive cell lines, while SJSA-1 and MNNG/HOS as the resistant cell line.

Supplementary Fig. 3

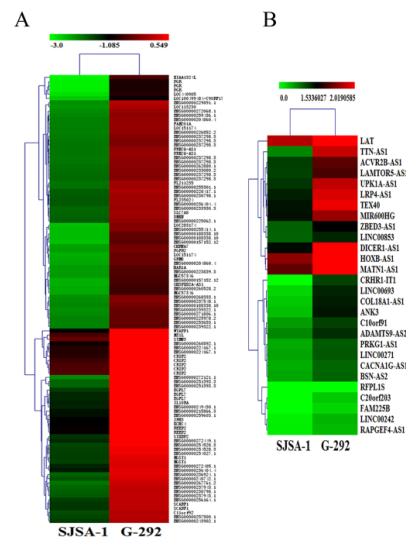


The promoter region, CG sites, BSP primers and the results of sequence of each site in four OS cell lines. (A).The 2000bp promoter region of DICER1-AS1, the PCR amplification sequence riched in CG island was marked with bold and underline.





**Supplementary Fig. 5**



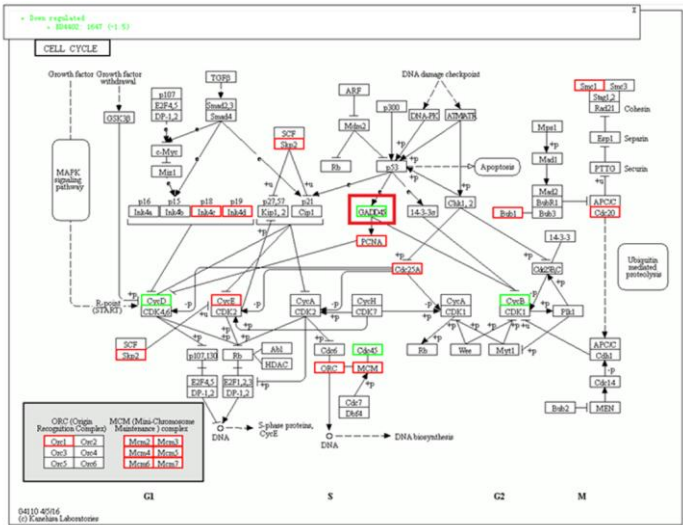
(A). Hierarchical clustering analysis of RNA-seq lncRNAs that were differentially expressed between G-292 and SJSA-1 cells, the coordinates on the right represent the expression of the lg(fpkm+1). (B). Hierarchical clustering analysis of RNA-seq lncRNAs that negatively correlates with miR-34a-5p, the coordinates on the right represent the expression of the lg(fpkm+1).

Supplementary Fig. 6

| Gene ID         | Name        | Position                  | Strand | SJSA-1 RPKM | G-292 RPKM | G-292/SJSA-1 |
|-----------------|-------------|---------------------------|--------|-------------|------------|--------------|
| ENST00000582491 | CRER1-IT1   | chr17:45638975-45646239   | +      | 0.008       | 0.135      | 17.290       |
| ENST00000582847 | TTN-AS1     | chr21:17852827-178620217  | +      | 0.052       | 0.824      | 15.787       |
| ENST00000554453 | LAT         | chr16:28994036-28999783   | +      | 0.763       | 9.807      | 12.858       |
| ENST00000425118 | LINC00693   | chr9:28575278-28758337    | +      | 0.018       | 0.202      | 11.327       |
| ENST00000397787 | COL1A1-AS1  | chr21:45419716-45425970   | -      | 0.021       | 0.227      | 10.835       |
| ENST00000414786 | ANK3        | chr10:60026298-60735526   | -      | 0.024       | 0.250      | 10.486       |
| ENST00000402383 | TERC        | chr3:169764810-169785060  | -      | 164.017     | 1033.610   | 6.302        |
| ENST00000439819 | DICER1-AS1  | chr14:95157888-95179933   | +      | 0.289       | 1.479      | 5.115        |
| ENST00000443196 | UPK1A-AS1   | chr19:35667948-35673291   | -      | 0.164       | 0.799      | 4.867        |
| ENST00000502049 | LRP4-AS1    | chr11:46846411-46874416   | +      | 0.217       | 0.890      | 4.110        |
| ENST00000328404 | TEX40       | chr11:64300391-64304767   | +      | 0.253       | 0.966      | 3.813        |
| ENST00000515356 | ZBED3-AS1   | chr5:77066798-77148351    | +      | 0.089       | 0.337      | 3.787        |
| ENST00000415531 | ACVR2B-AS1  | chr9:38451027-38454320    | -      | 0.144       | 0.512      | 3.559        |
| ENST00000392630 | C11orf91    | chr10:132443210-132448321 | +      | 0.039       | 0.136      | 3.458        |
| ENST00000608990 | C20orf203   | chr20:32631625-32651981   | -      | 0.006       | 0.021      | 3.458        |
| ENST00000460833 | ADAMTS9-AS2 | chr3:64684870-65011468    | +      | 0.052       | 0.180      | 3.458        |
| ENST00000598158 | LAMTOR3-AS1 | chr1:110407809-110416274  | +      | 0.136       | 0.471      | 3.458        |
| ENST00000592764 | HOOB-AS1    | chr17:4854351-48551241    | +      | 0.637       | 1.951      | 3.063        |
| ENST00000420193 | PRKG1-AS1   | chr10:5239848-52314128    | -      | 0.032       | 0.096      | 2.997        |
| ENST00000421178 | LINC00771   | chr1:135497801-135699338  | +      | 0.035       | 0.102      | 2.882        |
| ENST00000595495 | CACNA1G-AS1 | chr17:59596307-59562108   | -      | 0.050       | 0.082      | 2.690        |
| ENST00000414282 | SNHG7       | chr9:136724394-136728184  | -      | 102.005     | 258.103    | 2.530        |
| ENST00000421598 | B5N-AS2     | chr3:49549306-49543466    | -      | 0.033       | 0.084      | 2.497        |
| ENST00000449175 | MIR6080G    | chr9:123109494-123115477  | -      | 0.269       | 0.671      | 2.492        |
| ENST00000437615 | LINC00342   | chr9:169788790-169798825  | -      | 0.012       | 0.028      | 2.305        |
| ENST00000429328 | LINC00853   | chr1:47179250-47180339    | +      | 0.088       | 0.202      | 2.305        |
| ENST00000423546 | FAM223B     | chr9:113104723-113111677  | -      | 0.007       | 0.017      | 2.305        |
| ENST00000461386 | RFP1L5      | chr22:28437015-28442129   | -      | 0.003       | 0.007      | 2.305        |
| ENST00000414532 | MATN1-AS1   | chr1:30718772-30726746    | +      | 0.524       | 1.207      | 2.305        |
| ENST00000435328 | RAPGEF4-AS1 | chr2:172723189-172736206  | -      | 0.013       | 0.025      | 2.017        |

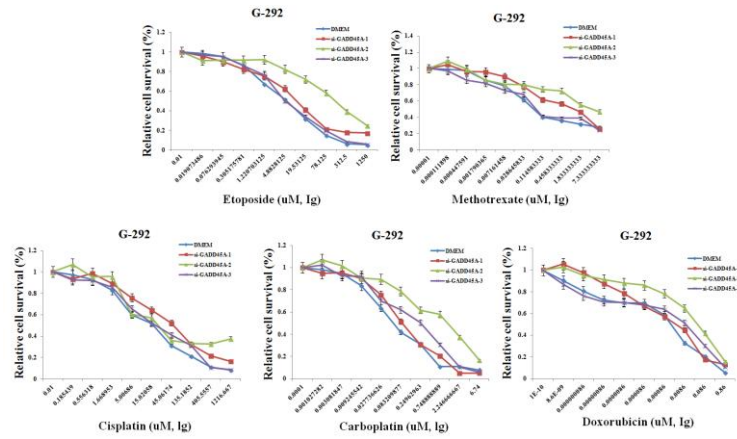
Using pathway enrichment with Kegg data base to screen the lncRNA genes (the ratio>2) related to miR-34a-5p, which is our previous focused.

Supplementary Fig. 7



The associated genes analysis of GADD45A in cells by pathway enrichment with Kegg data base.

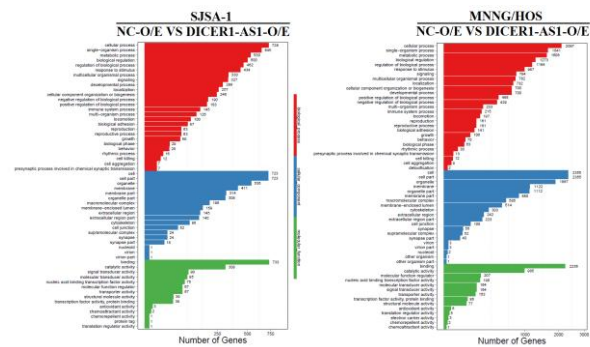
Supplementary Fig. 8



G-292 cells transfected 12hours with the three siRNA of GADD45A were seeded in triplicate in 96-well plates at a density of  $0.5 \times 10^4$  cells/well and treated with 4-fold serially diluted drugs concentration gradient for another 72 hours, then, cell viability was measured using CCK-8.

## Supplementary Fig. 9

Gene Ontology enrichment analyses with differentially expressed gene



## Supplementary Fig. 10

The snapshots of RNA-seq in GADD45A loci

