Abstract # 3221

Kevetrin induces p53-dependent and independent cell cycle arrest and apoptosis in ovarian cancer cell lines representing heterogeneous histologies

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Introduction

Ovarian cancer (OC) is a molecularly and histologically heterogeneous disease; however, standard treatment is the same for all subtypes. High-grade serous OC initially responds to chemotherapy; however, low-grade serous and clear cell OC are relatively chemo-resistant. Limited treatment options are available upon recurrence. p53 mutations are found in over 90% of high-grade serous OC. Low-grade serous OC harbor wild type p53, but contain other mutations. During later stages of OC, tumors are a heterogeneous population of mutant cells; thus development of a novel drug that addresses these molecular differences is highly desirable.

Previously, we showed that Kevetrin stabilized wild type p53 and induced transcriptional targets in human lung carcinoma. We sought to validate Kevetrin as a potential treatment for OC with varied p53 status. Endometrioid carcinoma (**A2780, wt p53**), high-grade serous (**OVCAR-3, mutant p53; OV-90, mutant p53**) and atypical non-serous clear cell (**SKOV-3, partially deleted p53**) OC cell lines and tumors were treated with Kevetrin for our studies.

Methodology

RNA Isolation and Quality Control: Human cell culture samples were submitted to Ocean Ridge Biosciences (ORB, Deerfield Beach, FL) for mRNA-Sequencing. Cell lines, A2780, OVCAR-3, OV90 and SKOV-3, were treated with 100 µM, 200 µM, or 400 µM Kevetrin (HCl salt) and compared to untreated controls. Xenograft tissue samples treated with Kevetrin were also submitted to ORB for mRNA-Sequencing. Total RNA was isolated from cell culture samples using the TRI Reagent® method. Following isolation, RNA was quantified by OD measurement, and assessed for quality on a 1% agarose / 2% formaldehyde RNA Quality Control (QC) gel. RNA was digested with RNase-free DNase I and re-purified using Agencourt RNAClean XP bead purification kit. The newly digested RNA samples were then quantified by OD measurement.

For small RNA: RNA was then digested with RNase free DNase I and filtered through Nanosep 100K Omega columns and re-purified on RNeasy MinElute columns using an alternative high-ethanol binding condition to preserve Low Molecular Weight (LMW) RNA. The newly prepared LMW RNA samples were then quantified by OD measurement.

Library Preparation (mRNA): Amplified cDNA libraries suitable for sequencing were prepared from 250-500 nanograms (ng) of DNA-free total RNA using the TruSeq Stranded Total RNA Library Prep Kit with Ribo-Zero Gold (Illumina Inc., San Diego, CA). The quality and size distribution of the amplified libraries were determined by chip-based capillary electrophoresis (LabChip GX microfluidic system, Caliper Life Sciences). Libraries were quantified using the KAPA Library Quantification Kit.

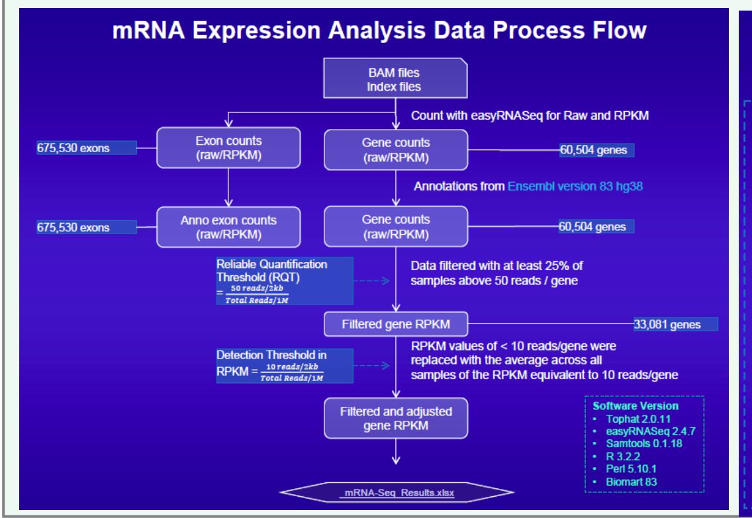
Library Preparation (small RNA): Template DNA molecules suitable for cluster generation were prepared from the isolated RNA samples using the CleanTag Small RNA Library Prep Kit according to the manufacturer's instructions. Following the CleanTag preparation, purified libraries were electrophoresed through a freshly cast 6% native acrylamide gel. Library fragments of 130-160 nt corresponding to inserts of 11-41 nt were excised from the gel and recovered by overnight agitation at 37°C at 200 RPM in elution buffer, passage of the eluate through a 0.45um filter, and ethanol precipitation. The quality and size distribution of the amplified libraries were determined by chip-based capillary electrophoresis

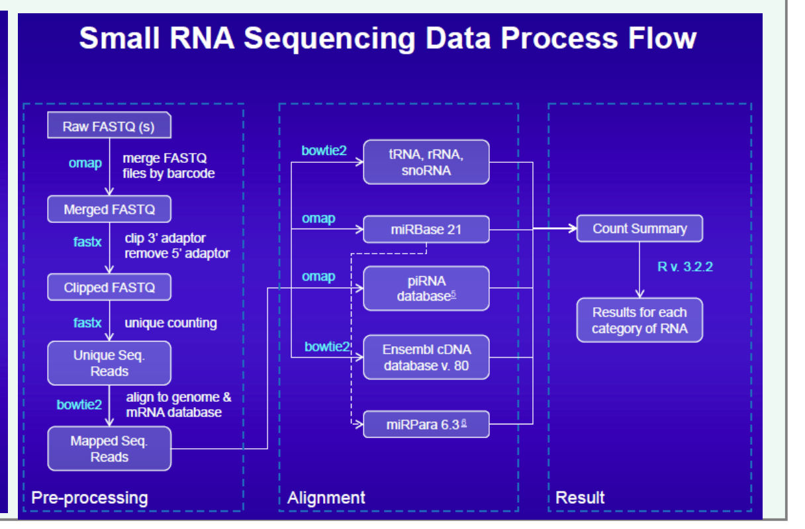
Sequencing: The libraries were pooled at equimolar concentrations and diluted prior to loading onto the flow cell of the Illumina cBot cluster station. The libraries were extended and bridge amplified to create sequence clusters using the Illumina HiSeq PE Cluster Kit v4 and sequenced on an Illumina HiSeq Flow Cell v4 with 50 nt paired-end reads plus dual index reads using the Illumina HiSeq SBS Kit v4. Real time image analysis and base calling were performed on the instrument using the HiSeq Sequencing Control Software version 2.2.58

Statistical Analysis

A two-way ANOVA was performed to compare the overall effects of the cell line, treatment and the interaction of cell line and treatment on gene expression. Replicates for each cell line were treated as random factors in the ANOVA. Tukey's Honest Significant Difference (HSD) tests were performed to compare cell lines within each treatment, and to compare treatments within each cell line. Fold changes were also calculated for the same comparisons as the Tukey tests using the mean of each group being compared. If the mean of both groups considered in the fold change comparison was below RQT, 'NA' was reported. All statistical analysis was performed using R version 3.2.2 statistical computing software.

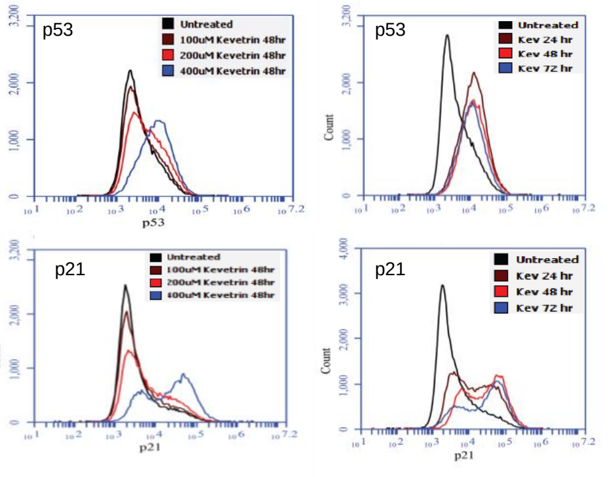
For xenograft samples, one-way ANOVA was performed to compare the effects of the number of days of Kevetrin dosing on mRNA expression. Tukey's HSD tests were used to identify significant differences among the individual treatment groups. The Tukey test generates P-values for all possible pair-wise comparisons and then adjusts the P-values upwards based on the total number of comparisons tested, which can be calculated as [n/2 * n-1], where n is the number of groups. Fold changes were also calculated for the same comparisons as the Tukey tests using the mean of each group being compared. If the mean of both groups considered in the fold change comparison was below RQT, 'NA' is reported. Linear regression on days of Kevetrin treatment was also performed. The strength of the regression for day is indicated by Regression Coef. Day, which is negative when mRNA expression decreases with time and positive when mRNA expression increases with time. The Regression Adjusted R^2 values indicates the fraction of variance explained by the model, taking into account the number of explanatory variables in the model.


mRNA Expression Analysis Data Process Flow

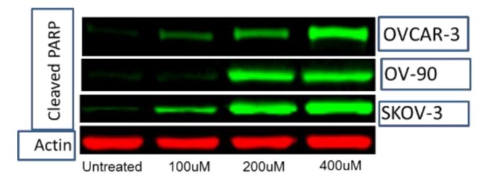

Small RNA Sequencing Data Process Flow

Key Findings

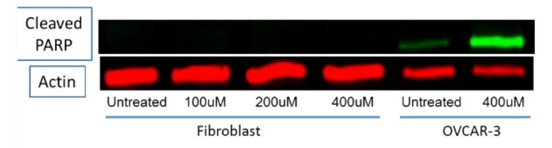
Kevetrin increased p53 level and induced p21 in A2780 cell line



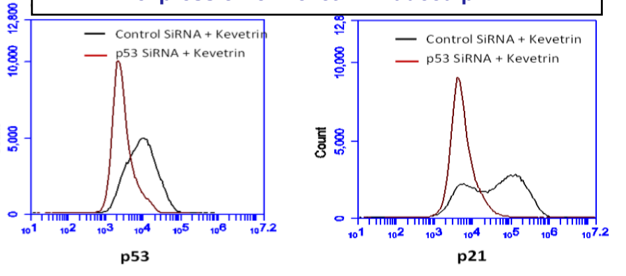
Kevetrin induced PARP cleavage in OVCAR-3, OV-90, SKOV-3 cell lines (48 hr)



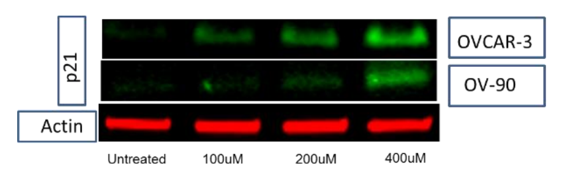
Kevetrin did *not* induce PARP cleavage in primary Fibroblast cells (48 hr)



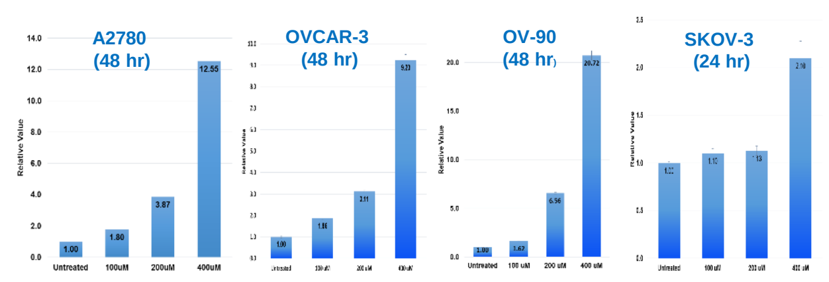
Knocking down p53 in A2780 reduced the expression of Kevetrin-induced p21



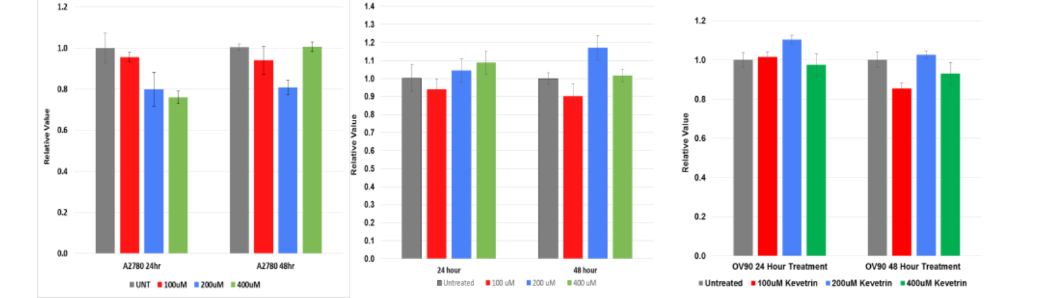
Kevetrin induced p21 in OVCAR-3 and OV-90 cell lines (48 hr)



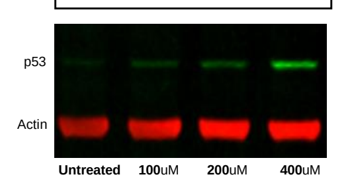
p21 mRNA expression increased with Kevetrin in ovarian cell lines



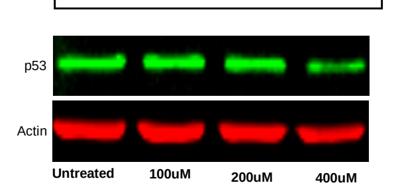
No significant change in p53 mRNA in A2780, OV-90 and OVCAR-3 cell lines in response to Kevetrin by qPCR



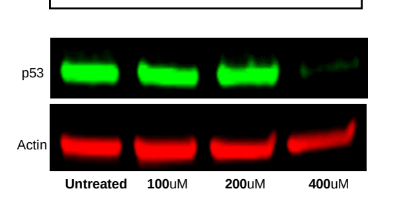
p53 protein up-regulated in A2780 cell line (48 hr)



p53 protein down-regulated in OVCAR-3 cell line (48 hr)



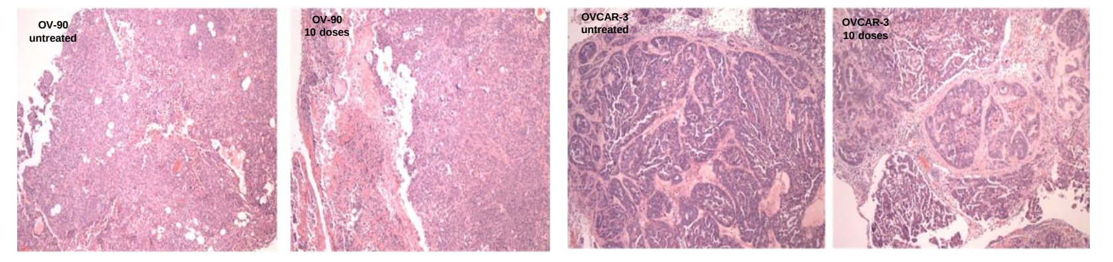
p53 protein down-regulated in OV-90 cell line (48 hr)



Kevetrin induced ~40% cell death in OV-90 or OVCAR-3 xenograft tumors by IHC

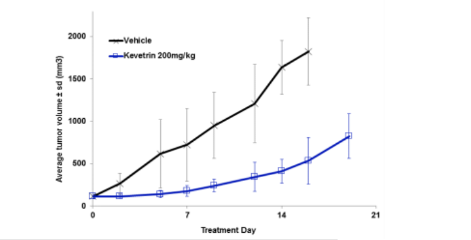
Kevetrin induced ~40% cell death after 10 doses in OV-90 tumors

Kevetrin induced ~40% cell death after 10 doses in OVCAR-3 tumors

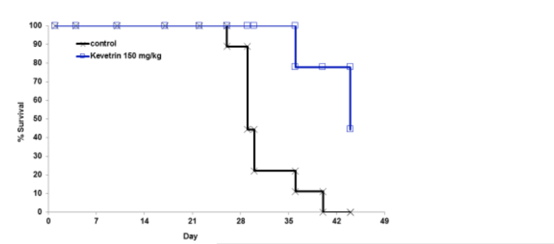


OV90 tumor (No Kevetrin) OV90 tumor (10 doses Kevetrin) OVCAR-3 (No Kevetrin) OVCAR-3 (10 doses Kevetrin)

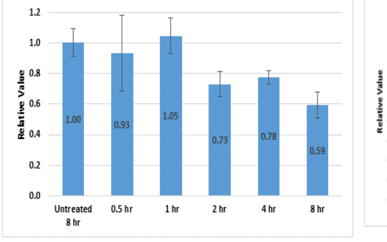
Significant reduction in tumor growth (p<0.05) with Kevetrin in A2780 xenograft tumor model



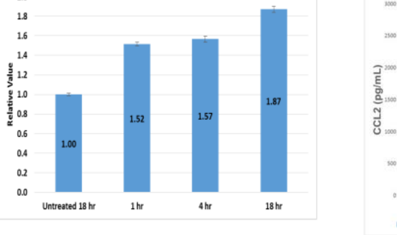
Significant increase in survival (p<0.05) with Kevetrin in SKOV-3 xenograft ascites model



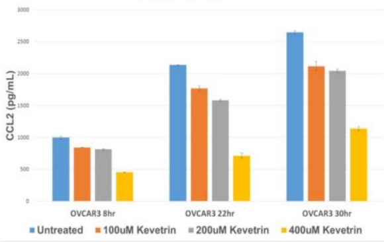
Kevetrin downregulates miRNA-27a in OVCAR-3



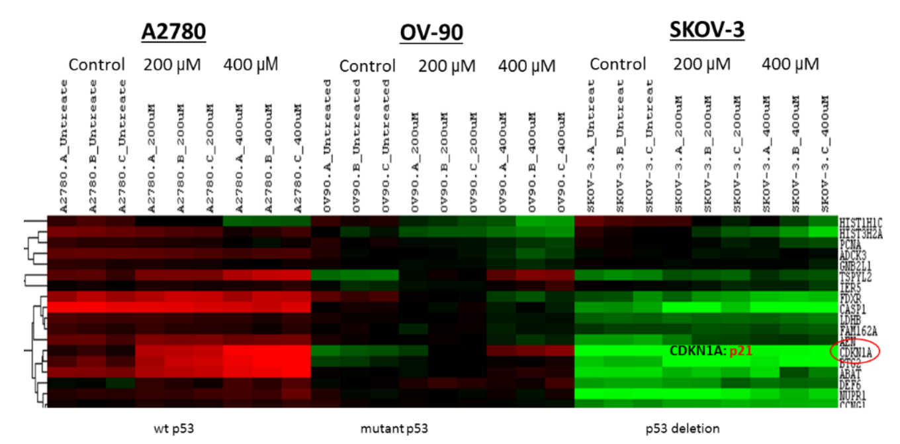
Kevetrin upregulates miRNA-1274b in OVCAR-3



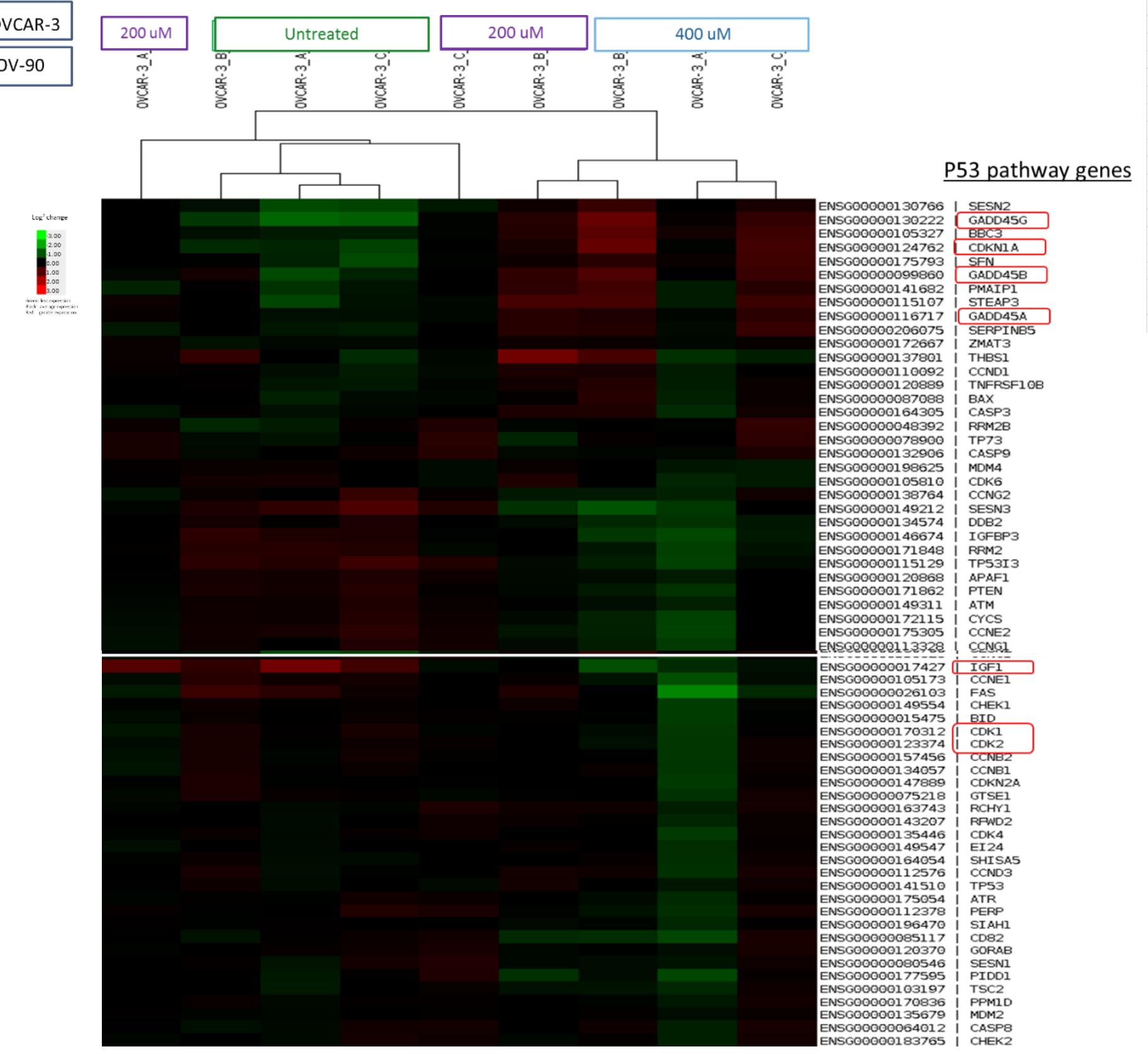
Kevetrin downregulates CCL2 (MCP-1) in OVCAR-3



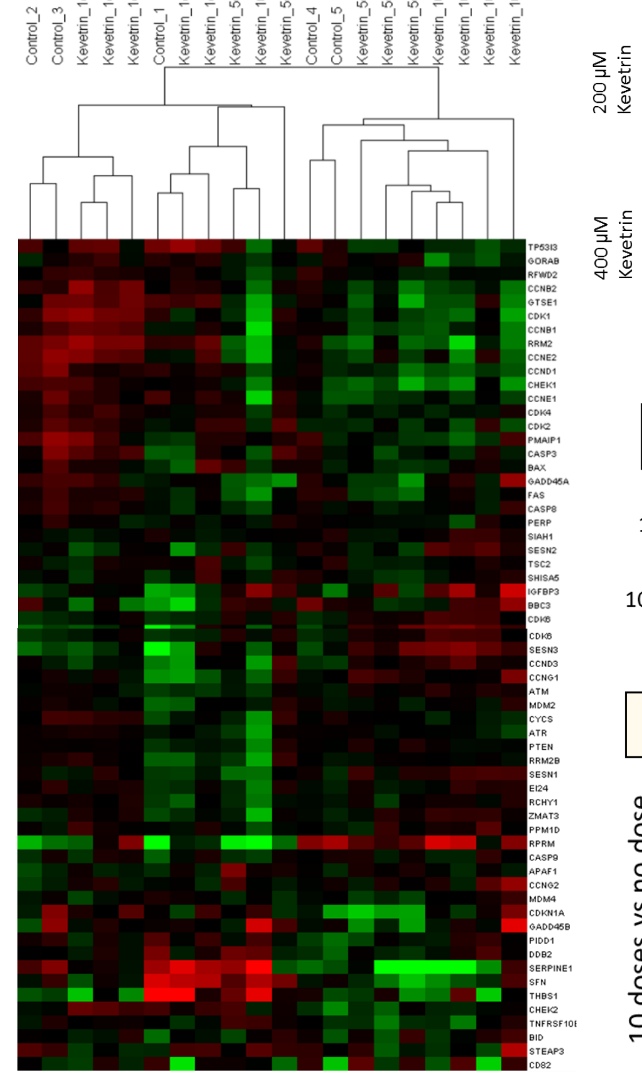
Heat map snapshot showing modulation of p53 pathway related genes differs among the different ovarian cancer cell lines in response to Kevetrin (48 hr)



OVCAR-3 (p53 mutant) KEGG p53 pathway clustering RNAseq analysis, shows strong GADD45x and CDKN1A signal in response to Kevetrin



SKOV-3 (p53 partial deletion) tumors mRNA KEGG p53 pathway clustering in response to Kevetrin



A2780 (p53 wild type) cell-line shows modulation of p53 pathway by Kevetrin (mRNAseq study; KEGG pathway analysis)

200 uM 48hr

PathwayName	#Gene	Statistics
p53 signaling pathway	6	adjP=6.38e-07

400 uM 48hr

PathwayName	#Gene	Statistics
Systemic lupus erythematosus	27	adjP=9.02e-21
DNA replication	15	adjP=4.92e-11
p53 signaling pathway	15	adjP=6.57e-07
Cell cycle	16	adjP=0.0005
ECM-receptor interaction	10	adjP=0.0021
Base excision repair	7	adjP=0.0021
Staphylococcus aureus infection	5	adjP=0.0113
Mismatch repair	5	adjP=0.0113
Glycine, serine and threonine metabolism	5	adjP=0.0123
Pyrimidine metabolism	10	adjP=0.0131
Purine metabolism	12	adjP=0.0246
Amoebiasis	8	adjP=0.0362
Small cell lung cancer	8	adjP=0.0454

Kevetrin (400 uM 48hr) modulates p53 signaling pathway in A2780 (p53 wt) cell line (mRNAseq study; KEGG pathway analysis)

Gene Symbol	Gene name	Pathway	Pathway name	Fold change
CCNE2	cyclin E2	KEGG	p53 signaling pathway	0.26
MDM2	Mdm2, p53 E3 ubiquitin protein ligase homolog (mouse)	KEGG	p53 signaling pathway	3.03
GADD45A	growth arrest and DNA-damage-inducible, alpha	KEGG	p53 signaling pathway	3.51
PIDD	p53-induced death domain protein	KEGG	p53 signaling pathway	2.55
RRM2B	ribonucleotide reductase M2 B (TP53 inducible)	KEGG	p53 signaling pathway	2.36
CD82	CD82 molecule	KEGG	p53 signaling pathway	2.45
PPM1D	protein phosphatase, Mg2+/Mn2+ dependent, 1D	KEGG	p53 signaling pathway	2.08
RRM2	ribonucleotide reductase M2	KEGG	p53 signaling pathway	0.38
APAF1	apoptotic peptidase activating factor 1	KEGG	p53 signaling pathway	2.39
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	KEGG	p53 signaling pathway	6.86
SESN1	sestrin 1	KEGG	p53 signaling pathway	3.51
TP53I3	tumor protein p53 inducible protein 3	KEGG	p53 signaling pathway	4.52
ZMAT3	zinc finger, matrin-type 3	KEGG	p53 signaling pathway	2.97
SFN	stratifin	KEGG	p53 signaling pathway	6.94
FAS	Fas (TNF receptor superfamily, member 6)	KEGG	p53 signaling pathway	4.59

Kevetrin modulates p53 signaling pathway in OVCAR-3 (p53mutant) (mRNAseq; KEGG pathway analysis)

PathwayName	#Gene	Statistics
Systemic lupus erythematosus	23	adjP=2.71e-09
Steroid biosynthesis	10	adjP=5.56e-05
p53 signaling pathway	15	adjP=0.0104
Butanoate metabolism	8	adjP=0.0104
Metabolic pathways	109	adjP=0.0104
Terpenoid backbone biosynthesis	6	adjP=0.0114
Cell adhesion molecules (CAMs)	15	adjP=0.0114
Metabolism of xenobiotics by cytochrome P450	8	adjP=0.0114
Salivary secretion	12	adjP=0.0114
Biosynthesis of unsaturated fatty acids	7	adjP=0.0114
Neuroactive ligand-receptor interaction	15	adjP=0.0123
Glycosaminoglycan degradation	6	adjP=0.0139
Hedgehog signaling pathway	10	adjP=0.0160
Calcium signaling pathway	17	adjP=0.0175
Gap junction	14	adjP=0.0175
Bile secretion	8	adjP=0.0175
Melanoma	11	adjP=0.0277
Steroid hormone biosynthesis	6	adjP=0.0277
ECM-receptor interaction	11	adjP=0.0307

Kevetrin modulates multiple genes in the p53 signaling pathway in OVCAR-3 (p53 mutant) (RNAseq; KEGG pathway analysis)

Gene symbol	Gene name	Pathway	Pathway name	P value	Fold change
CCNE2	cyclin E2	KEGG	p53 signaling pathway	0.046	0.617
DDB2	damage-specific DNA binding protein 2, 48kDa	KEGG	p53 signaling pathway	0.00223	0.631
SESN2	sestrin 2	KEGG	p53 signaling pathway	0.0278	1.907
SESN3	sestrin 3	KEGG	p53 signaling pathway	0.0407	0.462
PTEN	phosphatase and tensin homolog	KEGG	p53 signaling pathway	0.017	0.662
GADD45G	growth arrest and DNA-damage-inducible, gamma	KEGG	p53 signaling pathway	0.00589	3.183
IGF1	insulin-like growth factor 1 (somatomedin C)	KEGG	p53 signaling pathway	0.0247	0.369
RRM2	ribonucleotide reductase M2	KEGG	p53 signaling pathway	0.00304	0.57
BBC3	BCL2 binding component 3	KEGG	p53 signaling pathway	0.0149	1.984
CYCS	cytochrome c, somatic	KEGG	p53 signaling pathway	0.0477	0.657
CDKN1A	cyclin-dependent kinase inhibitor 1A (p21, Cip1)	KEGG	p53 signaling pathway	0.0206	2.439
CCNE1	cyclin E1	KEGG	p53 signaling pathway	0.0369	0.617
TP53I3	tumor protein p53 inducible protein 3	KEGG	p53 signaling pathway	0.00864	0.515
SFN	stratifin	KEGG	p53 signaling pathway	0.0296	1.832
IGFBP3	insulin-like growth factor binding protein 3	KEGG	p53 signaling pathway	0.00124	0.505

OVCAR-3 small RNAseq study strongly predicts the p53 pathway affected by Kevetrin (KEGG pathway analysis)

Top 30 MicroRNAs by ANOVA P Value for Treatment

Mature miRNA Name	Mature miRNA Accession	Mature miRNA Sequence	ANOVA P: Treatment	Regression P: Treatment	Tukey P: 200 vs UNT	Tukey P: 400 vs UNT	Fold Change: 200 / UNT	Fold Change: 400 / UNT
hsa-miR-548ay-5p	MIMAT0025452	AAAAGUAAUUGUGGUUUUUGC	5.45E-05	2.11E-01	7.95E-01	4.58E-01	1.35	1.49
hsa-miR-5092	MIMAT0021084	AAUCCACGCUGAGCUUGGCAUC	2.58E-04	2.99E-02	9.13E-01	7.26E-02	0.87	0.49
hsa-miR-1180-3p	MIMAT0005825	UUUCCGGCUCGCGUGGGUGUGU	6.12E-04	2.78E-03	1.83E-01	1.38E-02	0.86	0.74
hsa-let-7e-3p	MIMAT0004485	CUAUACGGCCUCCUAGCUUUCC	9.30E-04	4.26E-03	2.71E-02	9.49E-03	2.02	2.48
hsa-miR-152-5p	MIMAT0026479	AGGUUCUGUGAUACACUCCGACU	9.62E-04	5.66E-01	3.56E-02	5.07E-01	0.48	1.36
hsa-miR-873-3p	MIMAT0022717	GGAGACUGAUGAGUUCCCGGGA	1.03E-03	5.31E-05	8.83E-04	9.69E-05	0.59	0.46
hsa-miR-548av-3p	MIMAT0022304	AAAACUGCAGUUACUUUUGC	1.31E-03	6.31E-03	NA	2.21E-02	NA	12.22
hsa-miR-425-5p	MIMAT0003393	AAUGACACGAUCACUCCCGUUGA	1.47E-03	6.24E-01	9.58E-01	8.79E-01	0.93	1.11
hsa-let-7a-3p	MIMAT0004481	CUAUACAAUCUACUGUCUUUC	1.59E-03	4.20E-03	8.50E-02	1.63E-02	1.93	2.68
hsa-miR-28-3p	MIMAT0004502	CACUAGAUUGUGAGCUCCUGGA	1.95E-03	1.20E-01	6.85E-02	1.65E-01	0.73	0.78
hsa-miR-128-1-5p	MIMAT0026477	CGGGGCCGUAGCACUGUCUGAGA	2.14E-03	1.20E-01	6.36E-01	3.01E-01	0.84	0.72
hsa-miR-1292-5p	MIMAT0005943	UGGGAACGGGUUCCGGCAGACGCUG	2.37E-03	6.46E-02	1.38E-03	8.70E-03	0.39	0.51
hsa-miR-548d-5p	MIMAT0004812	AAAAGUAAUUGUGGUUUUUGCC	2.51E-03	2.12E-01	8.40E-01	4.58E-01	1.30	1.49
hsa-miR-625-3p	MIMAT0004808	GACUAUAGAACUUUCCCCCUCA	2.60E-03	4.28E-01	2.40E-01	6.45E-01	0.53	0.65
hsa-miR-26a-2-3p	MIMAT0004681	CCUAUUCUUGAUUACUUGUUUC	2.84E-03	9.94E-02	8.39E-01	2.55E-01	1.16	1.55
hsa-miR-1306-5p	MIMAT0022726	CCACCUCCCCUGCAAACGUCCA	6.77E-03	6.28E-02	6.90E-01	4.93E-02	1.13	0.66
hsa-miR-183-3p	MIMAT0004560	GUGAAUUACCGAAGGGCCAUAA	6.97E-03	3.82E-01	5.77E-01	6.61E-01	0.85	0.85
hsa-miR-345-5p	MIMAT0000772	GCUGACUCCUAGUCCAGGGCUC	7.13E-03	5.63E-04	3.27E-02	3.01E-03	0.64	0.48
hsa-miR-92b-3p	MIMAT0003218	UAUUGCACUCGUCCCGGCCUCC	7.77E-03	2.08E-03	1.12E-01	1.06E-02	0.77	0.62
hsa-miR-550a-5p	MIMAT0004800	AGUGCCUGAGGGAGUAAGAGCCC	7.79E-03	2.36E-02	7.61E-01	6.97E-02	0.93	0.75
hsa-miR-21-5p	MIMAT0000076	UAGCUUAUCAGACUGAUGUUGA	7.83E-03	1.50E-01	1.68E-01	2.68E-01	1.35	1.29
hsa-miR-331-3p	MIMAT0000760	GCCCCUGGGCCUAUCCUAGAA	8.11E-03	1.71E-01	9.28E-01	3.85E-01	1.09	1.42
hsa-miR-378c	MIMAT0016847	ACUGGACUUGGAGUCAGAAGAGUGG	8.30E-03	4.65E-02	2.06E-01	1.20E-01	1.39	1.50
hsa-miR-1910-5p	MIMAT0007884	CCAGUCCUGUGCCUGCCGCCU	8.55E-03	5.88E-03	8.18E-01	8.49E-03	0.85	0.36
hsa-miR-146a-5p	MIMAT0000449	UGAGAACUGAAUUCCAUGGGUU	8.71E-03	1.76E-01	5.67E-01	3.93E-01	0.76	0.66
hsa-miR-27b-5p	MIMAT0004588	AGAGCUUAGCUGAUUGGUGAAC	9.44E-03	1.60E-03	1.03E-01	8.55E-03	0.73	0.57
hsa-miR-503-5p	MIMAT0002874	UAGCAGCGGGAACAGUUCUGCAG	1.10E-02	1.19E-02	1.68E-02	1.35E-02	0.74	0.73
hsa-miR-3145-3p	MIMAT0015016	AGAUAUUUUGAGUGUUUGGAAUUG	1.17E-02	5.60E-01	8.42E-01	8.19E-01	0.96	1.27
hsa-miR-140-5p	MIMAT0000431	CAGUGGUUUUACCCUAUGGUAG	1.17E-02	1.29E-01	9.54E-01	3.01E-01	1.00	0.66
hsa-miR-191-5p	MIMAT0000440	CAACGGAAUCCCAAAAGCAGCUG	1.20E-02	1.78E-01	1.63E-01	3.04E-01	0.65	0.73

Kevetrin modulates cancer targeted pathways in SKOV-3 (p53 partial deletion) mRNAseq study (KEGG pathway analysis)

200 µM Kevetrin

PathwayName	#Gene	Statistics
Cytokine-cytokine receptor interaction	5	adjP=0.0237
Pathways in cancer	6	adjP=0.0527

400 µM Kevetrin

PathwayName	#Gene	Statistics
Systemic lupus erythematosus	20	adjP=9.92e-10
Steroid biosynthesis	6	adjP=0.0086
Pathways in cancer	28	adjP=0.0242
Bladder cancer	8	adjP=0.0242
Neuroactive ligand-receptor interaction	13	adjP=0.0388

SKOV-3 (p53 partial deletion) xenograft tumor small RNAseq analysis shows p53 pathway modulation (KEGG pathway analysis)

10 doses vs no dose

PathwayName	#Gene	Statistics
p53 signaling pathway	26	adjP=0.0114

10 doses vs one dose

PathwayName	#Gene	Statistics
p53 signaling pathway	19	adjP=0.0013

p53 pathway modulation was not observed in OV-90 (p53 mutant) xenograft tumor after treatment with Kevetrin (KEGG pathway analysis)

tumor small RNA

10 doses vs no dose

PathwayName	#Gene	Statistics
Colorectal cancer	2	adjP=0.0188
Bacterial invasion of epithelial cells	2	adjP=0.0188
Fc gamma R-mediated phagocytosis	2	adjP=0.0188
mTOR signaling pathway	2	adjP=0.0188
Chemokine signaling pathway	2	adjP=0.0414
Insulin signaling pathway	2	adjP=0.0488

Kevetrin modulates the p53 signaling pathway in SKOV-3 (p53 partial deletion) xenograft tumors (mRNAseq study; KEGG pathway analysis)

Gene Symbol	Gene name	Pathway	Pathway name	P value	Fold change
GTSE1	G-2 and S-phase expressed 1	KEGG	p53 signaling pathway	0.00163	0.644
RRM2	ribonucleotide reductase M2	KEGG	p53 signaling pathway	0.000155	0.533
CCNB2	cyclin B2	KEGG	p53 signaling pathway	0.00037	0.659
CDK1	cyclin-dependent kinase 1	KEGG	p53 signaling pathway	0.00447	0.626
TP53I3	tumor protein p53 inducible protein 3	KEGG	p53 signaling pathway	0.000746	0.66
IGFBP3	insulin-like growth factor binding protein 3	KEGG	p53 signaling pathway	0.0078	1.536

p53 signaling in SKOV-3 xenograft tumor (mRNAseq study; KEGG pathway analysis)

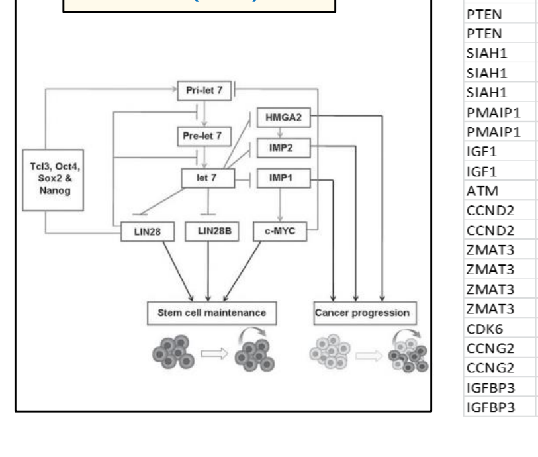
10 doses vs no dose

PathwayName	#Gene	Statistics
Cytokine-cytokine receptor interaction	10	adjP=0.0038
MAPK signaling pathway	15	adjP=0.0038
Protein digestion and absorption	6	adjP=0.0051
Hematopoietic cell lineage	5	adjP=0.0066
Antigen processing and presentation	5	adjP=0.0289
Pancreatic secretion	5	adjP=0.0320
Vascular smooth muscle contraction	6	adjP=0.0331

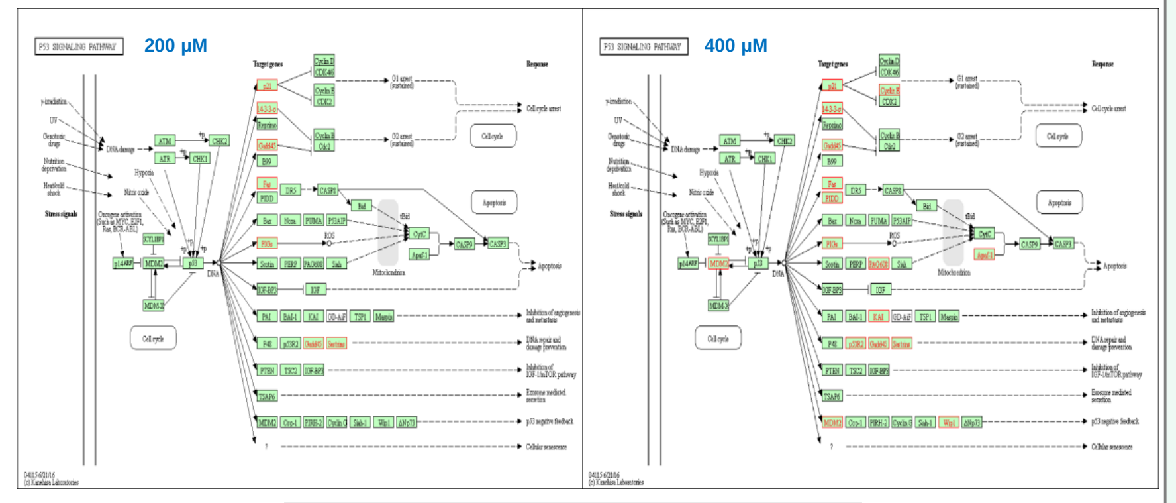
10 days vs one dose

PathwayName	#Gene	Statistics
Systemic lupus erythematosus	20	adjP=1.48e-16
p53 signaling pathway	6	adjP=0.0525

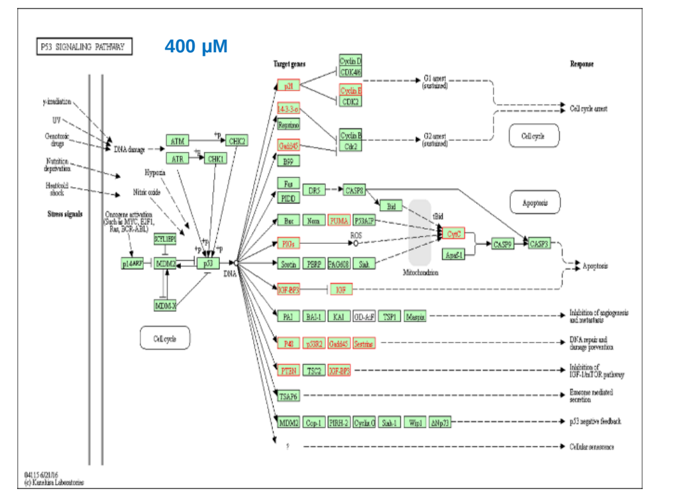
Regulatory circuits of microRNA (miRNA) let-7



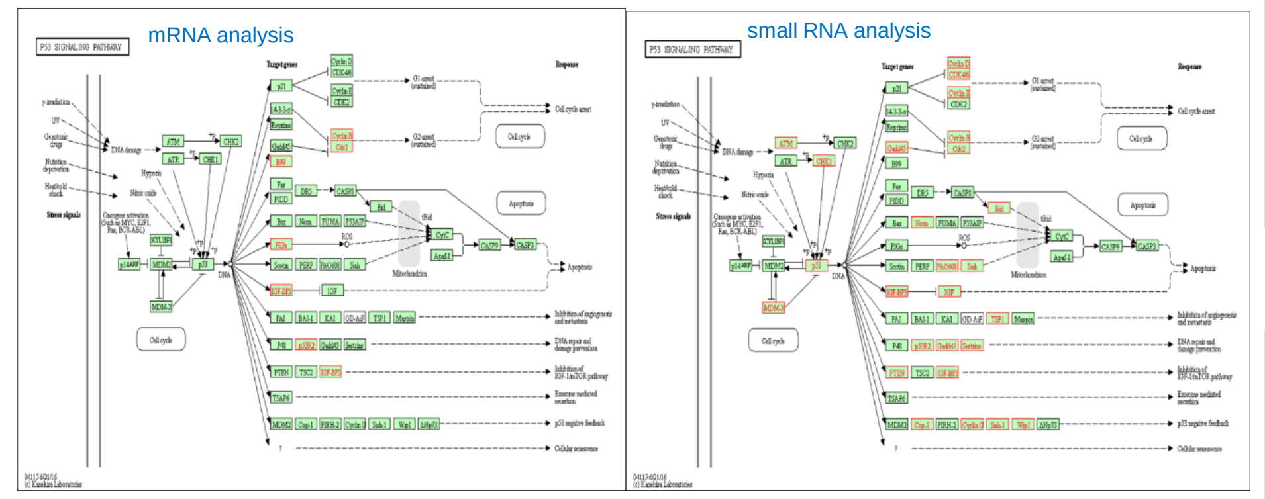
Dose dependent increase in p53 effectors in A2780 cell line – KEGG p53 pathway analysis



Kevetrin modulates multiple genes in the p53 signaling pathway in OVCAR-3 (p53 mutant) - KEGG pathway analysis



SKOV-3 (partial deletion) xenograft tumor shows p53 pathway modulation in both (mRNAseq and small RNAseq) analyses - KEGG pathway analysis)



p53 pathway genes modulated by microRNA changes in SKOV-3 (p53 partial deletion) xenograft tumor

Gene Symbol	Gene Name	Pathway	Pathway Name	Mature miRNA Name	p value	Fold Change (10 doses/ no dose)
CCNE2	cyclin E2	KEGG	p53 signaling pathway	hsa-miR-30a-5p	0.0663	1.706
CCNE2	cyclin E2	KEGG	p53 signaling pathway	hsa-miR-34a-5p	0.0738	0.543
CCNE2	cyclin E2	KEGG	p53 signaling pathway	hsa-miR-369-3p	0.0722	0.114
PPM1D	protein phosphatase, Mg2+/Mn2+ dependent, 1D	KEGG	p53 signaling pathway	hsa-miR-16-5p	0.00339	0.557
PPM1D	protein phosphatase, Mg2+/Mn2+ dependent, 1D	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
PPM1D	protein phosphatase, Mg2+/Mn2+ dependent, 1D	KEGG	p53 signaling pathway	hsa-miR-195-5p	0.0343	0.142
PPM1D	protein phosphatase, Mg2+/Mn2+ dependent, 1D	KEGG	p53 signaling pathway	hsa-miR-497-5p	0.0862	0.185
MDM4	Mdm4 p53 binding protein homolog (mouse)	KEGG	p53 signaling pathway	hsa-miR-34a-5p	0.0738	0.543
TP53	tumor protein p53	KEGG	p53 signaling pathway	hsa-miR-150-5p	0.00339	0.087
RRM2	ribonucleotide reductase M2	KEGG	p53 signaling pathway	hsa-miR-17-5p	0.0862	0.625
CHEK1	checkpoint kinase 1	KEGG	p53 signaling pathway	hsa-miR-16-5p	0.00339	0.557
CHEK1	checkpoint kinase 1	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
CHEK1	checkpoint kinase 1	KEGG	p53 signaling pathway	hsa-miR-195-5p	0.0343	0.142
CHEK1	checkpoint kinase 1	KEGG	p53 signaling pathway	hsa-miR-497-5p	0.0862	0.185
CCNB1	cyclin B1	KEGG	p53 signaling pathway	hsa-miR-548h-5p	0.0923	0.33
BID	BH3 interacting domain death agonist	KEGG	p53 signaling pathway	hsa-miR-26a-5p	0.0225	0.619
SESN1	sestrin 1	KEGG	p53 signaling pathway	hsa-miR-16-5p	0.00339	0.557
SESN1	sestrin 1	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
SESN1	sestrin 1	KEGG	p53 signaling pathway	hsa-miR-195-5p	0.0343	0.142
SESN1	sestrin 1	KEGG	p53 signaling pathway	hsa-miR-497-5p	0.0862	0.185
CDK1	cyclin-dependent kinase 1	KEGG	p53 signaling pathway	hsa-miR-16-5p	0.00339	0.557
CDK1	cyclin-dependent kinase 1	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
CDK1	cyclin-dependent kinase 1	KEGG	p53 signaling pathway	hsa-miR-195-5p	0.0343	0.142
CDK1	cyclin-dependent kinase 1	KEGG	p53 signaling pathway	hsa-miR-497-5p	0.0862	0.185
CCNE1	cyclin E1	KEGG	p53 signaling pathway	hsa-miR-16-5p	0.00339	0.557
CCNE1	cyclin E1	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
CCNE1	cyclin E1	KEGG	p53 signaling pathway	hsa-miR-195-5p	0.0343	0.142
CCNE1	cyclin E1	KEGG	p53 signaling pathway	hsa-miR-497-5p	0.0862	0.185
THBS1	thrombospondin 1	KEGG	p53 signaling pathway	hsa-miR-19b-3p	0.0517	0.447
THBS1	thrombospondin 1	KEGG	p53 signaling pathway	hsa-let-7c-5p	0.038	0.257
THBS1	thrombospondin 1	KEGG	p53 signaling pathway	hsa-miR-19a-3p	0.0862	0.471
RFWD2	ring finger and WD repeat domain 2, E3 ubiquitin protein	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
CCND1	cyclin D1	KEGG	p53 signaling pathway	hsa-miR-19a-3p	0.0862	0.471
SESN3	sestrin 3	KEGG	p53 signaling pathway	hsa-miR-433-3p	0.0663	0.102
GADD45A	growth arrest and DNA-damage-inducible, alpha	KEGG	p53 signaling pathway	hsa-miR-374b-5p	0.0179	0.612
PTEN	phosphatase and tensin homolog	KEGG	p53 signaling pathway	hsa-miR-19b-3p	0.0517	0.447
PTEN	phosphatase and tensin homolog	KEGG	p53 signaling pathway	hsa-miR-19a-3p	0.0862	0.471
SIAH1	siah E3 ubiquitin protein ligase 1	KEGG	p53 signaling pathway	hsa-miR-16-5p	0.00339	0.557
SIAH1	siah E3 ubiquitin protein ligase 1	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
SIAH1	siah E3 ubiquitin protein ligase 1	KEGG	p53 signaling pathway	hsa-miR-195-5p	0.0343	0.142
PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	KEGG	p53 signaling pathway	hsa-miR-26a-5p	0.0225	0.619
PMAIP1	phorbol-12-myristate-13-acetate-induced protein 1	KEGG	p53 signaling pathway	hsa-let-7c-5p	0.038	0.257
IGF1	insulin-like growth factor 1 (somatomedin C)	KEGG	p53 signaling pathway	hsa-miR-19b-3p	0.0517	0.447
IGF1	insulin-like growth factor 1 (somatomedin C)	KEGG	p53 signaling pathway	hsa-miR-19a-3p	0.0862	0.471
ATM	ataxia telangiectasia mutated	KEGG	p53 signaling pathway	hsa-miR-26a-5p	0.0225	0.619
CCND2	cyclin D2	KEGG	p53 signaling pathway	hsa-miR-19b-3p	0.0517	0.447
CCND2	cyclin D2	KEGG	p53 signaling pathway	hsa-miR-19a-3p	0.0862	0.471
ZMAT3	zinc finger, matrin-type 3	KEGG	p53 signaling pathway	hsa-miR-16-5p	0.00339	0.557
ZMAT3	zinc finger, matrin-type 3	KEGG	p53 signaling pathway	hsa-miR-15b-5p	0.056	0.529
ZMAT3	zinc finger, matrin-type 3	KEGG	p53 signaling pathway	hsa-miR-195-5p	0.0343	0.142
ZMAT3	zinc finger, matrin-type 3	KEGG	p53 signaling pathway	hsa-miR-497-5p	0.0862	0.185
CDK6	cyclin-dependent kinase 6	KEGG	p53 signaling pathway	hsa-miR-191-5p	0.00339	0.516
CCNG2	cyclin G2	KEGG	p53 signaling pathway	hsa-miR-142-5p	0.0663	0.278
CCNG2	cyclin G2	KEGG	p53 signaling pathway	hsa-miR-17-5p	0.0862	0.625
IGFBP3	insulin-like growth factor binding protein 3	KEGG	p53 signaling pathway	hsa-miR-19b-3p	0.0517	0.447
IGFBP3	insulin-like growth factor binding protein 3	KEGG	p53 signaling pathway	hsa-miR-19a-3p	0.0862	0.471

Results Summary

- Kevetrin induced apoptosis in wild type p53, mutant p53 and partially deleted p53 ovarian cancer cell lines as assayed by cleavage of PARP.
- p53 and p21 increased protein levels were seen in A2780 cells (p53 wild type) after 24 to 48 hr exposure to Kevetrin. Depletion of p53 by SiRNA reduced the p21 expression as assayed by FACS.
- Kevetrin downregulated oncogenic mutant p53 in OVCAR-3 and OV-90 (p53 mutant)
- Increase in p21 mRNA in response to Kevetrin was observed in all OC cell lines.
- No significant changes were observed in p53 mRNA.
- In xenograft models, Kevetrin inhibited A2780 tumor growth, and increased SKOV-3 animal survival.
- Transcriptomic analyses demonstrated that Kevetrin modulates p53 signaling pathways and induces apoptosis and cell cycle arrest in ovarian cancer cell lines as well as xenograft tumors as shown by RNAseq data. KEGG pathway analysis was used for these studies.
- Altered expression of miRNA-27a, miRNA-1274b, miRNA-25 (known to be dysregulated in ovarian cancer) in a time-dependent manner in OVCAR-3 cells provides possible biomarkers for Kevetrin response.
- In tumors where p53 is partially deleted (SKOV-3), Kevetrin modulates p53 pathways and induces apoptosis. Further detailed mechanism studies are ongoing.

Conclusions

- Kevetrin regulates multiple molecular targets and signaling pathways.
- Kevetrin induces wild type p53 and induces apoptosis in a non-genotoxic manner (previously shown). Most currently available chemotherapeutic drugs are genotoxic and damage DNA.
- Targeting multiple pathways such as the p53 signaling pathways has benefits of anti-tumor activity, reducing toxicity and overcoming development of drug resistance.
- In a completed Phase 1 clinical trial, Kevetrin was well tolerated with minimal adverse effects. A clinical trial in ovarian cancer is underway.

Further Information

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