Supplementary Data

Involvement of extracellular vesicle long non-coding RNA (linc-VLDLR) in tumor cell responses to chemotherapy

Kenji Takahashi, Irene K. Yan, Hiroaki Haga, Tushar Patel

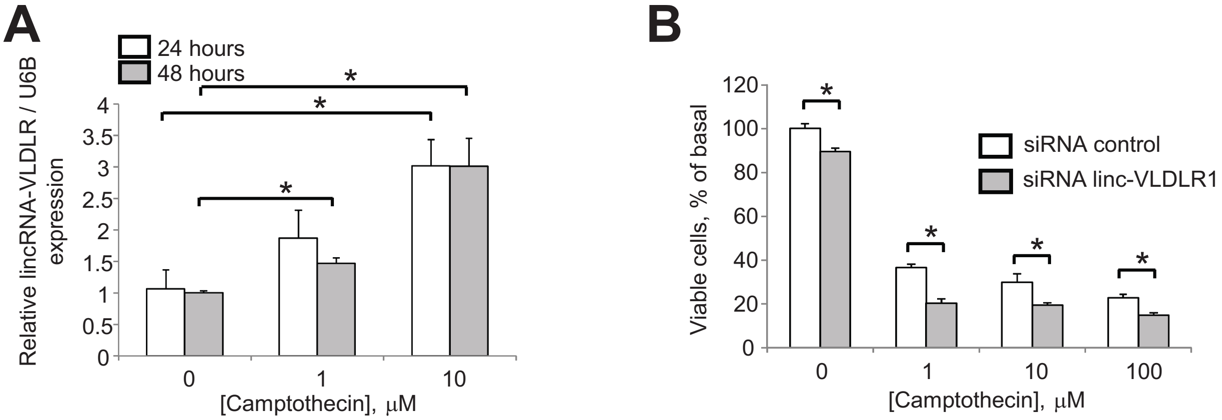

Figure 1. Linc-VLDLR expression and response to camptothecin in HepG2 cells. A, Cells were incubated with varying concentrations of camptothecin for 24 or 48 hours. Linc-VLDLR expression was determined by qRT-PCR, and normalized to that of RNU6B. Linc-VLDLR expression is represented relative to normalized expression in controls at each time point. B, Cells were transfected with siRNA to linc-VLDLR (siRNA VLDLR-1) or non-targeting siRNA controls. Transfected cells were then incubated with varying concentrations of camptothecin and cell viability was assessed after 48 hours.

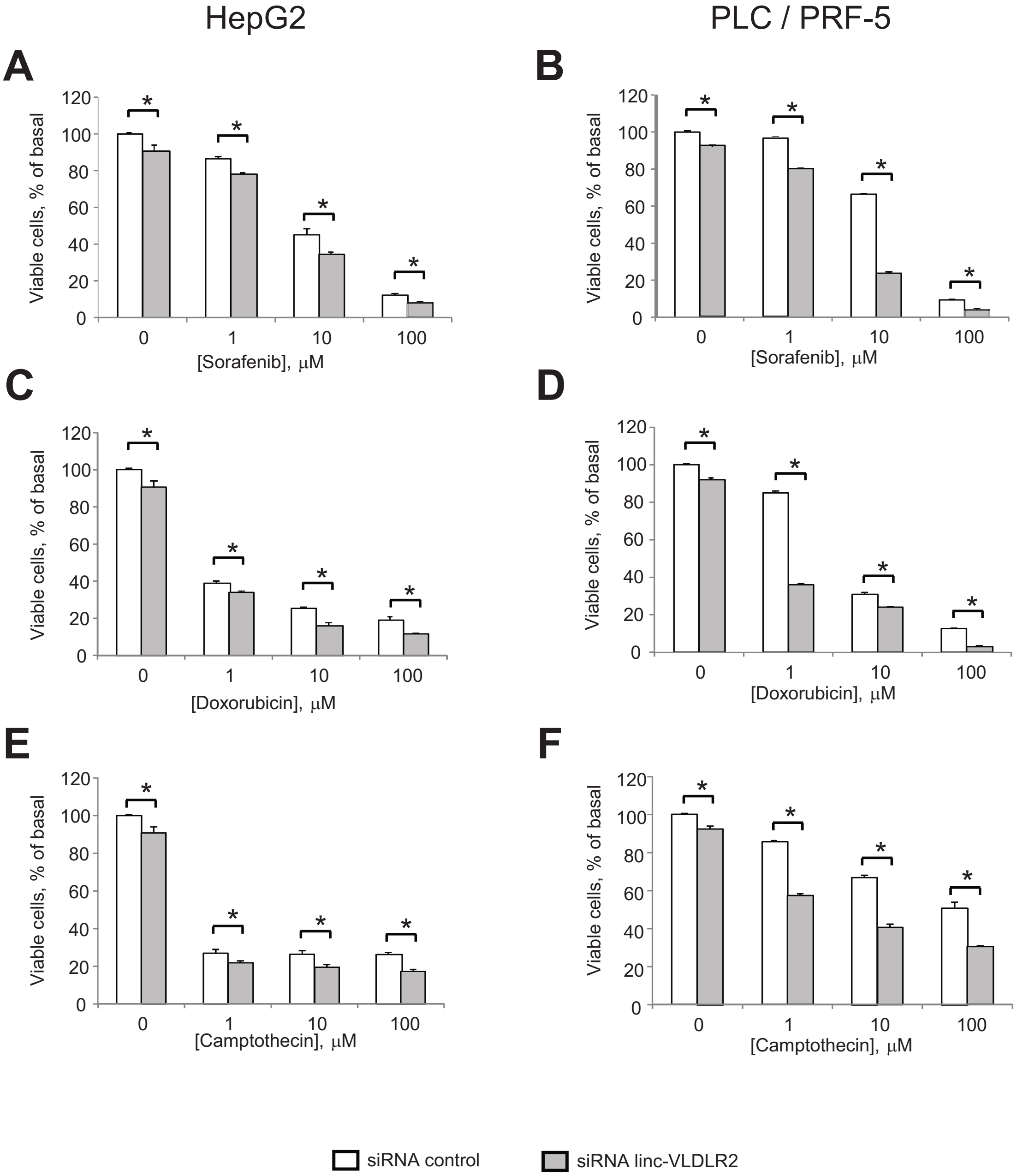

Figure 2. Linc-VLDLR modulates response to chemotherapy. HepG2 cells (A, C and E) or PLC/PRF-5 cells (B, D and F) were transfected with siRNA to linc-VLDLR (siRNA VLDLR-2) or non-targeting siRNA controls. Transfected cells were then incubated with varying concentrations of sorafenib (A, B), doxorubicin (C, D) or camptothecin (E, F) and cell viability was assessed after 48 hours. Bars represent the mean ± SEM of 3 separate studies. *, $p < 0.05$.

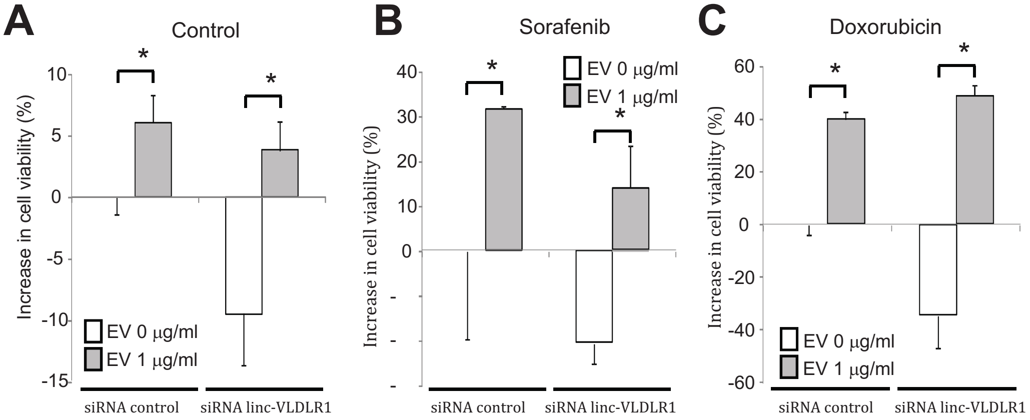

Figure 3. Involvement of extracellular vesicle linc-VLDLR in tumor cell responses to chemotherapy. A - C, HepG2 cells were transfected with siRNA to linc-VLDLR (siRNA VLDLR-1) or non-targeting controls. After 48 hours, cells were collected and plated (1 x 104/well) on 96 well-plates in vesicle-depleted medium and incubated with 0 or 1 μg/ml of HepG2 cell derived EVs. After 24 hours, cells were incubated with (A) diluent (DMSO) control, (B) 10 μM sorafenib, or (C) 10 μM doxorubicin. Cell viability was assessed after 72 hours using MTS assay. The bars represent the mean ± SEM of the increase in cell viability from 3 independent studies. *, $p < 0.05$.

Supplementary Table 1

LncRNA expression was examined in HepG2 HCC cells and non-malignant human hepatocytes (HH). Data represents Ct values and SE values from three independent replicates.

Gene name	HepG2 cells Average of Ct value	SE	HH cells Average of Ct value	SE
21A	23.32	1.12	21.57	0.43
7SK	22.83	1.26	21.54	0.75
7SL	21.94	1.11	20.25	0.47
Air	39.30	1.29	39.06	0.49
AK023948	32.71	1.50	31.78	0.62
Alpha 280	31.68	2.20	34.42	0.87
ANRIL	44.41	2.96	43.60	0.83
anti-NOS2A	35.31	4.32	44.20	3.19
BACE1AS (family)	33.54	1.67	31.48	0.20
CAR Intergenic 10	37.13	2.29	34.23	0.62
DHFR upstream transcripts (family)	44.48	0.71	40.72	0.40
DISC2 (family)	43.81	1.86	38.95	0.41
E2F4 antisense	28.15	1.54	30.72	0.67
EgoA	35.11	1.93	33.73	0.87
EGO B	33.55	2.17	30.56	0.57
Emx2os	39.59	1.76	37.31	0.55
GAS5-family	26.91	1.94	24.33	0.28
Gomafu	36.91	1.66	37.09	2.80
H19 antisense	26.36	1.26	25.91	0.41
H19 upstream conserved 1 & 2	26.31	1.28	24.85	0.62
HAR1A	32.78	1.10	31.23	0.70
HAR1B	38.06	2.16	37.54	1.47
HOTAIR	44.77	1.83	43.54	0.93
HOTAIRM1	31.88	1.45	29.23	0.66
HOTTIP	37.72	1.56	36.31	0.75
Hoxa11as	27.04	1.17	26.54	0.12
HOXA3as	33.39	1.57	34.92	0.34
HOXA6as	29.54	1.75	28.23	0.41
IGF2AS (family)	36.32	2.56	33.58	0.68
IPW	45.41	1.93	43.82	0.96
Jpx	36.15	1.40	34.05	0.72
Kcnq1ot1	35.46	1.49	34.20	0.39
KRASP1	42.67	1.62	41.19	0.30
lincRNA-p21	34.35	1.40	31.19	0.16
lincRNA-RoR	29.73	2.74	33.61	0.92
lincRNA-SFMBT2	29.91	2.44	37.95	3.04
lincRNA-VLDLR	29.07	2.09	31.66	0.68
LOC285194	38.59	2.37	38.08	0.58

LUST	24.43	1.41	25.27	2.13
Malat1	28.86	1.76	25.82	0.49
mascRNA	39.26	5.64	31.31	0.33
MEG3 (family)	37.15	1.89	33.19	0.34
MEG9	45.10	1.96	42.90	1.42
MER11C	33.33	1.20	32.57	0.38
ncR-uPAR	25.88	1.56	24.62	0.45
NDM29	24.88	1.37	23.45	0.84
NEAT1 (family)	30.01	1.33	29.20	0.45
Nespas	33.30	1.25	32.58	0.62
NRON	33.76	1.49	32.94	0.47
NTT	34.47	1.81	32.13	0.47
p53 mRNA	27.49	1.63	27.28	2.23
PR antisense transcripts	47.61	1.31	47.05	1.57
PRINS	33.96	1.47	30.58	0.43
PSF inhibiting RNA	38.93	1.01	37.46	0.47
RNCR3	43.61	1.44	39.02	1.84
SAF	35.52	1.24	34.91	0.44
SCA8	36.78	2.35	34.92	0.27
snaR	23.98	1.30	25.16	0.63
SNHG1	32.48	1.40	31.36	0.33
SNHG3	38.26	2.01	35.87	0.81
SNHG4	25.99	1.28	26.47	2.35
SNHG5	25.99	1.38	25.55	0.24
SNHG6	29.54	1.31	28.27	0.54
Sox2ot	38.27	2.22	35.25	0.41
SRA	33.21	1.37	31.69	0.40
ST7OT	32.55	1.53	31.65	0.12
TEA ncRNAs (family)	43.68	0.52	41.31	1.09
Tmevpg1	36.61	1.96	34.04	0.23
TncRNA	30.85	1.29	28.98	0.60
Tsix	27.06	0.68	23.79	0.35
TUG1 (family)	33.35	1.53	32.75	0.31
UCA1	47.33	1.40	45.48	1.07
UM9-5	34.20	1.51	35.00	1.51
WT1-AS	35.77	1.48	31.69	0.14
Xist	34.87	1.74	28.82	0.31
Y RNA-1	22.83	1.20	21.07	0.60
Zeb2NAT	33.71	1.55	32.06	0.28
Zfas1	25.31	1.23	25.25	0.30
Zfhx2as	33.11	1.37	33.72	0.35
18S rRNA	17.66	1.69	15.87	0.51
RNU43 (snoRNA)	25.88	1.69	23.40	0.65
GAPDH	20.79	1.40	19.55	0.30
LAMIN A/C	30.16	1.38	30.03	0.33
U6 snRNA	23.15	1.19	21.61	0.83

Supplementary Table 2

LncRNA expression was examined in HepG2 cells and EV derived from these cells. Data represents Ct values and SE values from three independent replicates.

Gene name	HepG2 derived EV Average of Ct value	SE	HepG2 cells Average of Ct value	SE
21A	38.31	5.99	23.32	1.12
7SK	36.46	1.33	22.83	1.26
7SL	31.84	1.32	21.94	1.11
Air	46.31	2.75	39.30	1.29
AK023948	38.34	1.33	32.71	1.50
Alpha 280	41.04	4.62	31.68	2.20
anti-NOS2A	42.89	1.75	35.31	4.32
BACE1AS (family)	43.45	3.29	33.54	1.67
CAR Intergenic 10	39.00	1.57	37.13	2.29
DHFR upstream transcripts (family)	46.94	1.70	44.48	0.71
DISC2 (family)	46.08	1.98	43.81	1.86
E2F4 antisense	38.61	5.69	28.15	1.54
EgoA	45.44	2.60	35.11	1.93
EGO B	44.09	2.04	33.55	2.17
GAS5-family	36.19	0.93	26.91	1.94
H19 antisense	38.13	1.52	26.36	1.26
H19 upstream conserved 1 & 2	33.10	0.97	26.31	1.28
HAR1A	46.49	1.85	32.78	1.10
HAR1B	40.81	3.35	38.06	2.16
HOTAIRM1	41.51	2.05	31.88	1.45
Hoxa11as	35.79	0.42	27.04	1.17
HOXA6as	40.54	1.66	29.54	1.75
Kcnq1ot1	42.88	3.65	35.46	1.49
L1PA16	44.79	2.64	35.78	1.92
lincRNA-p21	39.17	0.59	34.35	1.40
lincRNA-RoR	34.00	0.71	29.73	2.74
lincRNA-SFMBT2	39.16	5.43	29.91	2.44
lincRNA-VLDLR	31.98	0.44	29.07	2.09
LUST	34.68	1.82	24.43	1.41
Malat1	40.95	1.91	28.86	1.76
mascRNA	45.24	2.41	39.26	5.64
MEG3 (family)	47.55	1.23	37.15	1.89
MER11C	41.22	1.34	33.33	1.20
ncR-uPAR	39.27	1.72	25.88	1.56
NDM29	33.18	1.13	24.88	1.37
NEAT1 (family)	35.11	1.11	30.01	1.33
Nespas	38.60	0.58	33.30	1.25

NRON	42.52	1.65	33.76	1.49
NTT	43.91	2.62	34.47	1.81
SAF	42.37	2.41	35.52	1.24
SCA8	43.61	1.32	36.78	2.35
snaR	34.08	0.62	23.98	1.30
SNHG1	41.66	2.07	32.48	1.40
SNHG4	33.72	1.28	25.99	1.28
SNHG5	37.72	2.08	25.99	1.38
SNHG6	35.41	0.72	29.54	1.31
Sox2ot	45.16	3.04	38.27	2.22
SRA	44.95	2.60	33.21	1.37
ST7OT	40.55	1.68	32.55	1.53
TncRNA	43.40	3.30	30.85	1.29
Tsix	30.90	2.56	27.06	0.68
TUG1 (family)	39.34	3.49	33.35	1.53
UM9-5	48.33	0.84	34.20	1.51
Y RNA-1	29.48	1.17	22.83	1.20
Zeb2NAT	42.49	1.23	33.71	1.55
Zfas1	40.72	2.10	25.31	1.23
Zfhx2as	39.66	2.44	33.11	1.37
18S rRNA	27.33	0.96	14.98	1.69
RNU43 (snoRNA)	34.62	4.08	22.96	1.69
GAPDH	36.88	4.32	19.64	1.40
U6 snRNA	33.37	0.69	20.72	1.19

Supplementary Table 3

LncRNA expression was examined in MzChA1 cells and EV derived from these cells. Data represents Ct values and SE values from three independent replicates.

Gene name	Mz-ChA-1 derived EV		Mz-ChA-1 cells	
	Average of Ct value	SE	Average of Ct value	SE
7SK	32.28	1.02	20.86	0.96
7SL	27.20	1.42	20.75	1.12
AK023948	38.17	2.34	33.51	0.23
Alpha 280	34.55	0.71	34.70	0.70
anti-NOS2A	44.08	3.19	38.52	1.66
BACE1AS (family)	38.62	2.45	32.86	0.93
CAR Intergenic 10	37.64	1.84	38.03	1.27
DHFR upstream transcripts (family)	44.75	2.71	43.77	0.33
DISC2 (family)	42.20	1.98	44.48	0.52
DLG2AS (family)	44.90	2.65	34.51	1.26
E2F4 antisense	38.16	5.94	30.16	0.43
EgoA	36.50	1.37	35.95	0.85
EGO B	35.83	0.87	33.16	1.18
Emx2os	43.56	2.09	39.28	1.05
GAS5-family	29.87	1.69	25.25	0.52
Gomafu	45.37	2.00	36.52	1.37
H19 antisense	35.45	1.27	27.30	0.69
H19 upstream conserved 1 & 2	38.74	5.75	25.86	0.89
HAR1A	43.41	1.87	33.12	0.31
HAR1B	35.72	1.90	39.82	1.38
HOTAIR	41.74	4.21	44.59	0.99
HOTAIRM1	39.84	0.38	32.51	0.75
HOTTIP	49.12	0.46	38.53	0.96
Hoxa11as	35.03	2.19	27.53	0.64
HOXA6as	35.64	0.20	29.43	1.08
IPW	44.93	3.23	47.69	0.72
Jpx	44.37	3.39	35.65	0.47
Kcnq1ot1	43.46	3.37	38.17	1.10
L1PA16	44.69	2.77	41.60	1.61
lincRNA-p21	37.75	0.93	33.18	0.85
lincRNA-RoR	32.48	0.99	34.74	0.68
lincRNA-SFMBT2	31.45	0.99	34.44	0.67
lincRNA-VLDLR	29.90	0.89	33.51	0.50
LUST	30.00	2.86	23.82	0.98
mascRNA	38.07	2.48	34.05	0.80

MEG9	45.59	2.78	45.28	1.91
MER11C	38.73	0.35	33.48	0.44
ncR-uPAR	33.77	2.72	25.57	0.60
NDM29	30.87	0.97	25.09	0.54
NEAT1 (family)	32.15	0.86	31.09	0.67
Nespas	36.68	1.79	34.07	0.86
NRON	41.66	4.18	34.27	0.66
NTT	42.47	4.14	34.18	0.54
p53 mRNA	47.68	2.07	26.59	0.29
RNCR3	44.10	3.34	42.15	0.64
SAF	43.28	2.72	36.00	0.60
SCA8	40.66	4.76	36.58	1.83
snaR	31.66	1.51	27.44	0.80
SNHG3	46.30	1.28	38.23	1.19
SNHG4	31.23	2.07	25.36	1.06
SNHG5	40.99	4.27	26.56	0.47
SNHG6	40.60	4.71	27.90	0.41
Sox2ot	43.72	3.20	38.28	1.10
SRA	42.31	4.50	33.57	0.53
ST7OT	39.28	3.32	32.89	0.49
TncRNA	41.01	4.51	29.95	0.58
Tsix	27.69	1.04	25.71	0.78
TUG1 (family)	37.21	5.90	36.24	0.36
UM9-5	41.01	3.71	35.14	0.88
Y RNA-1	26.80	0.98	21.82	0.44
Zeb2NAT	43.91	3.13	33.20	1.07
Zfas1	35.00	1.07	25.24	0.39
Zfhx2as	39.17	1.27	33.18	0.53
18S rRNA	22.76	1.01	17.43	1.22
GAPDH	38.75	3.77	20.67	0.22
LAMIN A/C	43.16	2.96	31.43	0.66
U6 snRNA	28.80	1.33	21.82	0.85