APPENDICES

Appendix 1: Solutions, formulations and commercial kits

Dulbecco's Modified Eagle Medium (DMEM)

The cell culture medium was prepared by adding 10.0 % (v/v) fetal bovine serum (FBS) (Lonza Inc., USA) to Dulbecco's Modified Eagle Medium (DMEM) (Hyclone Laboratories Inc., USA). The cell culture medium was stored at 4°C until further use.

Keratinocyte-serum free medium (KSFM)

The cell culture medium was prepared by adding 5.0 % (v/v) fetal bovine serum (FBS) (Lonza Inc., USA) to keratinocyte-serum free medium (KSFM) (Invitrogen, USA). The cell culture medium was stored at 4°C until further use.

1× Phosphate buffered saline (PBS) solution

The 1× PBS solution was prepared by diluting 10× Calcium- and Magnesium-Free Phosphate buffered saline (PBS) (Mediatech Inc., USA) at 1/10 with dH_20 and autoclaved at 121°C, 15 psi for 15 min before being stored at room temperature.

1× Phosphate buffered saline (PBS) solution with EDTA

The 1× PBS solution with EDTA consisted of 39.4 mg of EDTA powder (Gibco, USA) in 200.0 ml of 1× PBS. The solution was then autoclaved at 121°C, 15 psi for 15 min before being stored at room temperature.

0.25% Trypsin-EDTA solution

The 0.25% Trypsin-EDTA was prepared by diluting 2.5% trypsin (SAFC Biosciences, USA) at 1/10 with 1× PBS with EDTA before being stored at room temperature.

0.4% Trypan blue solution

The trypan blue solution consisted of 40.0 mg of trypan blue powder (Sigma-Aldrich, USA) dissolved in 10.0 ml of 1× PBS. The solution was mixed well and stored at room temperature.

MTT [3-(4,5-dimethylthiazol-2-γl)-2,5-diphenyl-tetrazoliumbromide] solution

The MTT solution was prepared by dissolving 5.0 mg of MTT powder (Merck, Germany) to 1.0 ml of 1× PBS. The solution was mixed well and stored at 4°C in the dark until further use.

10 mM Tris-Cl, pH 7.5

The 10.0 mM Tris-Cl, pH 7.5 was prepared by dissolving 75.7 mg of powdered Tris base (Promega, USA) in 50.0 ml of DEPC-treated water. The pH of the solution was then adjusted to pH 7.5 with HCl and stored in room temperature.

Commercial kits

Commercial kits used in this study were:

1. Agilent RNA 6000 Nano Kit (Agilent Technologies, USA)
2. FlashTag™ Biotin RNA Labelling Kit for Affymetrix® GeneChip® miRNA Arrays (Genisphere, USA)
3. GeneChip® miRNA Array (Affymetrix, USA)
4. GeneChip® Eukaryotic Hybridization Control Kit (Affymetrix, USA)
5. GeneChip® Hybridization, Wash and Stain Kit (Affymetrix, USA)
6. TaqMan® MicroRNA Reverse Transcription Kit (Applied Biosystems, USA)
7. TaqMan® MicroRNA Assays (Applied Biosystems, USA)

Appendix 2: MTT assays on Ca Ski cells for standalone ACA

Standalone ACA – 12 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	8.0
2.0	89.2	1.3
4.0	75.6	6.7
6.0	51.1	6.7
8.0	45.5	2.7
10.0	37.5	4.0
12.0	20.5	4.0

Standalone ACA – 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	6.7
2.0	80.9	1.7
4.0	68.7	1.7
6.0	48.1	3.3
8.0	39.7	3.3
10.0	29.0	1.7
12.0	20.2	5.0

Standalone ACA – 36 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	3.1
2.0	80.6	2.0
4.0	62.1	3.1
6.0	40.3	4.1
8.0	26.6	5.1
10.0	15.3	4.1
12.0	9.7	4.1

Standalone ACA – 48 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100	3
2.0	73.3	3.7
4.0	38.2	0.7
6.0	16.8	2.2
8.0	11.5	0.7
10.0	5.3	0.7
12.0	0	1.5

Appendix 3: MTT assays on Ca Ski cells for standalone CDDP

Standalone CDDP – 12 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	3.9
33.3	85.3	1.5
66.7	62.5	3.9
100.0	61.0	3.9
133.3	56.6	3.9
166.6	47.8	2.0
200.0	43.4	2.0

Standalone CDDP – 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	1.4
33.3	66.4	5.7
66.7	38.7	4.3
100.0	33.6	4.3
133.3	30.7	1.4
166.6	33.6	1.4
200.0	32.1	2.9

Standalone CDDP – 36 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	3.9
33.3	47.4	3.9
66.7	24.8	3.9
100.0	17.5	3.9
133.3	11.7	2.6
166.6	10.2	1.3
200.0	5.8	1.3

Standalone CDDP – 48 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	3.2
33.3	36.4	6.4
66.7	7.0	1.1
100.0	2.3	5.3
133.3	0.0	2.1
166.6	0.0	1.1
200.0	0.0	1.1

Appendix 4: MTT assays on Ca Ski cells for ACA in combination with CDDP

Simultaneous treatment with ACA at constant concentration for 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100	8.1
16.7	43.5	8.1
33.3	38.7	3.2
50.0	29.0	3.2
66.7	29.0	4.8
83.3	27.0	9.7
100.0	30.6	4.8

Simultaneous treatment with CDDP at constant concentration for 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100	2.6
2.0	57.3	0.9
4.0	25	3.4
6.0	29	0.9
8.0	16.9	3.4
10.0	15.3	2.6
12.0	8.1	5.2

Sequential pre-treatment with ACA for 12 h followed by CDDP for 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	6.5
16.7	37.8	1.2
33.3	33.3	0.6
50.0	35.1	0.6
66.7	18.9	0.6
83.3	23.4	2.4
100.0	14.4	0.6

Sequential pre-treatment with CDDP for 12 h followed by ACA for 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	3.4
2.0	60.9	0.9
4.0	53.6	9.5
6.0	43.6	2.6
8.0	35.4	6.9
10.0	38.2	9.5
12.0	30.9	3.4

Appendix 5: MTT assays on NP69 cells for standalone ACA

Standalone ACA – 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	3.0
6.0	83.0	6.3
12.0	75.0	17.2

Appendix 6: MTT assays on NP69 cells for standalone CDDP

Standalone CDDP – 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100.0	9.5
100.0	64.0	2.8
200.0	45.0	3.7

Appendix 7: MTT assays on NP69 cells for ACA in combination with CDDP

Simultaneous treatment with ACA at constant concentration for 24 h

Concentration (µM)	Total viable cells (%)	Standard deviation (%)
0.0	100	7.4
50.0	79.0	17.1
100.0	82.0	17.6

Appendix 8: Predicted gene targets using TargetScanHuman v5.2

Genes	hsa-miR-138	hsa-miR-210	hsa-miR-744
ABL1	n/a	n/a	-0.10
AKT1	-0.17	n/a	-0.04
AKT2	-0.09	n/a	n/a
ARAF	-0.12	n/a	n/a
ARNT2	-0.30	n/a	n/a
ATP2B2	n/a	n/a	-0.09
ATP2B3	n/a	-0.42	n/a
ATP2B4	n/a	n/a	-0.02
BAD	n/a	n/a	-0.04
BCL2	-0.08	n/a	n/a
BCR	-0.15	n/a	n/a
BIRC5	-0.12	n/a	n/a
CACNA1A	n/a	-0.02	n/a
CACNA1C	n/a	-0.18	n/a
CACNA1D	n/a	n/a	-0.10
CACNA1E	n/a	n/a	-0.11
CACNA1H	n/a	n/a	-0.12
CASP3	-0.29	n/a	n/a
CCND1	-0.01	n/a	n/a
CCNE1	-0.21	n/a	n/a
CDK6	-0.28	n/a	-0.04
CDKN2B	-0.26	n/a	n/a
CREBBP	-0.02	n/a	n/a
DVL2	-0.32	n/a	n/a
DVL3	-0.25	n/a	n/a
E2F2	-0.11	n/a	n/a
E2F3	-0.17	-0.27	n/a
EP300	-0.17	n/a	n/a
FZD7	-0.07	n/a	n/a
GRIN2D	n/a	n/a	-0.19
HIF1A	-0.18	n/a	n/a
IKBKB	-0.06	n/a	n/a
ITGA2	n/a	n/a	-0.02
JUN	n/a	n/a	-0.10
LEF1	-0.01	n/a	n/a
MAP2K2	n/a	n/a	-0.12
MAPK1	n/a	-0.1	n/a
PDGFA	n/a	n/a	-0.05
PDGFRB	-0.06	n/a	-0.18
PIK3R1	-0.11	n/a	n/a
PIK3R2	n/a	n/a	-0.13
PIK3R3	-0.18	n/a	n/a
PIK3R5	n/a	-0.13	n/a

Genes	hsa-miR-138	hsa-miR-210	hsa-miR-744
PTK2	-0.30	n/a	n/a
SLC8A3	n/a	-0.23	n/a
SMAD3	n/a	n/a	-0.09
SMAD4	-0.14	-0.02	n/a
SOS2	-0.26	n/a	n/a
TCF7	-0.17	n/a	-0.17
TCF7L1	-0.17	n/a	-0.13
TRAF2	-0.07	n/a	n/a
WNT1	-0.02	n/a	n/a
WNT2B	-0.20	n/a	n/a
WNT3	-0.11	n/a	n/a
WNT3A	-0.11	n/a	-0.02
WNT4	-0.10	n/a	-0.01
WNT5A	n/a	n/a	-0.17

† Values represent the total context score as predicted using TargetScanHuman v5.2, whereby the lower the value, the higher chance of it being an actual target.