Abstract #832

Suicide gene therapy is effective for treating chemo-resistant small cell lung cancer cells

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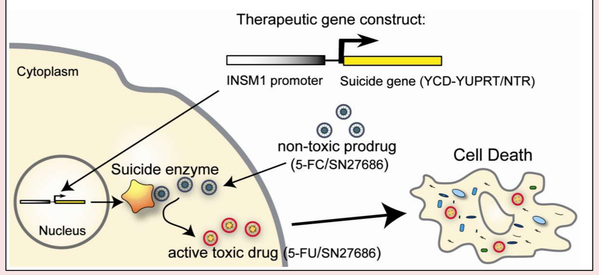
Background

- Small Cell Lung Cancer (SCLC) is a highly aggressive cancer and SCLC patients have a very poor prognosis
- Resistance arises in patients with SCLC following treatment with chemotherapy
- Suicide gene (SG) therapy driven by the SCLC specific Insulinoma-associated 1 (INSM1) promoter makes it possible to target suicide toxin production and cytotoxicity exclusively to SCLC
- This study examined INSM1 promoter-driven SG therapy using different systems: Yeast cytosine deaminase-uracil phosphoribosyl transferase (YCD-YUPRT) in combination with the prodrug 5-fluorocytosine (5-FC) and E.coli nitroreductase (NTR) together with the bromomustard prodrug SN27686 (figure below)

SG-therapy strategy:



Aim

To test the influence of chemo-resistance on the therapeutic potential of transcriptional targeted SG therapy

Methods

- SCLC cell lines:
  - NCI-H69-derived cell lines resistant to etoposide (H69-VP), cisplatin (H69-CPR), BCNU (H69-BCNU) and daunorubicin (H69-DAU).
  - GLC-14, GLC-16, GLC-19; cell lines derived from the same patient before treatment, after chemotherapy with cyclophosphamide, doxorubicin and etoposide or after subsequent radiation therapy, respectively .
- Sensitivity profiles were obtained by MTT assay after treating the cells with etoposide (VP-16), cisplatin (CP), carmustine (BCNU) or daunorubicin (DAU).
- Protein expression was determined by Western blot (WB) analysis and Human apoptosis or phospho-kinase array kits
- INSM1 promoter activity was evaluated directly by transient transfection of cells with luciferase (LUC) reporter gene plasmids carrying the INSM1 promoter or the unspecific SV40 promotor or indirectly by real-time PCR quantification of INSM1 mRNA level.
- *In vivo* INSM1 promoter activity was evaluated by immunohistochemistry (IHC) of SCLC tumor xenografts stably expressing EGFP under the control of the INSM1 promoter
- The INSM1-YCD-YUPRT/5-FC and INSM1-NTR/SN27686 SG systems were tested by MTT assay after plasmid transfection by lipofectamine, and subsequent prodrug exposure.

Results

Chemo-sensitivity profiles of a panel of SCLC cell lines

Cell Line	Drug (μM) VP-16	CP	DAU	BCNU
NCI-H69	0.42 (0.35-0.50)	0.30 (0.22-0.42)	0.055 (0.042-0.073)	5.1 (3.2-8.1)
H69-VP	6.2 (5.6-6.8)	0.41 (0.29-0.58)		
H69-DAU			0.93 (0.30-2.9)	1.6 (0.96-2.6)
H69-CPR	0.37 (0.31-0.45)	3.1 (1.9-5.1)		
H69-BCNU			0.045 (0.036-0.055)	10.82 (5.7-20.6)
GLC-14	0.062 (0.051-0.076)	0.42 (0.29-0.60)	0.020 (0.013-0.030)	5.93 (5.05-6.96)
GLC-16	0.48 (0.46-0.51)	0.30 (0.26-0.34)	0.048 (0.039-0.059)	25.40 (21.8-29.6)
GLC-19	0.24 (0.19-0.31)	0.35 (0.22-0.55)	0.029 (0.026-0.032)	3.97 (3.28-4.82)

Table 1: Chemo-sensitivity of 8 SCLC cell lines to VP-16 (Etoposide), CP (Cisplatin), DAU (Daunorubicin) and BCNU (Carmustine) measured by MTT assay after 7 days of exposure. Table shows IC_{50} values with (95% CI)

The panel of chemo-resistant cell lines display a heterogeneous expression profile of proteins involved in response to chemotherapy

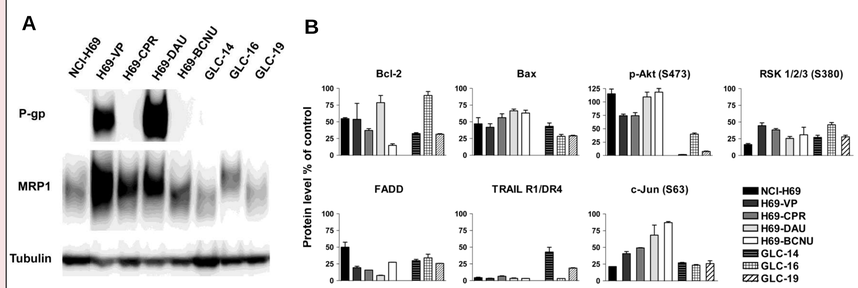


Figure 1: Expression of molecules involved in multi-drug resistance (MDR), apoptosis and survival pathways
A) Expression of MDR proteins. Detected by WB.
B) Expression of molecules involved in apoptosis and survival pathways. Detected by Human protein profiler arrays.

INSM1 promoter activity is high in both chemo-sensitive and -resistant SCLC cells and tumors

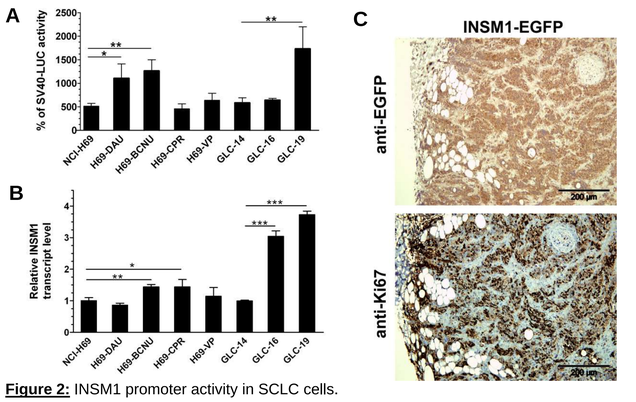


Figure 2: INSM1 promoter activity in SCLC cells.
A) Cells were transiently transfected with INSM1-LUC or SV40-LUC and luciferase activity was measured.
B) INSM1 mRNA transcript level measured by real-time qPCR.
C) IHC detection of EGFP (indirectly measurement of INSM1 activity) and Ki67 (proliferation) expression in xenografts of GLC-16 cells stably transfected with INSM1-EGFP plasmid.

- The utilized panel of SCLC cell lines exhibit resistance to a broad range of chemotherapeutic agents with varying IC_{50} values (table 1)
- Drug resistance facilitates changes in expression of proteins involved in regulation of MDR, apoptosis and kinase growth pathways (table 1 & figure 1)
- The INSM1 promoter activity is maintained in vitro regardless of chemo-resistance and changes in expression of proteins involved in response to chemotherapy (figure 1-2, table 1)
- The INSM1 promoter is highly active *in vivo* in chemo-resistant SCLC xenografts (figure 2)
- The chemo-resistant cell lines were equally sensitive to YCD-YUPRT/5-FC gene therapy as their chemo-sensitive variants (figure 3)
- Despite reduced sensitivity to NTR/SN27686 therapy in cells resistant to alkylating agents, equal cytotoxicity were obtained by exposing cells to higher prodrug doses, still not inducing off-target toxicity in NTR-negative cells (mock transfected) (figure 4)
- Additive cytotoxic effects were achieved in chemo-resistant SCLC cells exposed to combination therapy as compared to single agent therapy (figure 5)

Resistance to chemotherapy does not reduce the sensitivity to INSM1-YCD-YURPT/5-FC therapy

INSM1-YCD-YUPRT/5-FC sensitivity (μM)		
Cell line	IC_{50}	95% CI
NCI-H69	10.9	5.36 to 22.0
H69-CPR	6.26	4.82 to 8.14
H69-VP	15.3	7.33 to 32.0
H69-DAU	15.7	5.98 to 41.2
H69-BCNU	28.3	13.8 to 58.0
GLC-14	147.9	103.7 to 211.1
GLC-16	23.74	13.61 to 41.43
GLC-19	313.2	162.9 to 602.2

Figure 3: Effects of INSM1-YCD-YUPRT therapy. Cells were transiently transfected with INSM1-YCD-YUPRT or mock plasmid followed by exposure to series of 5-FC concentrations for 7 days.

INSM1-driven NTR/SN27686 therapy can effectively eliminate both chemo-sensitive and -resistant cells

INSM1-NTR/SN27686 sensitivity (μM)		
Cell line	IC_{50}	95% CI
NCI-H69	0.35	0.26 to 0.48
H69-CPR	4.7	3.8 to 5.7
H69-BCNU	1.2	0.92 to 1.6
GLC-16	0.64	0.51 to 0.81

Figure 4: Effect of INSM1-NTR/SN27686 therapy. Cells were transiently transfected with INSM1-NTR or mock plasmid followed by exposure to series of SN27686 concentrations for 7 days.

Combination suicide gene therapy and chemotherapy is superior to single agent therapy in chemo-resistant cells

Figure 5: Cytotoxic effect of combination therapy
A) Viability of the cells after transient transfection with INSM1-YCD-YUPRT plasmid and exposure to 5-FC, VP-16 and CP in indicated doses for 7 days.
B) Viability of cells transiently transfected with mock or the combination of INSM1-YCD-YUPRT and INSM1-FLAG.NTR plasmid and exposed to either 5-FC or SN27686 or a combination in indicated doses for 7 days

Conclusion

Targeted suicide gene therapy is a potent therapeutic approach for chemo-resistant SCLC with highest efficacy achieved when applied as combination therapy