Resistance to common chemotherapeutic agents do not influence the cytotoxic effects of suicide gene therapy in small cell lung cancer cell lines

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Background

- Small Cell Lung Cancer (SCLC) is a highly aggressive cancer and SCLC patients are characterized by having a very poor prognosis
- Resistance arises in patients with SCLC following treatment with chemotherapeutics
- Suicide gene (SG) therapy is a novel treatment strategy for cancer in which the introduced therapeutic gene encodes an enzyme capable of transforming a non-toxic prodrug into a cell poison
- Using the SCLC specific promoter Insulinoma-associated 1 (INSM1) in combination with the suicide gene encoding the super cytosine deaminase (SCD) enzyme, which converts the prodrug 5-fluorocytosine (5-FC) into the toxin 5-fluorouracil (5-FU), it is possible to control SG expression and resulting cytotoxicity exclusive to SCLC cells

Aim

To test the ability of transcriptionally targeted SG therapy to overcome chemo-resistance in SCLC cells

Material and Methods

- SCLC cell lines:
  - NCI-H69-derived cell lines resistant to etoposide (NCI-H69-VP), cisplatin (NCI-H69-CPR), BCNU (NCI-H69-BCNU) and daunorubicin (NCI-H69-DAU).
  - GLC-14, GLC-16, GLC-19; cell lines derived from the same patient respectively before treatment, after chemotherapy with cyclophosphamide, doxorubicin and etoposide and after subsequent radiation therapy.
- Resistence profiles were obtained by MTT assay and clonogenic assay after treating the cells with etoposide, cisplatin, BCNU, daunorubicin and 5-FU.
- The INSM1-SCD/5-FC SG system was tested by MTT assay (plasmid transfection using lipofectamine) and clonogenic assay (adeno-virus transduction).
- INSM1 promoter activity was evaluated by transient transfection of cells with luciferase (LUC) reporter gene plasmids carrying the INSM1 promoter or the unspecific SV40 promotor.
- Protein expression was determined by Western blot (WB) analysis

Results

Phenotypic changes of chemo-resistant vs. chemo-naive cells

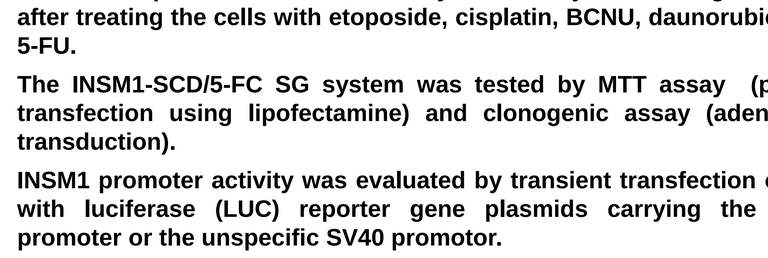

Figure 1 a) WB detection of the proteins Bax, Bcl-2 and p-Akt in all chemo-resistant and chemo-naive cell lines used b) Pictures illustrating the growth changes of chemo-resistant NCI-H69 derived cells compared to chemo-naive NCI-H69 cells

Results

Chemo-resistant cell lines are equally sensitive to INSM1-regulated suicide gene therapy compared to the chemo-naive cells

Drug		NCI-H69	NCI-H69-VP	NCI-H69-CPR	NCI-H69-BCNU	NCI-H69-DAU
Etoposide	IC50	0,42	6,2	0,37		
	RF		15	0,9		
Cisplatin	IC50	0,30	0,41	3,1		
	RF		1,4	10,3		
BCNU	IC50	5,1			10,8	1,6
	RF				2,1	0,3
Daunorubicin	IC50	0,06			0,04	0,43
	RF				0,7	7,2
5-FU	IC50	4,3	2,6	8,1	2,8	2,1
	RF		0,6	1,9	0,7	0,5

Table 1 Resistance profiles of H69 and derived cell lines towards a number of chemotherapeutics measured by MTT assay after 7 days of exposure. IC50 is the concentration were 50% reduction in cell viability is seen. Resistance factor (RF)= IC50 derived cell line/IC50 parental cell line (NCI-H69)

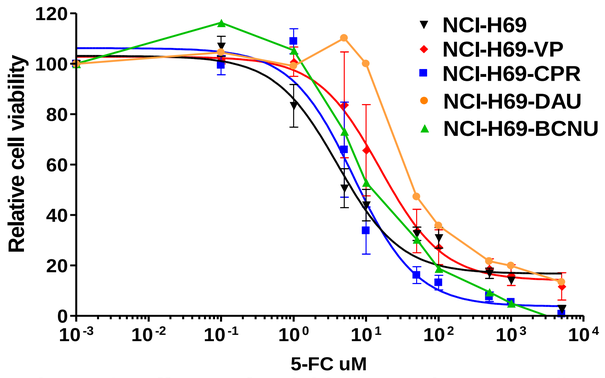

Figure 2. Effects of suicide gene therapy. Cells were transiently transfected with INSM1-SCD followed by exposure to series of 5-FC concentrations for 7 days.

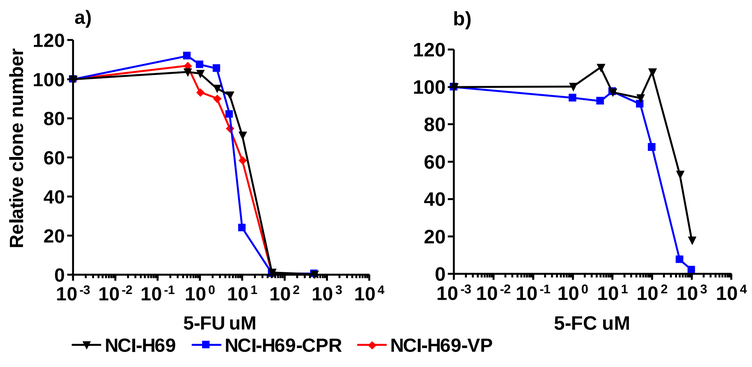

Figure 3. Confirmation of MTT results by clonogenic assays. Single cells plated in soft agar with varying concentrations of (a) 5-FU or (b) 5-FC together with adeno-INSM1-YCD-YUPRT-FLAG virus on a enriched feeder layer. Number of colonies was evaluated after 3 weeks .

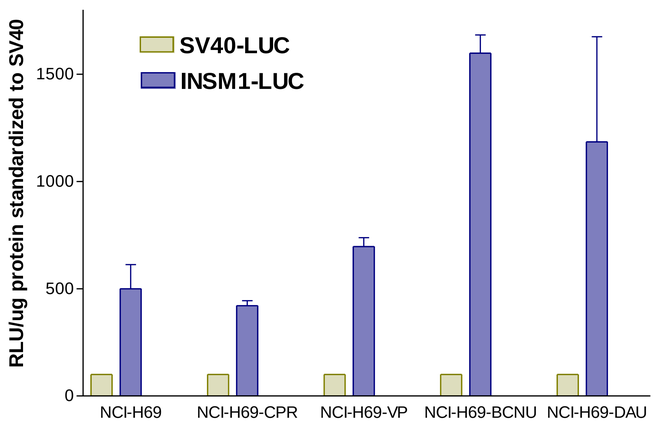

Figure 4. INSM1 promoter activity relative to SV40. Cells were transiently transfected with INSM1-LUC or SC40-LUC. Luciferase activity was measured relative to total cellular protein.

Resistance to common chemotherapeutics can even facilitate increased sensitivity to 5-FU

Drug		GLC-14	GLC-16	GLC-19
Etoposide	IC50	0,03	0,43	0,25
	RF		13,3	8,3
Cisplatin	IC50	0,74	0,34	0,86
	RF		0,5	1,2
BCNU	IC50	4,5	23,3	3,1
	RF		5,2	0,7
Daunorubicin	IC50	0,05	0,08	0,03
	RF		1,6	0,6
5-FU	IC50	10,3	1,9	2,4
	RF		5,4	4,2

Table 2 Resistance profile for GLC-14, -16 and -19 cell lines

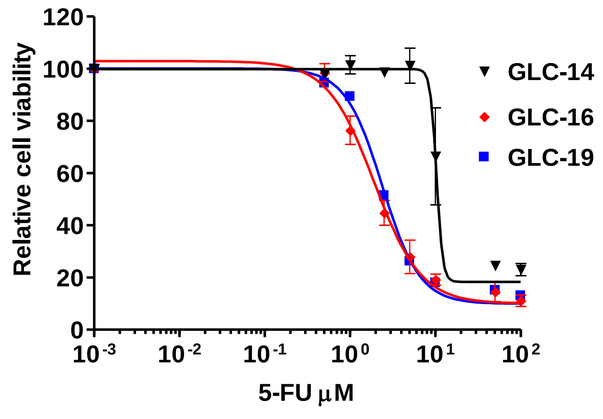

Figure 5. Cellular sensitivity towards 5-FU. Cells were treated with increasing 5-FU concentrations for 7 days and evaluated by MTT.

- Drug resistance facilitates changes in the growth pattern and expression of proteins involved in apoptosis and survival (figure 1)
- The used SCLC cell lines exhibit resistance to a broad range of chemotherapeutics with varying IC50 values (table 1 and 2)
- The H69 derived chemo-resistant cell lines were as sensitive to 5-FU treatment and SCD/5-FC gene therapy as their parental chemo-naive cell line (table 1, figure 2 and 3)
- The INSM1 promoter stays active regardless of drug resistance in NCI-H69 derived cells and even have a tendency to increase in some cell lines (figure 4)
- GLC-16 and GLC-19 are more sensitive to 5-FU than GLC-14, suggesting that this will also apply for SCD/5-FC gene therapy (figure 5)

Conclusion

Chemo-resistant SCLC cells are highly sensitive to transcriptionally targeted suicide gene therapy