The suicide fusion gene YCD-YUPRT induces significant cytotoxicity in small cell lung cancer cell lines and tumor growth delay in xenografts when regulated from a cancer specific promoter

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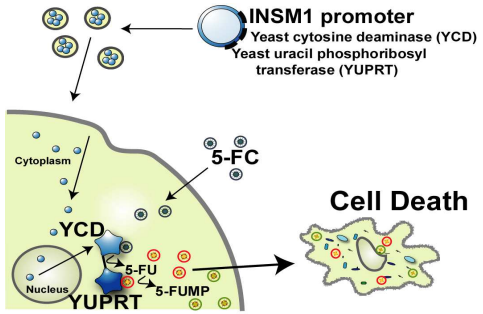
Aim

To identify efficient suicide gene therapy to use in combination with cancer specific promoters for the treatment of SCLC

Background

- Small cell lung cancer (SCLC) is characterized by aggresive progression, early dissemination and the fact that presently available treatment regimes are unable to cure patients.
- To develop a gene therapeutic strategy for systemic treatment of SCLC a high degree of targeting is needed
- We have identified SCLC specific promoter regions for the regulated expression of suicide genes in SCLC cells – the most efficient promoter identified to date is the insulinoma-associated 1 (INSM1) promoter
- A promising suicide gene strategy is the Yeast Cytosine Deaminase (YCD) gene fused with the Yeast Uracil Phosphoribosyl Transferase (YUPRT) gene in combination with the prodrug 5-fluorocytosin (5-FC)



- YCD converts 5-FC into the chemotherapeutic agent 5-fluorouracil (5-FU) while YUPRT augments the conversion of 5-FU into active cytotoxins (e.g. 5-FUMP)
- 5-FU and active cytotoxins can spread to nearby cancer cells and induce cytotoxicity (known as bystander effect)

Results

• A panel of SCLC cell lines are highly sensitive towards YCD-YUPRT/5-FC suicide gene therapy regulated from the SCLC-specific promoter INSM1

• The YCD-YUPRT/5-FC therapy is superior to therapy with the well-established suicide gene system consisting of the Herpes Simplex Virus Thymidine Kinase (HSVTK) gene and prodrug Ganciclovir (GCV) in all tested cell lines

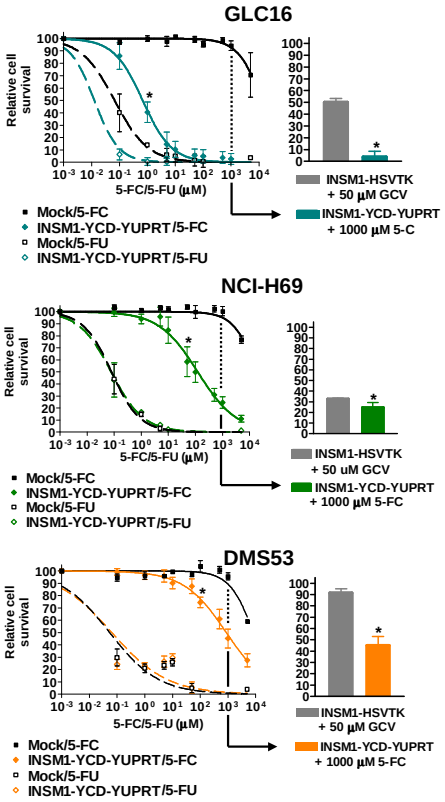


Figure 1. SCLC cell lines were transiently transfected with (left graph) mock or INSM1-YCD-YUPRT vector and exposed to series of 5-FC or 5-FU concentrations or (right graph) INSM1-YCD-YUPRT or INSM1-HSVTK vector and exposed to maximum prodrug doses (non-toxic to mock cells) of 1000 uM 5-FC and 50 uM GCV respectively. Relative cell survival was measured with an MTT assay. * represents significant difference between INSM1-YCD-YUPRT/5-FC and mock/5-FC treated cells (left) or INSM1-HSVTK/GCV and INSM1-YCD-YUPRT/5-FC treated cells (right).

• Combining YCD-YUPRT/5-FC therapy with HSVTK/GCV increased cytotoxicity only at low 5-FC doses - no additional cytotoxicity could be achieved at high 5-FC doses

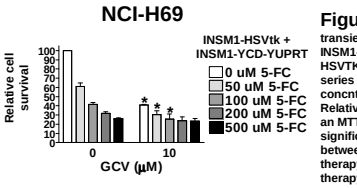


Figure 2. A SCLC cell line was transiently transfected with both INSM1-YCD-YUPRT and INSM1-HSVTK vector and exposed to series of 5-FC and GCV concntrations in combination. Relative cell death is measured by an MTT assay. * represents significant difference (P>0.05) between double suicide gene therapy and YCD-YUPRT/5-FC therapy alone (0 uM GCV)

• INSM1-YCD-YUPRT/5-FC gene therapy induces high bystander cytotoxicity in a panel of SCLC cell lines

• Cells not initially greatly affected from the suicide gene therapy (DMS53 - Figure 1) are highly sensitive to bystander toxins

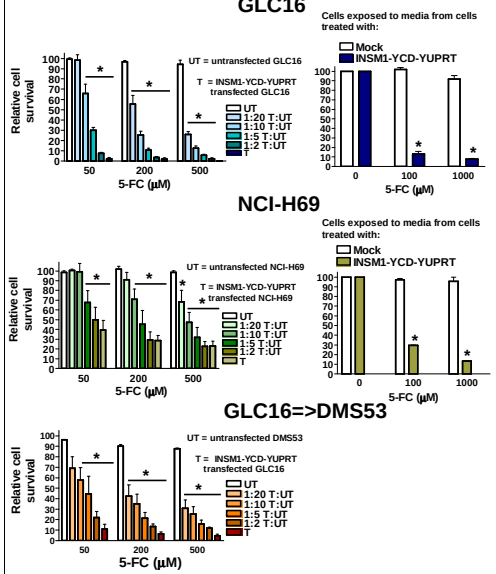


Figure 3. SCLC cell lines were (left graph) transiently transfected with INSM1-YCD-YUPRT vector (T) and mixed with untransfected cells (UT) in the specified ratios and exposed to series of 5-FC concentrations or (right graph) exposed to media from cells transiently transfected with mock or INSM1-YCD-YUPRT vector and exposed to series of 5-FC concentrations. Relative cell survival was measured with an MTT assay. * represents significant difference (P<0.05) compared to untransfected cells alone (left) or medium from mock/5-FC treated cells (right).

• Cell lines of other origin than SCLC do not respond to INSM1-YCD-YUPRT/5-FC gene therapy although highly sensitive to 5-FU treatment – confirms the specificity of the strategy

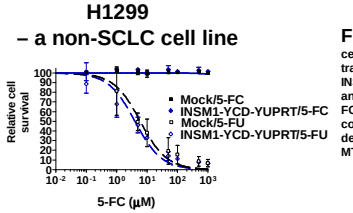


Figure 4. A non-SCLC cell line was transiently transfected with mock or INSM1-YCD-YUPRT vector and exposed to series of 5-FC or 5-FU concntrations in combination. Relative cell death is measured by an MTT assay.

• Treatment of SCLC xenografts on nude mice with INSM1-YCD-YUPRT/5-FC results in significant tumor growth delay compared to mock treated xenografts

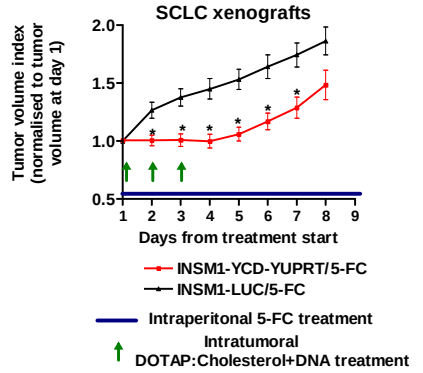


Figure 5. Xenografts were established from the SCLC cell line NCI-H69 by injection of cells on both flanks on nude mice. When tumor volume reached between 400-700 mm^3 intratumoral injektions with plasmid DNA pre-complexed with the DOTAP:Cholesterol lipid formulation was performed on 3 consecutive days. From day 1 of treatment start daily intraperitonal injections of 500 mg/kg 5-Fc was given until day 10 where animals were sacrified due to tumor size. * represents significant difference (P>0.05) between INSM1-YCD-YUPRT/5-FC and mock treated xenografts.

Conclusion

The suicide fusion gene YCD-YUPRT in combination with 5-FC is highly efficient for the treatment of SCLC when regulated from a SCLC specific promoter