

Identification of novel tumor suppressor genes specific for small cell lung cancer and primary glioblastoma multiforme



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Background

- Small cell lung cancer (SCLC) and primary glioblastoma multiforme (GBM) are two forms of cancer to which, at present, there are no satisfactory treatments.
- SCLC is a highly metastatic disease and is generally disseminated at the time of diagnosis.
- Less than 10% of the SCLC patients have a 5-year survival rate.
- Primary GBM is the most common and aggressive primary brain tumor and it is also the most fatal.
- The median survival time for optimally treated GBM patients is limited to ~15 months, and the two year survival rate is ~27%.
- There is an urgent need for development of more efficient treatment modalities for patients with SCLC and primary GBM.
- A promising strategy to treat SCLC and GBM patients is cancer gene therapy and one of the commonly used approaches is *tumor suppressor restoration therapy*.
- As different types of cancers have different tumor suppressor deficiencies, the strategy must be customized to each cancer type.

Aim

To identify and characterize novel tumor suppressor gene candidate(s) for SCLC and primary GBM, which eventually can be tested for use in cancer gene therapy.

Materials and Methods

Affymetrix Microarray Analysis and Validation:

Total RNA was isolated from SCLC, GBM, NIH3T3 and Ras-transformed NIH3T3 cell lines, SCLC and GBM xenografts and SCLC tumors or obtained from commercial sources (Ambion/Clontech). Labeled cRNA was analyzed on the Affymetrix[®] human U95Av2 and murine U74A,B and Cv2 GeneChips.

The microarray data was verified by semi-quantitative RT-PCR analysis.

Western Blot analysis

Cells were lysed in RIPA lysis buffer and equal amounts of protein (10 µg) resolved by SDS-PAGE and electroblotted onto nitrocellulose membranes (Invitrogen). Proteins of interest were detected by immunoblotting in an Autochemi System (UVP).

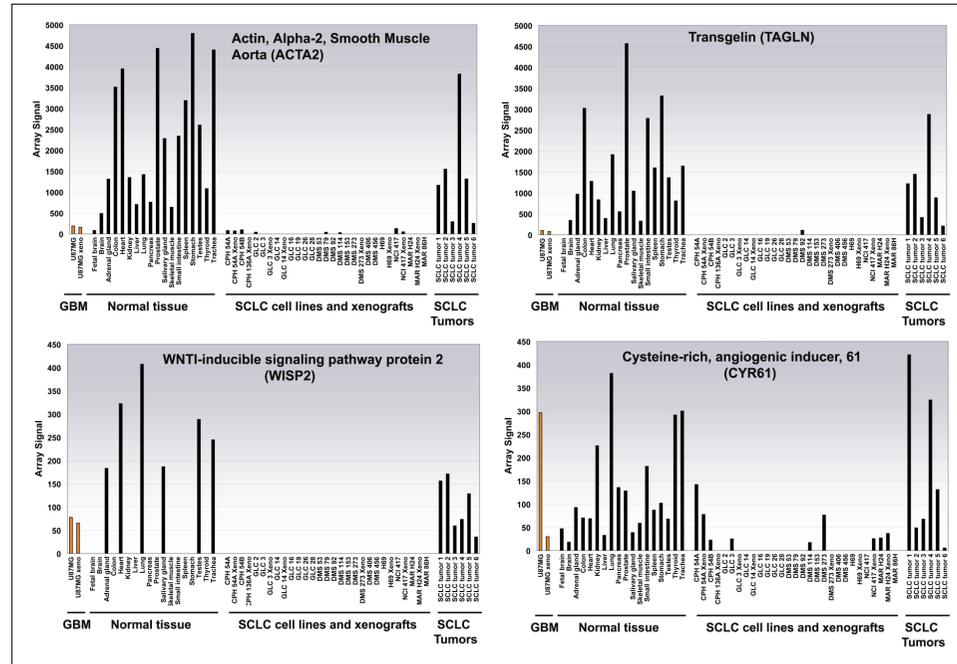


Figure 1. CYR61, WISP2, ACTA2 and TAGLN mRNA levels in GBM and SCLC cell lines, GBM and SCLC xenografts and SCLC tumors as compared to normal tissues assessed by Affymetrix microarray.

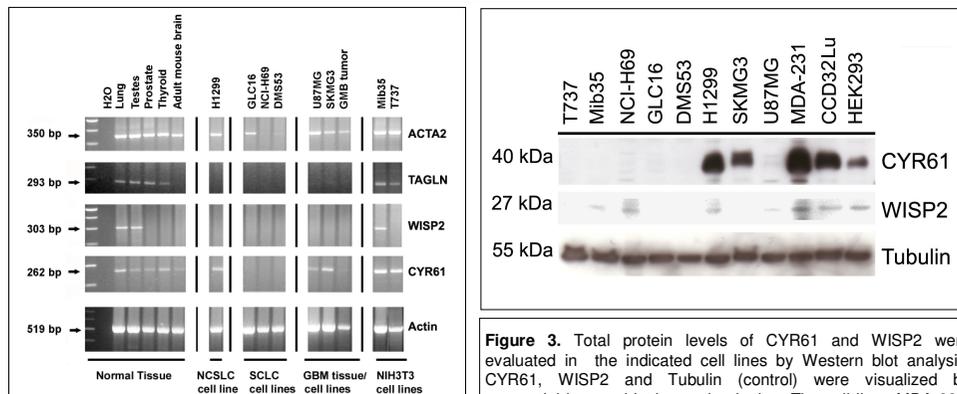


Figure 2. The microarray data was verified by semi-quantitative RT-PCR. NSCLC denotes Non Small Cell Lung Cancer. Mib35 and T737 are untransformed and Ras-transformed mouse NIH3T3 fibroblast cell lines, respectively.

Results

- By a global gene expression analysis (Affymetrix)¹ we have identified four genes, for which the mRNA levels are markedly downregulated in SCLC and GBM cell lines, SCLC and GBM xenografts and SCLC tumors compared to normal cells and tissues (**Figure 1**).
- The four genes are: ACTA2, TAGLN, WISP2 and CYR61 (**Figure 1**).
- The microarray data was verified by semi-quantitative RT-PCR showing similar results (**Figure 2**).
- Some of these genes are also downregulated in the Ras-transformed mouse NIH3T3 cell line (T737) compared to the parental cell line (Mib35) (**Figure 2**).
- The decreased expression levels of CYR61 and WISP2, were also reflected at the protein level, as assessed by Western Blot analysis (**Figure 3**). However, total protein levels of TAGLN and ACTA2 have not yet been evaluated.
- Contrary to the other genes, CYR61 is highly expressed in the glioma cell lines U87MG and SKMG3 (**Figure 1-3**). CYR61 has been found overexpressed in glioma cell lines and tumors from primary GBM², which is in agreement with our findings.
- CYR61 and WISP2 have been shown to act both as tumor suppressors and oncoproteins depending on the tissues in which they are expressed²⁻⁵.
- ACTA2 and Transgelin have been shown to be downregulated in transformed and cancer cell lines compared to normal cells^{6,7}. However, it is still unclear whether they can act as tumor suppressors.

Conclusions

- The expression levels of WISP2, ACTA2 and TAGLN are markedly down-regulated in GBM, SCLC and Ras transformed NIH3T3 cells as compared to normal cells and tissues.
- CYR61 expression level is highly downregulated in SCLC cells compared to normal tissues.
- CYR61, WISP2, ACTA2 and TAGLN are TSG candidates in SCLC and/or GBM.

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Figure 3. Total protein levels of CYR61 and WISP2 were evaluated in the indicated cell lines by Western blot analysis. CYR61, WISP2 and Tubulin (control) were visualized by sequential immunoblotting and stripping. The cell lines MDA-231, CCD32Lu and HEK293 are human breast cancer, human lung fibroblast and human embryonal kidney epithelial cell lines, respectively.