Identification of tumour-suppressor-gene candidates in small cell lung cancer, which alone or combined with a p53-reactivating molecule, can be used for cancer gene therapy

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Background

Small cell lung cancer (SCLC) is a deadly disease with no current satisfactory treatments. It is a highly disseminated disease and is generally disseminated at the time of diagnosis.

There is therefore an urgent need for development of more efficient treatment modalities for patients with SCLC.

One intriguing modality is tumour-suppressor-restoration-therapy.

As different types of cancers have different tumour suppressor deficiencies, the strategy must be customized to each cancer type, either by using tumour specific tumour suppressor genes (TSG) or by combining different TSG.

p53 is mutated in up to 90% of SCLC tumours, and as mutant p53 tends to accumulate in tumour cells, an effective approach would be to reactivate mutant p53 in cancer cells combined with reintroduction of another TSG, frequently inactivated in SCLC.

PRIMA-1<sup>Met</sup> (p53-dependent reactivation and induction of massive apoptosis) is a small molecule reported to have the capacity of restoring tumour-suppressor function to mutant p53 in various cancer cell lines and hence induce cancer cell death.

Whether PRIMA-1<sup>Met</sup> is capable of reactivating mutant p53 in SCLC cells are not yet known.

Aim

To test the effect of PRIMA-1<sup>Met</sup> on SCLC cell lines with mutant p53.

To identify TSG candidates in SCLC which can be combined with PRIMA-1<sup>Met</sup> to be used for reactivation of SCLC gene therapy.

Materials and Methods

Detection of p53 mutations: RNA was extracted from various SCLC cell lines and p53 cDNA was synthesized using RT-PCR and sequenced. The results were compared to a normalised genome to detect p53 mutations.

In some of the SCLC cell lines, high levels of mutant p53 was detected (Figure 1).

PRIMA-1<sup>Met</sup>, a methylated form of the original compound PRIMA-1 (Figure 2), inhibited the growth of the SCLC cell lines with high levels of mutant p53 markedly, but only caused a minor reduction in growth rate in the control cell lines H1299 (p53 null) and CCD32lu (wt-p53).

The IC50 values for PRIMA-1<sup>Met</sup> varied depending on cell lines tested. However, SCLC cell lines expressing mutant p53 had a lower IC50 value than those with wt-p53 (CCD32lu or p53 null) (Table 2).

MTT assay: Cells were seeded in 96-well plates and incubated with various concentrations of PRIMA-1<sup>Met</sup> for 72 h. The plates were incubated for 4 h with MTT, after which a solubilization buffer (10% SDS, 0,03 M H2O2) was added.

Transient transfection: Cells were transiently transfected with expression vectors encoding either Cyr61 or WISP2 using Lipofectamine 2000.

Results

The mutation status of p53 in a panel of SCLC cell lines was previously evaluated in our laboratory (Table 1).

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Discussion

PRIMA-1<sup>Met</sup> seems to reactivate mutant p53 in SCLC, leading to transactivation of target genes, and subsequently apoptosis.

CyR61 and WISP2 do not seem to be effective alone but might induce pronounced growth suppression when combined with PRIMA-1<sup>Met</sup>.

Conclusion

The non-transformed diploid human lung fibroblast cell line, CCD32Lu, DMS273, GLC14, GLC16 and GLC19) was studied using MTT assay.

The growth suppression effect of different concentrations of PRIMA-1<sup>Met</sup> on a panel of SCLC cell lines with p53 mutation (DMS273, GLC14, GLC16 and GLC19) was studied using MTT assay. The non-transformed diploid human lung fibroblast cell line, CCD32Lu, expressing wild type p53 and the p53 null NSCLC cell line H1298, were used as control cell lines.

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