

### In-vitro combination of arsenic trioxide and chemotherapy in small-cell lung cancer

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**Introduction:** Arsenic trioxide (ATO), an anti-tumour agent with multi-faceted mechanisms of action, has become a breakthrough treatment for acute promyelocytic leukaemia in recent years. There have been preliminary data about the potential activity of ATO in solid tumours, including small-cell lung cancer (SCLC). As SCLC is considered a chemo-sensitive malignancy, we conducted an in-vitro study examining the cytotoxic effects of ATO, clinically effective chemotherapeutic agents, or a combination of both in a SCLC cell line model.

**Methods:** Drug treatment (ATO, cisplatin, etoposide) experiments were performed in 4 SCLC cell lines (H-187, DMS-79, H-526, H-69) obtained from ATCC. The cancer cell viability was assessed by 3-(4,5-dimethyl-thiazoyl-2-yl)-2,5-diphenyl-tetrazolium bromide (MTT) assay. The proportion of apoptotic cell death was detected by Annexin-V/Propidium iodide assay with flow cytometry. The effects of combination treatment were determined by isobologram analysis using standard computer software (CalcuSyn, Biosoft, US).

**Results:** All the SCLC cell lines reacted to ATO and traditional chemotherapeutic reagents in time- and dose-dependent way. Two of them (H-187, H-526) were sensitive to ATO (eg  $IC_{50}[48\text{ hr}]=2.4\pm 0.35\ \mu\text{M}$  and  $IC_{50}[48\text{ hr}]=2\pm 0.21\ \mu\text{M}$ ), while the other two SCLC cell lines, DMS-79 and H-69, were resistant to either ATO (eg  $IC_{50}[48\text{ hr}]=12.3\pm 1.5\ \mu\text{M}$  and  $10\pm 3.7\ \mu\text{M}$ ) or chemotherapeutic agents ( $IC_{50}=10-50\ \mu\text{M}$ ). Moderate synergistic cytotoxicity or additive effect were found in ATO and cisplatin combination treatment either in sensitive or resistant cell lines ( $CI=0.5-0.9$ ). On the other hand, antagonistic interaction was shown in the combination of ATO and etoposide in all 4 cell lines ( $CI=0.9-2$ ).

**Conclusion:** Combination of ATO and cisplatin was synergistic in chemo-sensitive and additive in chemo-resistant SCLC models. However, the combination of ATO and etoposide has resulted in antagonism. Further study is needed to determine the possibility and best schedule of combination treatment of ATO with existing standard chemotherapy.

### 'Yin-Yang' regulation of insulin signalling by APPL1 and APPL2 in skeletal muscle cells

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**Introduction:** APPL1 and APPL2 are two intracellular adapter proteins containing a PH domain, a PTB domain, and a Leucine zipper motif. A growing body of evidence suggests that APPL1 acts as a key signalling molecule integrating multiple signalling stimuli. We have recently demonstrated that APPL1 potentiates insulin actions in liver and adipose tissue. However, the physiological functions and the underlying molecular mechanisms of APPL1 and APPL2 in regulating insulin actions in skeletal muscle have not been explored. The objective of this study was to investigate the role of APPLs in insulin signalling and downstream glucose uptake in both cultured myocytes and rodent models.

**Methods:** Proteins physically associated with APPL1 or APPL2 were retained by affinity purification and co-immunoprecipitation, followed by mass spectrometry-based proteomic identification. The key domains of APPL2 involved in its interaction with APPL1 and TBC1D1 were determined by progressive truncation and site directed mutagenesis. Effects of APPL1 and APPL2 in regulating insulin signalling were measured by Akt phosphorylation and in-vitro or ex-vivo glucose uptake assay.

**Results:** Overexpression of APPL2 inhibits insulin-stimulated Akt phosphorylation leading to down-regulation of glucose uptake in  $C_2C_{12}$  myotubes and in skeletal muscle of the APPL2 transgenic mice. In contrast, suppressing APPL2 expression by RNAi significantly enhances insulin-stimulated Akt phosphorylation and glucose uptake in  $C_2C_{12}$  myotubes. However, APPL1 exerts opposite effects in regulating insulin signalling in muscles compared to APPL2. Co-immunoprecipitation assay followed by western blot analysis revealed the interaction of TBC1D1 with APPL2 but not APPL1. Furthermore, the interaction occurs in the N-terminal PTB domain of TBC1D1 and N-terminal BAR domain of APPL2. The binding of APPL2 to TBC1D1 was enhanced by insulin-stimulated Akt activation, and suppressed by overexpression of APPL1.

**Conclusion:** APPL1 and APPL2 act as a pair of 'Yin-and-Yang' molecules critically involved in the regulation of insulin signalling and glucose uptake in skeletal muscle. Further investigations on these two proteins might lead to the identification of novel regulatory mechanisms that underlie insulin resistance and diabetes.