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Using a 3-D model system to screen for drugs effective on solid tumors

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen
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ABSTRACT

There is a large medical need for the development of effective anticancer agents with minimal side effects. The present thesis represents an attempt to identify potent drugs for treatment of solid tumors. We used a strategy where 3-D multicellular tumor spheroids (cancer cells grown in three dimensional culture) were utilized as *in vitro* models for solid tumors. Drug libraries were screened using spheroids as targets and using apoptosis induction and loss of cell viability as endpoints. The hanging drop method for production of spheroids was modified to allow spheroid generation in the 96-well format. Initial studies showed that the screening of multicellular spheroids resulted in the identification of different hit compounds compared to screening of monolayer cultures. Interestingly, we found that spheroid screening enriched for hydrophobic compounds (XlogP >4), a finding of considerable interest for chemical library design and lead optimization in the field of anticancer drug development.

An approach based on the analysis of drug-induced gene profiles was used to unravel the mechanism of action of hits identified in the screen. The proposed mechanisms of action were subsequently confirmed by specific *in vitro* assays.

The generation of a caspase-cleaved product of cytokeratin 18 was used to determine apoptosis of carcinoma cells in spheroids. The same method could be used as a plasma biomarker to evaluate whether candidate compounds induced apoptosis in xenograft tumor models. The antibodies used in the assay recognize human but not mouse cytokeratin 18 – an advantage when xenograft models are used since tumor apoptosis can be specifically measured in blood samples.

The screening work resulted in the identification of a novel topoisomerase inhibitor (thaspine), a novel iron chelator (CB21) and a number of microtubuli inhibitors.

- Thaspine (an alkaloid from *Croton lechleri*) was identified in both monolayer and spheroid screening experiments. Thaspine was found to inhibit both topoisomerase I and II.

Interestingly, thaspine was effective on cell lines overexpressing drug efflux transporters and showed *in vivo* activity.

- CB21 was of particular interest, since it was toxic to the hypoxic quiescent cell population in spheroid cores which are known to be resistant to many chemotherapeutic drugs. The compound was not toxic to quiescent immortalized cells. CB21 was shown to be a very potent iron chelator. The compound induced marked induction of autophagy both in the outer and inner layers of spheroids. Interestingly, CB21 increased glucose uptake and reduced cellular oxygen consumption. The cytotoxicity of the compound was found to be increased during low glucose conditions, known to occur in the cores of spheroids. The compound showed a significant inhibitory effect in tumor xenografts.

- A number of novel microtubuli inhibitors were identified in the spheroid screen. This result was unexpected since such compounds are expected to be preferentially active on dividing cells.

We conclude that drug screening using multicellular spheroids is a promising approach for anticancer drug discovery. A number of novel compounds were identified by screening, and some may be possible to develop for clinical use.