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Classification of anti-cancer drugs based on tissue penetration using a novel *in vitro* screening assay

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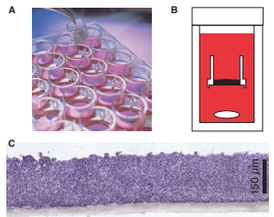
Multilayered cell culture

A 3-D model of the tumour extravascular compartment

Multilayered cell culture (MCC)

MCC is a three-dimensional tissue culture which models the tumour extravascular compartment and can be used to study drug penetration in a controlled environment.

Similar to spheroids, MCCs are grown from tumour cells as discs of tissue under controlled conditions using standard tissue culture inserts.

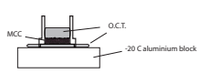


A| MCCs are grown from a single cell suspension seeded in tissue culture inserts. **B**| Once cells attach cultures are transferred to a stirred growth apparatus. **C**| H&E stained cryosection of a ~150 µm thick MCC showing the permeable support membrane.

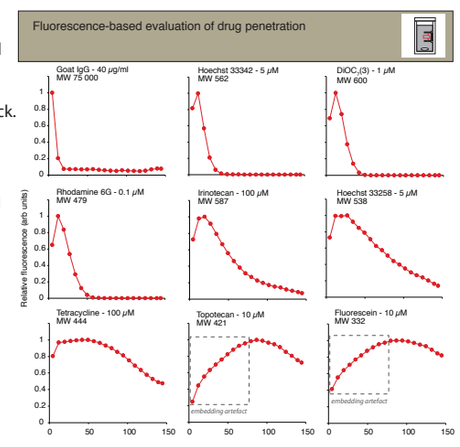
Direct visualisation of drug profiles

The relative distribution of a panel of fluorescent molecules was compared following 1-h exposure in HCT-116 MCCs 15 cell layers thick.

Results indicate a wide variation possible in drug distribution that does not solely follow molecular weight. Molecules that distributed best appeared to be subject to wash out during the freezing process.



Embedding process used to prepare MCCs for cryosectioning



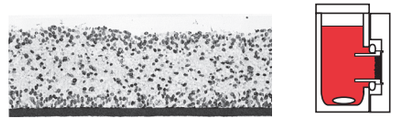
Effect-based evaluation of drug penetration

Effect-based screening assay

In addition to allowing direct visualisation of labelled drugs, the symmetrical growth that occurs in MCCs allows drug penetration to be evaluated by comparing a drug's effect on the cells on either edge of the cultures following exposure to one side.

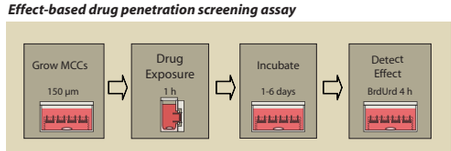
During growth the cultures have access to oxygen and nutrients from top and bottom and both edges exhibit high proliferation rates.

During drug exposure cultures are temporarily closed-off to allow drug build up.



BrdUrd immunostained MCC showing symmetrical S-phase cell distribution & the apparatus used to perform drug penetration experiments

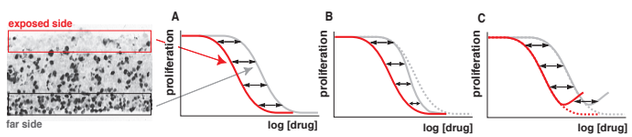
Effect-based drug penetration screening assay



Estimating drug penetration using an effect based assay

The shift in dose response curves obtained from the exposed and far sides of MCCs will yield an estimate of the difference in drug exposure that occurs on the two sides.

Examples of hypothetical dose response curves for exposed and far sides of MCCs following drug exposure for the cases of **A**| concentration independent drug penetration, **B**| drug penetration improves with driving concentration and **C**| cell loss or cell cycle perturbation occurs at higher concentrations.



Hypothesis

The symmetrical proliferation profile exhibited in MCCs can be applied to measure the ability of anti-cancer drugs to penetrate and distribute within tissue using a biological endpoint.

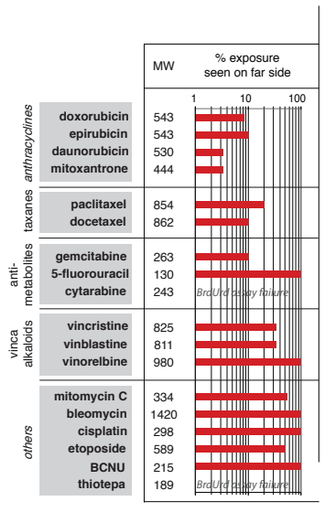
Conclusions

Using the effect-based assay the penetration of a panel of anti-cancer drugs was assessed in HCT-116 MCCs. Based on the results drugs could be grouped into four classes:

- near uniform tissue distribution (cisplatin, 5-FU and vinorelbine)
- 1-5 fold decrease in drug exposure to cells on the far side versus the exposed side of the cultures (vincristine, vinblastine, paclitaxel, mitomycin C and etoposide)
- ~10-fold decrease (doxorubicin, epirubicin, docetaxel and gemcitabine)
- more than 10-fold decrease (daunorubicin and mitoxantrone).

In the case of the anthracyclines and taxanes, the MCC-based assay was validated by comparing the predicted drug distributions with direct visualization of the drugs themselves (data previously presented).

This model could be applied as a screening system for the discovery of biologically active drugs which exhibit desirable penetration properties.



Acknowledgements

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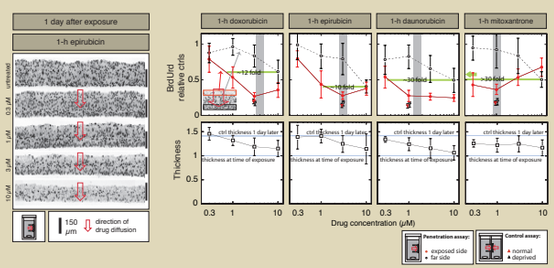
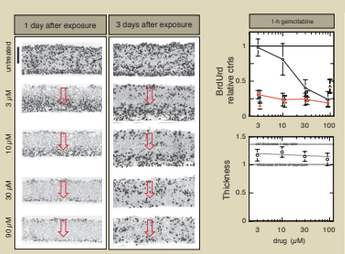
In detail:

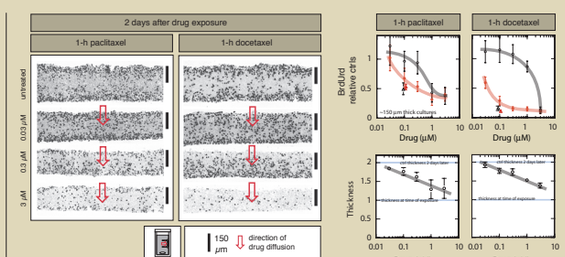
anthracyclines



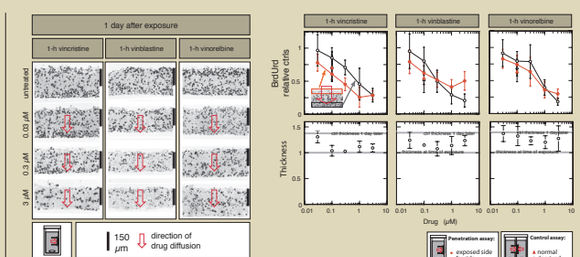
gemcitabine



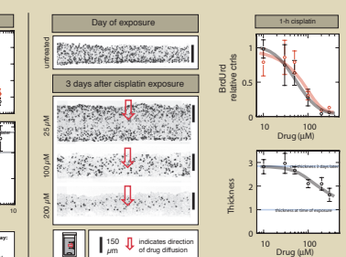
taxanes



vinca alkaloids



cisplatin

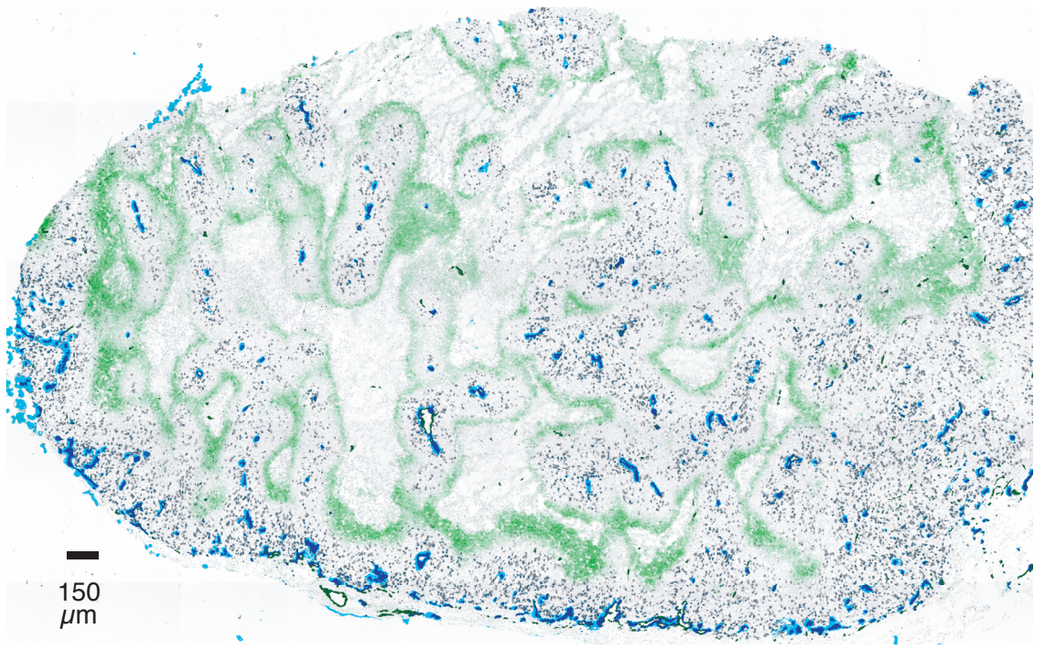


Tumour architecture & drug delivery

Tumour mapping

Tumour biology

Solid tumours contain a network of blood vessels supplying oxygen and nutrients. Tumour growth is limited by the balance struck between the consumption of nutrients & oxygen by the cells comprising the tumour and their supply from an expanding vasculature. Cells within tumours can be located up to ~15-20 cell layers (~150-250 µm) from a blood vessel, beyond which necrosis occurs.

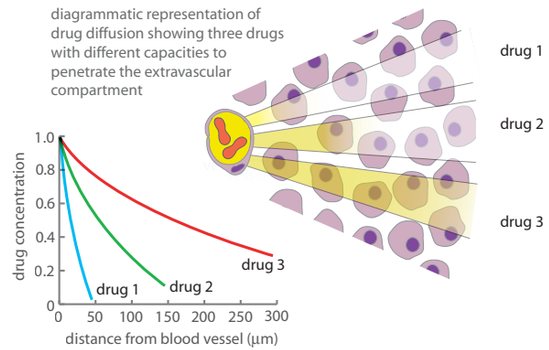


staining key:
- blood vessels (CD31)
- blood flow ($DiOC_7$)
- S-phase cells (BrdUrd)
- hypoxic areas (Pimonidazole)

Extravascular delivery of drugs

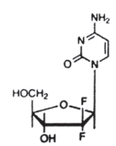
Diffusion limited tissue penetration

The penetration of drugs into solid tumours is limited by the balance between drug diffusion and tissue consumption in a fashion similar to that of oxygen. Drug binding, metabolism and sequestration within tissue all act to limit penetration.

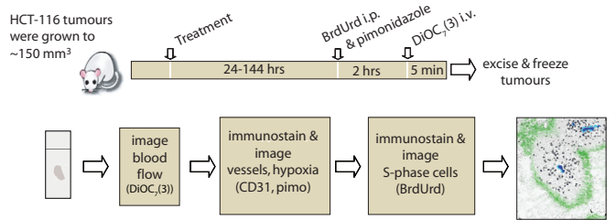


Mapping the effects of drugs within solid tumours

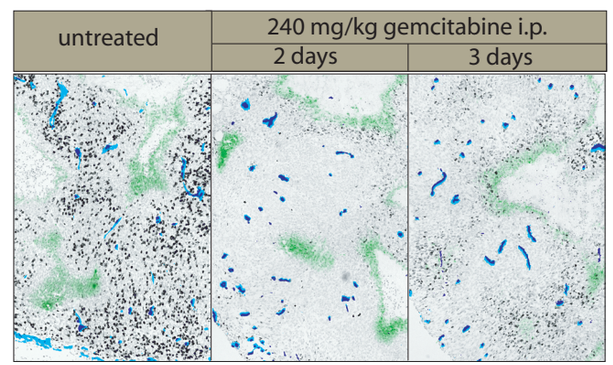
We mapped the effect of the pyrimidine analogue gemcitabine on tumor cell proliferation in relation to vasculature and hypoxia in HCT-116 xenografts.



gemcitabine



Following treatment proliferation appeared to recur in tumour cells distant from vasculature; due either to inadequate penetration or differing intrinsic sensitivity.



HCT-116 tumour xenografts showing the effect of gemcitabine 2 & 3 days after treatment.