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Caco-2 adenocarcinoma on chicken embryos chorioallantoic membrane (CAM) as an alternative to mammalian models for preclinical tests of new cytostatic drug formulations

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In the development of new cytostatic drug formulations targeting plays a prominent role. To test the efficacy of targeting strategies a frequent approach is to use immune-deficient nude mice grafted with tumors. The chicken embryo's chorioallantoic membrane (CAM) is a known alternative to mammalian *in vivo* models (Armstrong et al., 1982; Kunzi-Rapp et al., 2001) and a boarder-case between *in vitro* and *in vivo* model. It combines the advantages of *in vivo* models, that are to allow (simultaneous) investigating activity, biodistribution, pharmacokinetics, biocompatibility and toxicity of the drug and/or drug carrier, with some *in vitro* model advantages, like being quicker, less labor-intensive and pain-causing. However, the CAM model have not yet attracted that much interest in its role as test system for tumor-targeting drug delivery systems. This is possibly due to two major limitations: First, the difficulties to induce well-defined, reproducible damage of the CAM epithelium to enable reproducible tumor invasion and -growth also by moderately or well-differentiated tumor cell lines. Second the cumbersome quantization of tumor cells in contrast to CAM cells.

Caco-2 cell line, clone C2BBE1, was chosen as model for colorectal adenocarcinoma with limited tumorigenic potential. Without damaging treatment of the CAM epithelium Caco-2 cells were not able to penetrate into the CAM mesenchym. Mechanical treatment of a small CAM area and the supplementation of the inoculation sample with Matrigel® (BD Biosciences) lead to a reproducible tumor growth. Histological sections of these tumors showed typical growth patterns for colon cancer. In order to quantify the number of tumor cells, the tumors with surrounding CAM were cut, treated mechanically and subsequently enzymatically to produce single cell suspensions. A staining with tumor-specific EpCAM (CD326) antibody allowed distinguishing between tumor- and CAM cells in flow cytometer analysis.

In conclusion, the developed method enables the culturing and quantization of Caco-2 adenocarcinomas on the CAM. Therefore, this model should be a very useful tool to investigate drug delivery systems for cytostatic drugs.

References

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