Applying the chicken embryo chorioallantoic membrane assay to study treatment approaches in urothelial carcinoma.

Rapid development of novel treatment options demands valid preclinical screening models for urothelial carcinoma (UC). The translational value of high-throughput drug testing using 2-dimensional (2D) cultures is limited while for xenograft models handling efforts and costs often become prohibitive for larger-scale drug testing. Therefore, we investigated to which extent the chicken chorioallantoic membrane (CAM) assay might provide an alternative model to study antineoplastic treatment approaches for UC. The ability of 8 human UC cell lines (UCCs) to form tumors after implantation on CAMs was investigated. Epithelial-like RT-112 and mesenchymal-like T-24 UCCs in cell culture or as CAM tumors were treated with cisplatin alone or combined with histone deacetylase inhibitors (HDACi) romidepsin and suberanilohydroxamic acid. Tumor weight, size, and bioluminescence activity were monitored; tumor specimens were analyzed by histology and immunohistochemistry. Western blotting and quantitative real time polymerase chain reaction were used to measure protein and mRNA expression. UCCs were reliably implantable on the CAM, but tumor development varied among cell lines. Expression of differentiation markers...
(E-cadherin, vimentin, CK5, CK18, and CK20) was similar in CAM tumors and 2D cultures. Cellular phenotypes also remained stable after recultivation of CAM tumors in 2D cultures. Bioluminescence images correlated with tumor weight. Cisplatin and HDACi decreased weight and growth of CAM tumors in a dose-dependent manner, but HDACi treatment acted less efficiently as in 2D cultures, especially on its typically associated molecular markers. Synergistic effects of HDACi and subsequent cisplatin treatment on UCCs were neither detected in 2D cultures nor detected in CAM tumors. Our results demonstrate that the CAM assay is a useful tool for studying tumor growth and response to conventional anticancer drugs under 3D conditions, especially cytotoxic drugs as cisplatin. With some limitations, it might serve as a cost- and time-effective preclinical screening assay for novel therapeutic approaches before further assessment in expensive and cumbersome animal models.