CAM Issue in Cancers

Kunze, P.; Kreiss, L.; Novosadová, V.; Roehe, A.; Steinmann, S.; Prochazka, J.; Geppert, C.; Hartmann, A.; Schürmann, S.; Friedrich, O.; Schneider-Stock, R. Multiphoton Microscopy Reveals DAPK1-Dependent Extracellular Matrix Remodeling in a Chorioallantoic Membrane (CAM) Model. Cancers 2022, 14(10), 2364; https://doi.org/10.3390/cancers14102364.
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Cancer cells facilitate tumor growth by creating favorable tumor micro-environments (TME), altering homeostasis and immune response in the extracellular matrix (ECM) of surrounding tissue. A potential factor that contributes to TME generation and ECM remodeling is the cytoskeleton-associated human death-associated protein kinase 1 (DAPK1). Increased tumor cell motility and de-adhesion (thus, promoting metastasis), as well as upregulated plasminogen-signaling, are shown when functionally analyzing the DAPK1 ko-related proteome. However, the systematic investigation of how tumor cells actively modulate the ECM at the tissue level is experimentally challenging since animal models do not allow direct experimental access while artificial in vitro scaffolds cannot simulate the entire complexity of tissue systems. Here, we used the chorioallantoic membrane (CAM) assay as a natural, collagen-rich tissue model in combination with all-optical experimental access by multiphoton microscopy (MPM) to study the ECM remodeling potential of colorectal tumor cells with and without DAPK1 in situ and even in vivo. This approach demonstrates the suitability of the CAM assay in combination with multiphoton microscopy for studying collagen remodeling during tumor growth. Our results indicate the high ECM remodeling potential of DAPK1 ko tumor cells at the tissue level and support our findings from proteomics.

Wang, Y.; Rousset, X.; Prunier, C.; Garcia, P.; Dosda, E.; Leplus, E.; Viallet, J. PD-1/PD-L1 Checkpoint Inhibitors Are Active in the Chicken Embryo Model and Show Antitumor Efficacy In Ovo. Cancers 2022, 14(13), 3095; https://doi.org/10.3390/cancers14133095.
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Purpose: To assess the use of the chicken embryo (in ovo) model as an alternative in vivo model for immuno-oncology (IO) drug development, focusing on programmed cell death protein-1 (PD-1)/programmed cell death-ligand 1 (PD-L1) immune checkpoint inhibitors. (2) Methods: First, the presence of immune cells in the model was detected through the immunophenotyping of chicken peripheral blood mononuclear cells (PBMCs) based on fluorescence activated cell sorting (FACS) analysis and the immunohistochemistry (IHC) analysis of in ovo tumor-infiltrating lymphocytes. Second, the cross-reactivity between one anti-human PD-1 Ab, pembrolizumab (KEYTRUDA®), and chicken PD-1 was verified through the labelling of chicken splenocytes with pembrolizumab by FACS analysis. Third, the blockade effect of pembrolizumab on chicken PBMCs was assessed in vitro through cytotoxicity assay based on MTT. Fourth, the CAM assay was used to estimate the anti-tumor performance of pembrolizumab through the analyses of tumor growth and chicken immune cell infiltration in tumors. Finally, the efficacy of several PD-1 or PD-L1 inhibitors (nivolumab, atezolizumab and avelumab) on tumor growth was further assessed using the CAM assay. (3) Results: The presence of CD3+, CD4+, CD8+ T lymphocytes and monocytes was confirmed by FACS and IHC analyses. During in vitro assays, pembrolizumab cross-reacted with chicken lymphocytes and induced PD-1/PD-L1 blockade, which permitted the restoration of chicken T-cell's cytotoxicity against human lung cancer H460 tumor cells. All these in vitro results were correlated with in ovo findings based on the CAM assay: pembrolizumab inhibited H460 tumor growth and induced evident chicken immune cell infiltration (with significant chicken CD45, CD3, CD4, CD8 and CD56 markers) in tumors. Furthermore, the potency of the CAM assay was not limited to the application of pembrolizumab. Nivolumab, atezolizumab and avelumab also led to tumor growth inhibition in ovo, on different tumor models. (4) Conclusions: The chicken embryo affords a physiological, immune reactive, in vivo environment for IO research, which allows observation of how the immune system defense against tumor cells, as well as the different immune tolerance mechanisms leading to tumor immune escape. The encouraging results obtained with PD-1/PD-L1 inhibitors in this study reveal the potential use of the chicken embryo model as an alternative, fast, and reliable in vivo model in the different fields of IO drug discovery.

Buschmann, J.; Heuberger, D.; Kivrak Pfiffner, F.; Wolint, P.; Jang, J.; Jungraithmayr, W.; Giovanoli, P.; Calcagni, M.; Waschkies, C. Probing Vasoreactivity and Hypoxic Phenotype in Different Tumor Grafts Grown on the Chorioallantoic Membrane of the Chicken Embryo In Ovo Using MRI. Cancers 2022, 14(13), 3114; https://doi.org/10.3390/cancers14133114.
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Tumor grafts grown on the chorioallantoic membrane (CAM) of chicken embryos represent a transition between cell culture and mammalian in vivo models. Magnetic resonance imaging (MRI) started to harness this potential. Functional gas challenge is feasible on the CAM. Using quantitative T1 and T2* mapping, we characterized the response of MC-38 colon, A549, and H460 adeno-carcinoma cell grafts to hypercapnic (HC) and hypercapnic-hyperoxic (HCHO) gas challenges, pertaining to the grafts' vascular and oxygenation phenotypes. MR imaging revealed that larger T1 and T2* were located in the center of H460 and MC-38 tumors. Quantitative analysis showed a significant reduction in T1 and a significant increase in T2* in response to HCHO for A549 grafts, while H460 and MC-38 tumors did not respond to either gas challenge. Different tumor grafts respond differentially to HC and HCHO conditions. A549 tumor grafts, with higher vessel density and smaller tumor diameter compared with H460 and MC-38 grafts, had a significant response in T1 for HCHO and T2* increased slightly during HC and significantly under HCHO, consistent with a normoxic phenotype and functional vasoreactivity. Therefore, gas challenges enable differential characterization of tumor grafts with respect to their vascular and oxygenation status.

Rupp, T.; Legrand, C.; Hunault, M.; Genest, L.; Babin, D.; Froget, G.; Castagné, V. A Face-To-Face Comparison of Tumor Chicken Chorioallantoic Membrane (TCAM) In Ovo with Murine Models for Early Evaluation of Cancer Therapy and Early Drug Toxicity. Cancers 2022, 14(14), 3548; https://doi.org/10.3390/cancers14143548. https://www.mdpi.com/2072-6694/14/14/3548

Ethical considerations, cost, and time constraints have highlighted the need to develop alternatives to rodent in vivo models for evaluating drug candidates for cancer. The tumor chicken chorioallantoic membrane (TCAM) model provides an affordable and fast assay that permits direct visualization of tumor progression. Tumors from multiple species including rodents and human cell lines can be engrafted. In this study, we engrafted several tumor models onto the CAM and demonstrated that the TCAM model is an alternative to mouse models for preliminary cancer drug efficacy testing and toxicity analysis. Tumor cells were deposited onto CAM, and then grown for up to an additional 10 days before chronic treatments were administered. The drug response of anticancer therapies was screened in 12 tumor cell lines including glioblastoma, melanoma, breast, prostate, colorectal, liver, and lung cancer. Tumor-bearing eggs and tumor-bearing mice had a similar chemotherapy response (cisplatin and temozolomide) in four human and mouse tumor models. We also demonstrated that lethality observed in chicken embryos following chemotherapies such as cisplatin and cyclophosphamide were associated with corresponding side-effects in mice with body weight loss. According to our work, TCAM represents a relevant alternative model to mice in early preclinical oncology screening, providing insights for both the efficacy and the toxicity of anticancer drugs.

Löffler, J.; Herrmann, H.; Scheidhauer, E.; Wirth, M.; Wasserloos, A.; Solbach, C.; Glatting, G.; Beer, A.; Rasche, V.; Winter, G. Blocking Studies to Evaluate Receptor-Specific Radioligand Binding in the CAM Model by PET and MR Imaging. Cancers 2022, 14(16), 3870; https://doi.org/10.3390/cancers14163870. https://www.mdpi.com/2072-6694/14/16/3870

Inhibition studies in small animals are the standard for evaluating the specificity of newly developed drugs, including radiopharmaceuticals. Recently, it has been reported that the tumor accumulation of radiotracers can be assessed in the chorioallantoic membrane (CAM) model with similar results to experiments in mice, such contributing to the 3Rs principles (reduction, replacement, and refinement). However, inhibition studies to prove receptor-specific binding have not yet been performed in the CAM model. Thus, in the present work, we analyzed the feasibility of inhibition studies in ovo by PET and MRI using the PSMA-specific ligand [18F]siPSMA-14 and the corresponding inhibitor 2-PMPA. A dose-dependent blockade of [18F]siPSMA-14 uptake was successfully demonstrated by pre-dosing with different inhibitor concentrations. Based on these data, we conclude that the CAM model is suitable for performing inhibition studies to detect receptor-specific binding. While in the later stages of development of novel radiopharmaceuticals, testing in rodents will still be necessary for biodistribution analysis, the CAM model is a promising alternative to mouse experiments in the early phases of compound evaluation. Thus, using the CAM model and PET and MR imaging for early pre-selection of promising radiolabeled compounds could significantly reduce the number of animal experiments.

Rousset, X.; Maillet, D.; Grolleau, E.; Barthelemy, D.; Calattini, S.; Brevet, M.; Balandier, J.; Raffin, M.; Geiguer, F.; Garcia, J.; Decaussin-Petrucci, M.; Peron, J.; Benzerdjeb, N.; Couraud, S.; Viallet, J.; Payen, L. Embryonated Chicken Tumor Xenografts Derived from Circulating Tumor Cells as a Relevant Model to Study Metastatic Dissemination: A Proof of Concept. Cancers 2022, 14(17), 4085; https://doi.org/10.3390/cancers14174085. https://www.mdpi.com/2072-6694/14/17/4085

Patient-Derived Xenografts (PDXs) in the Chorioallantoic Membrane (CAM) are a representative model for studying human tumors. Circulating Tumor Cells (CTCs) are involved in cancer dissemination and treatment resistance mechanisms. To facilitate research and deep analysis of these few cells, significant efforts were made to expand them. We evaluated here whether the isolation of fresh CTCs from patients with metastatic cancers could provide a reliable tumor model after a CAM xenograft. We enrolled 35 patients, with breast, prostate, or lung metastatic cancers. We performed microfluidic-based CTC enrichment. After 48–72 h of culture, the CTCs were engrafted onto the CAM of embryonated chicken eggs at day 9 of embryonic development (EDD9). The tumors were resected 9 days after engraftment and histopathological, immunochemical, and genomic analyses were performed. We obtained in ovo tumors for 61% of the patients.

Dedifferentiated small tumors with spindle-shaped cells were observed. The epithelial-to-mesenchymal transition of CTCs could explain this phenotype. Beyond the feasibility of NGS in this model, we have highlighted a genomic concordance between the in ovo tumor and the original patient's tumor for constitutional polymorphism and somatic alteration in one patient. Alu DNA sequences were detected in the chicken embryo's distant organs, supporting the idea of dedifferentiated cells with aggressive behavior. To our knowledge, we performed the first chicken CAM CTC-derived xenografts with NGS analysis and evidence of CTC dissemination in the chicken embryo.

Demcisakova, Z.; Luptakova, L.; Tirpakova, Z.; Kvasilova, A.; Medvecky, L.; De Spiegelaere, W.; Petrovova, E. Evaluation of Angiogenesis in an Acellular Porous Biomaterial Based on Polyhydroxybutyrate and Chitosan Using the Chicken Ex Ovo Chorioallantoic Membrane Model. Cancers 2022, 14(17), 4194; https://doi.org/10.3390/cancers14174194. https://www.mdpi.com/2072-6694/14/17/4194

The chorioallantoic membrane (CAM) is a highly vascularized avian extraembryonic membrane widely used as an in vivo model to study angiogenesis and its inhibition in response to tissues, cells, or soluble factors. In recent years, the use of CAM has become an integral part of the biocompatibility testing process for developing biomaterials intended for regenerative strategies and tissue engineering applications. In this study, we used the chicken ex ovo CAM assay to investigate the angiogenic potential of innovative acellular biopolymer polyhydroxybutyrate/chitosan (PHB/CHIT) scaffold, which is intended for the treatment of hard tissue defects, depending on treatment with pro- and anti-angiogenic substances. On embryonic day (ED) 7, the experimental biomaterials were placed on the CAM alone or soaked in vascular endothelial growth factor (VEGF-A), saline solution (PHY), or tyrosine kinase inhibitor (SU5402). After 72 h, the formation of vessels was analyzed in the surrounding area of the scaffold and inside the pores of the implants, using markers of embryonic endothelium (WGA, SNA), myofibroblasts (α-SMA), and macrophages (KUL-01). The morphological and histochemical analysis showed strong angiogenic potential of untreated scaffolds without additional effect of the angiogenic factor, VEGF-A. The lowest angiogenic potential was observed in scaffolds soaked with SU5402. Gene expression of pro-angiogenic growth factors, i.e., VEGF-A, ANG-2, and VE-CAD, was upregulated in untreated scaffolds after 72 h, indicating a pro-angiogenic environment. We concluded that the PHB/CHIT has a strong endogenous angiogenic potential and could be promising biomaterial for the treatment of hard tissue defects.

Faihs, L.; Firouz, B.; Slezak, P.; Slezak, C.; Weißensteiner, M.; Ebner, T.; Ghaffari Tabrizi-Wizsy, N.; Schicho, K.; Dungel, P. A Novel Artificial Intelligence-Based Approach for Quantitative Assessment of Angiogenesis in the Ex Ovo CAM Model. Cancers 2022, 14(17), 4273; https://doi.org/10.3390/cancers14174273. https://www.mdpi.com/2072-6694/14/17/4273

Angiogenesis is a highly regulated process. It promotes tissue regeneration and contributes to tumor growth. Existing therapeutic concepts interfere with different steps of angiogenesis. The quantification of the vasculature is of crucial importance for research on angiogenetic effects. The chorioallantoic membrane (CAM) assay is widely used in the study of angiogenesis. Ex ovo cultured chick embryos develop an easily accessible, highly vascularised membrane on the surface. Tumor xenografts can be incubated on this membrane enabling studies on cancer angiogenesis and other major hallmarks. However, there is no commonly accepted gold standard for the quantification of the vasculature of the CAM. We compared four widely used measurement techniques to identify the most appropriate one for the quantification of the vascular network of the CAM. The comparison of the different quantification methods suggested that the CAM assay application on the IKOSA platform is the most suitable image analysis application for the vasculature of the CAM. The new CAM application on the IKOSA platform turned out to be a reliable and feasible tool for practical use in angiogenesis research. This novel image analysis software enables a deeper exploration of various aspects of angiogenesis and might support future research on new anti-angiogenic strategies for cancer treatment.

Heuberger, D.; Wolint, P.; Jang, J.; Itani, S.; Jungraithmayr, W.; Waschkies, C.; Meier-Bürgisser, G.; Andreoli, S.; Spanaus, K.; Schuepbach, R.; Calcagni, M.; Fahrni, C.; Buschmann, J. High-Affinity Cu(I)-Chelator with Potential Anti-Tumorigenic Action—A Proof-of-Principle Experimental Study of Human H460 Tumors in the CAM Assay. Cancers 2022, 14(20), 5122; https://doi.org/10.3390/cancers14205122. https://www.mdpi.com/2072-6694/14/20/5122

Human lung cancer ranks among the most frequently treated cancers worldwide. As copper appears critical to angiogenesis and tumor growth, selective removal of copper represents a promising strategy to restrict tumor growth. To this end, we explored the activity of the novel high-affinity membrane-permeant Cu(I) chelator PSP-2 featuring a low-zeptomolar dissociation constant. Using H460 human lung cancer cells, we generated small tumors on the chorioallantoic membrane of the chicken embryo (CAM assay) and studied the effects of topical PSP-2 application on their weight and vessel density after one week. We observed a significant angiosuppression along with a marked decrease in tumor weight under PSP-2 application compared to controls. Moreover, PSP-2 exposure resulted in lower ki67+ cell numbers at a low dose but increased cell count under a high dose. Moreover, HIF-1α+ cells were significantly reduced with low-dose PSP-2 exposure compared to high-dose and control. The total copper content was considerably lower in PSP-2 treated tumors, although statistically not significant. Altogether, PSP-2 shows promising potential as an anti-cancer drug. Nevertheless, further animal experiments and application to different tumor types are mandatory to support these initial findings, paving the way toward clinical trials.

Benčurová, K.; Friske, J.; Anderla, M.; Mayrhofer, M.; Wanek, T.; Nics, L.; Egger, G.; Helbich, T.H.; Hacker, M.; Haug, A.; Mitterhauser, M.; Balber, T. CAM-Xenograft Model Provides Preclinical Evidence for the Applicability of [68Ga]Ga-Pentixafor in CRC Imaging. Cancers 2022, 14, 5549. https://doi.org/10.3390/cancers14225549

Colorectal cancer is one of the leading causes of cancer-related deaths worldwide. Increased expression of CXCR4 has been associated with liver metastasis, disease progression, and shortened survival. Using in vitro cell binding studies and the in ovo model, we aimed to investigate the potential of [68Ga]Ga-Pentixafor, a radiotracer specifically targeting human CXCR4, for CRC imaging. Specific membrane binding and internalisation of [68Ga]Ga-Pentixafor was shown for HT29 cells, but not for HCT116 cells. Accordingly, [68Ga]Ga-Pentixafor accumulated specifically in CAM-xenografts derived from HT29 cells, but not in HCT116 xenografts, as determined by μPET/MRI. The CAM-grown xenografts were histologically characterised, demonstrating vascularisation of the graft, preserved expression of human CXCR4, and viability of the tumour cells within the grafts. In vivo viability was further confirmed by μPET/MRI measurements using 2-[18F]FDG as a surrogate for glucose metabolism. [68Ga]Ga-Pentixafor μPET/MRI scans showed distinct radiotracer accumulation in the chick embryonal heart, liver, and kidneys, whereas 2-[18F]FDG uptake was predominantly found in the kidneys and joints of the chick embryos. Our findings suggest that [68Ga]Ga-Pentixafor is an interesting novel radiotracer for CRC imaging that is worth further investigation. Moreover, this study further supports the suitability of the CAM-xenograft model for the initial preclinical evaluation of targeted radiopharmaceuticals.