Collection of CAM associated papers and arguments why to citate

Regine Schneider-Stock

Kunze P, Kreiss L, Novosadová V, Roehe AV, Steinmann S, Prochazka J, Geppert CI, Hartmann A, Schürmann S, Friedrich O, Schneider-Stock R. Multiphoton Microscopy Reveals DAPK1-Dependent Extracellular Matrix Remodeling in a Chorioallantoic Membrane (CAM) Model. Cancers (Basel). 2022 May 10;14(10):2364. doi: 10.3390/cancers14102364. PMID: 35625969; PMCID: PMC9139596.

The ECM-DAPK related article is already included in our recent CAM issue.

We demonstrated:
- the ECM remodeling capability of colon tumor cells that have lost the cytoskeletal DAPK protein
- we performed multiphotone microscopy to image the CAM ECM

Schneider-Stock R, Ribatti D. The CAM Assay as an Alternative In Vivo Model for Drug Testing. Handb Exp Pharmacol. 2021;265:303-323. doi: 10.1007/164_2020_375. PMID: 32776283.

Bueno-Fortes S, Muenzner JK, Berral-Gonzalez A, Hampel C, Lindner P, Berninger A, Huebner K, Kunze P, Bäuerle T, Erlenbach-Wuensch K, Sánchez-Santos JM, Hartmann A, De Las Rivas J, Schneider-Stock R. A Gene Signature Derived from the Loss of CDKN1A (p21) Is Associated with CMS4 Colorectal Cancer. Cancers (Basel). 2021 Dec 28;14(1):136. doi: 10.3390/cancers14010136. PMID: 35008299; PMCID: PMC8750372.

We demonstrated:
- the rather loosely packed tumor masses of ovografts derived from p21neg EMT HCT116 cell line whereas colon tumor cell line HCT116 shows a clear pushing front typical for microsatellite tumors
- in suppl. Figure 1 we document the tumor cell dissemination, using an in vivo imaging system for fluorescence labelled tumor cells

Kerstin Huebner

Huebner K, Erlenbach-Wuensch K, Prochazka J, Sheraj I, Hampel C, Mrazkova B, Michalcikova T, Tureckova J, Iatsiuk V, Weissmann A, Ferrazzi F, Kunze P, Nalli E, Sammer E, Gehring A, Cheema MM, Eckstein M, Paap EM, Soederberg A, Fischer C, Paul S, Mahadevan V, Ndreshkjana B, Meier MA, Muehlich S, Geppert CI, Merkel S, Grutzmann R, Roehe A, Banerjee S, Hartmann A, Sedlacek R, Schneider-Stock R. ATF2 loss promotes tumor invasion in colorectal cancer cells via upregulation of cancer driver TROP2. Cell Mol Life Sci. 2022 Jul 15;79(8):423. doi: 10.1007/s00018-022-04445-5. PMID: 35838828; PMCID: PMC9287261.

We demonstrated
- that we can recapitulate tumor heterogeneity in CAM ovografts of colorectal cancer (CRC) cell lines
- that the tumor-specific invasion pattern of MSI tumors, i.e. the distinct pushing front margin, is retained in CAM ovografts of MSI cells
- a cell-line specific growth pattern which is preserved independent of the inoculated tumor cell number
- a gene-knockout-specific dissemination potential by Alu-qPCR of CRC CAM ovografts

Marta Teixeira Pinto

Baptista-Silva S, Bernardes BG, Borges S, Rodrigues I, Fernandes R, Gomes-Guerreiro S, Pinto MT, Pintado M, Soares R, Costa R, Oliveira AL. Exploring Silk Sericin for Diabetic Wounds: An In Situ-Forming Hydrogel to Protect against Oxidative Stress and Improve Tissue Healing and Regeneration. Biomolecules. 2022 Jun 8;12(6):801.

Main findings:
- We develop a novel in situ-forming silk sericin-based hydrogel (SSH) as an advanced dressing for wound healing;
- CAM assay made it possible to observe that SSH led to an increase in the number of newly formed vessels without inducing an inflammatory reaction.
- (Graphic abstract: https://www.mdpi.com/2218-273X/12/6/801)

Tokarchuk I, Janser FA, Schläfli AM, Pinto MT, Humbert M, Niklaus NJ, Berezowska S, Langer R, Tschan MP. Increased LAMP2A levels correlate with a shorter disease-free survival of HER2 negative breast cancer patients and increased breast cancer cell viability. Biochem Biophys Res Commun. 2021 Sep 10;569:47-53.

- We demonstrated the importance of LAMP2A, a marker of Chaperone Mediated Autophagy (CMA), in HER2 negative breast cancer;
- We utilized the CAM to assessed the tumorigenic properties of LAMP2A-manipulated breast cancer cells. Validation was performed by immunohistochemistry (IHC) using human-specific KU80 staining, plus LAMP2A staining and proliferation activity marker Ki67 staining.

Pinto F, Santos-Ferreira L, Pinto MT, Gomes C, Reis CA. The Extracellular Small Leucine-Rich Proteoglycan Biglycan Is a Key Player in Gastric Cancer Aggressiveness. Cancers (Basel). 2021 Mar 16;13(6):1330.

Main findings:
- We report the oncogenic role of biglycan, an extracellular proteoglycan, in gastric carcinogenesis;
- In vivo experiments with the CAM, demonstrated that biglycan knock-out GC cells display lower angiogenic potential when compared with biglycan expressing cells;
- Ki-67 expression analysis on CAM xenographed tumors showed that tumors formed by KO cells present a less cohesive-like tumor mass with increased extracellular matrix stiffness.
  (CAM figure: https://www.mdpi.com/2072-6694/13/6/1330)

Gião T, Saavedra J, Vieira JR, Pinto MT, Arsequell G, Cardoso I. Neuroprotection in early stages of Alzheimer's disease is promoted by transthyretin angiogenic properties. Alzheimers Res Ther. 2021 Aug 24;13(1):143. doi: 10.1186/s13195-021-00883-8. PMID: 34429155; PMCID: PMC8385857.

Main findings:
- We demonstrate for the first time the involvement of protein transthyretin (TTR) in angiogenesis, particularly as a modulator of vascular alterations occurring in Alzheimer's disease;
- TTR is angiogenic in vivo and the neovessels formed are functional;
- Two different chick chorioallantoic membrane (CAM) assays were used: one to determine the angiogenic potential and other to evaluated the vascular permeability of newly formed vessels.
  (CAM figure: https://pubmed.ncbi.nlm.nih.gov/34429155/#&gid=article-figures&pid=fig-2-uid-1)

MT Pinto, AS Ribeiro, I Conde, R Carvalho, J Paredes (2021) The Chick Chorioallantoic Membrane Model: A New In Vivo Tool to Evaluate Breast Cancer Stem Cell Activity. Int. J. Mol. Sci. 2021,22, 334.

Mains findings:
- We established a CAM-Limiting Dilution Assay (LDA) able to rapidly reproduce tumor specificities in particular, the ability of the small population of Cancer Stem Cells (CSCs) to form tumors;
- CAM xenografted tumors presented the same cytoarchitecture as mice tumors and display clear CSC immunostaining.
  (figures: https://www.mdpi.com/1422-0067/22/1/334/htm; https://www.mdpi.com/1422-0067/22/1/334)

M Dionisio, A Vieira, R Carvalho, I Conde, M Oliveira, M Gomes, M Pinto, P Pereira, J Pimentel, C Souza, M Silveira, V da Silva, A Barroso, D Preto, J Cameselle-Teijeiro, F Schmitt, A Ribeiro and J Paredes (2020) BR-BCSC Signature: The Cancer Stem Cell Profile Enriched in Brain Metastases that Predicts a Worse Prognosis in Lymph Node-Positive Breast Cancer. Cells 2020, 9(11), 2442;

Mains findings:

- Brain-tropic breast cancer cells show increased stem cell activity and tumorigenic capacity in the chick embryo choriallantoic membrane (CAM) when compared to the parental cell line;
- An enrichment of a BCSC signature was found in brain metastases, which can be used as a new prognostic factor in the clinic practice.
  (CAM figure: https://www.mdpi.com/2073-4409/9/11/2442)

C Coelho, T Padrão, L Costa, M Pinto, P Costa, V Domingues, P Quadros, F Monteiro, S Sousa. (2020) The antibacterial and angiogenic effect of magnesium oxide in a hydroxyapatite bone substitute. Sci Rep 10, 19098 (2020).

Mains findings:

- We developed a new potential bone substitute composed of hydroxyapatite and MgO (HAp/MgO) spherical granules;
- The in vivo chicken embryo chorioallantoic membrane (CAM) model was used to shoe that spherical granules containing MgO stimulated angiogenesis without increasing inflammation.
  (CAM figure: https://pubmed.ncbi.nlm.nih.gov/33154428/#&gid=article-figures&pid=figure-8-uid-7)

M Leite, MS Marques, J Melo, MT Pinto, B Cavadas, M Aroso, M Gomez-Lazaro, R Seruca, C Figueiredo. Helicobacter Pylori Targets the EPHA2 Receptor Tyrosine Kinase in Gastric Cells Modulating Key Cellular Functions" Cells. 2020 Feb 24;9(2). pii: E513.

Main findings:

- We show that H. pylori targets the receptor tyrosine kinase EPHA2, contributing to the unraveling of the underlying mechanisms of H. pylori–host interactions and associated diseases;
- CAM assay was used to evaluate the angiogenic potential of EPHA2 siRNA silenced gastric cancer cells, demonstrating the participation of the TK receptor in the angiogenic process.
  (CAM figure: https://pubmed.ncbi.nlm.nih.gov/32102381/#&gid=article-figures&pid=figure-5-uid-7)

Ward De Spiegelaere

Tay H, Du Cheyne C, Demeyere K, De Craene J, De Bels L, Meyer E, Zijlstra A, Spiegelaere WD. Depletion of Embryonic Macrophages Leads to a Reduction in Angiogenesis in the Ex Ovo Chick Chorioallantoic Membrane Assay. Cells. 2021; 10(1):5. https://doi.org/10.3390/cells10010005

We demonstrated:

- Intravenous clodronate liposome injection in the CAM depletes chicken embryonic macrophages by 3-4 fold
- Albeit reducing viability in the ex vivo CAM model, a survival of 56% is still obtained compared to 82.5% which were PBS injected
- Clodronate treatment reduces vascularization of collagen onplants on the CAM

Michela Corsini

Simultaneously characterization of tumoral angiogenesis and vasculogenesis in stem cell-derived teratoma.
Exp Cell Research 2021 Jan.
doi: 10.1016/j.yexcr.2021.112490.

We used the CAM model to study teratomas vascularization.
We demonstrated that:
- CAM supports the growth and differentiation of embryonic stem cells into teratomas.
- In teratomas, embryonic stem cell-derived endothelial cells form chimeric vessels with stromal endothelial cells.
- CAM allows the simultaneous characterization of vasculogenesis and angiogenesis processes.

Nicole Dünker

Mol Oncol. 16(4):957-981 (2022) https://doi.org/10.1002/1878-0261.13054
Role of L1CAM in retinoblastoma tumorigenesis: identification of novel therapeutic targets
Oliver Dräger, Klaus Metz, Maike Busch, Nicole Dünker

We demonstrated that
- depletion of the neuronal cell adhesion molecule L1 (L1CAM) decreases the tumorigenic and migration potential of retinoblastoma cells in ovo
- L1CAM depletion likewise decreases viability and tumor growth of etoposideresistant retinoblastoma cells upon etoposide treatment in ovo
- L1CAM is a potential novel target for future retinoblastoma therapeutic strategies

Methods Protoc. 2022 Mar 2;5(2):21. DOI: 10.3390/mps5020021
Retinoblastoma Cell Growth In Vitro and Tumor Formation In Ovo-Influence of Different Culture Conditions
Annika Doege , Rebecca Steens Nicole Dünker 1 and Maike Anna Busch

We demonstrated that
- compared to inoculation of retinoblastoma (RB) cells on the CAM at embryonic development day (EDD) 10, inoculation at EDD 8 leads to decreased tumor formation and chicken embryo viability
- different RB cell concentrations did not significantly change size and weight of developing CAM tumors
- the starting point for CAM inoculation significantly influences the experimental outcome of investigations using RB cell lines

Tumor Biology 43: 11–26 (2021) https://doi.org/10.3233/tub-200072
Impact of RARα and miR-138 on retinoblastoma etoposide resistance
Maike Busch, Natalia Miroschnikov, Jaroslaw Thomas Dankert, Marc Wiesehöfer, Klaus Metz, Harald Stephan and Nicole Dünker

We demonstrated that
- overexpression of retinoic acid receptor alpha (RARalpha) decreases growth of etoposide resistant retinoblastoma (RB) cells in ovo
- RARalpha acts as a tumor suppressor in retinoblastoma and is downregulated upon etoposide resistance in RB cells
- RARalpha may contribute to the development and progression of RB chemoresistance

Tami Green

Palaniappan TK, Šlekienė L, Jonasson AK, Gilthorpe J, Gunhaga L. CAM-Delam: an in vivo approach to visualize and quantify the delamination and invasion capacity of human cancer cells. Sci Rep. 2020 Jun 26;10(1):10472. doi: 10.1038/s41598-020-67492-7. PMID: 32591581; PMCID: PMC7320147.

Green T, Šlekienė L, Gunhaga L. CAM-Delam Assay to Score Metastatic Properties by Quantifying Delamination and Invasion Capacity of Cancer Cells. J Vis Exp. 2022 Jun 2;(184). doi: 10.3791/64025. PMID: 35723486.

We have developed the CAM-Delam assay, an innovative approach to evaluate the metastatic aggressiveness of cancer cells. By visualizing the degree of damage (intact, altered, damaged or invasion) to the laminin layer of the CAM that cultured cancer cells induce, their potential risk for metastases can be estimated.