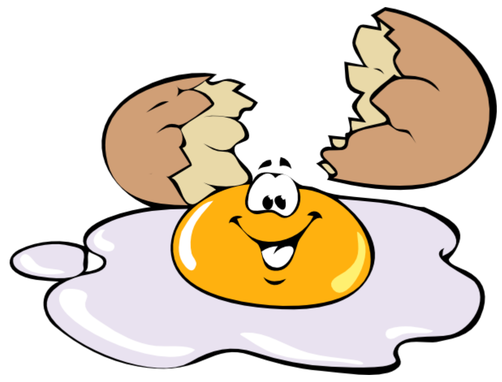
The *in ovo* model as a potential alternative to rodents for CRC research



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Objective

Development of novel radiotracers for cancer research involves costly and time-demanding animal testing. In accordance with the 3R principle[1], rodents can be replaced by immature forms of non-mammalian vertebrates, such as chick embryos (*in ovo*). We aimed at establishing this model for colorectal cancer (CRC) with the focus on C-X-C motif chemokine receptor type 4 (CXCR4) expression, as this receptor is of prognostic value for CRC[2,3].

Materials and Methods

EDD1
- incubate fertilized, pathogen-free chicken eggs horizontally at 37°C and ~60% humidity

EDD3
- rotate eggs 180° and puncture a small hole in the egg shell

EDD5
- enlarge the hole

EDD9
- inoculate human CRC cancer cells expressing CXCR4 (HT29 and HCT116)[4] onto the intersection of CAM vessels

EDD14-18
- inject [^{68}Ga]Ga-Pentixafor or [^{18}F]FDG into CAM-vessel
- anesthetize the chick embryo with isofluran
- perform multimodal imaging (µPET/CT/MR)

EDD19
- terminate chick embryos (shock freezing)
- harvest xenografts
- preserve xenografts for further histological analysis

Results

Protocols for incubation, tumor cell inoculation (Fig. 1), *i.v.* injection and anaesthesia were optimized. Immunohistochemical (IHC) analysis using an antibody against desmin proved vascularization of the grafts (Fig. 2B). Staining of cells of epithelial origin (cytokeratin 19 IHC) differentiated between the inoculated cancer cells and the CAM (Fig. 2C). µMRI showed superior soft tissue contrast compared to µCT (Fig. 3). Tumor accumulation of the radiolabeled glucose-analogue, [^{18}F]FDG (Fig. 4), indicated glucose turnover and viability of the grafts. The latter was proven with IHC (ki67, cleaved caspase 3) (Fig. 2D,E). Preliminary results showed accumulation of [^{68}Ga]Ga-Pentixafor, PET-tracer targeting CXCR4, in xenografts derived from HT29 and HCT116 cells (Fig. 5A,D). Specificity was shown by co-injection of an excess of CXCR4 Antagonist I (blocking) (Fig. 5B,D). Target expression was confirmed immunohistochemically (Fig. 2F).

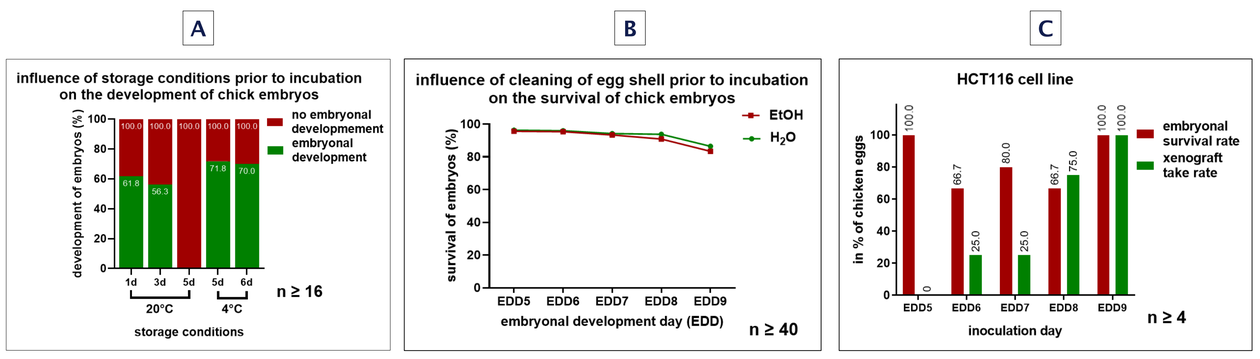


Figure 1: optimization of the *in ovo* model for CRC research
Temperature and duration of storage of chicken eggs prior to incubation influenced development of chick embryos (A). There was no significant difference when cleaning the egg shell using sterile, distilled water or 70% ethanol prior to start of incubation (B). Using HCT116, EDD9 was identified as an ideal day for inoculation of CRC cells (C).

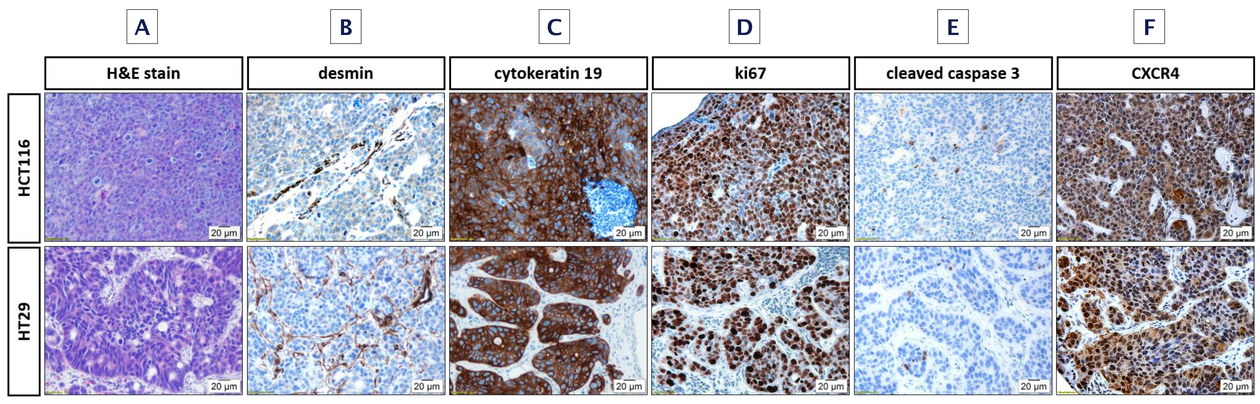


Figure 2: characterization of HCT116 and HT29 xenografts grown on the CAM
HCT116 (upper row) and HT29 (lower row) CAM-xenografts were characterized using H&E stain (A, (n≥9)). IHC analysis was performed using antibody against desmin (B, pericytes in the vascular network (n≥3)), cytokeratin 19 (C, epithelial cells (n≥3)), ki67 (D, proliferating cells (n≥3)), cleaved caspase 3 (E, apoptotic cells (n≥3)), CXCR4 (F, target of interest (n≥3)). Magnification: 200-fold.

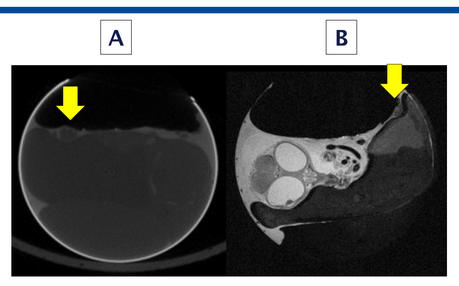


Figure 3*: in ovo* µCT vs. µMRI
CT image (80kV, 500µA) of EDD16 chick embryo performed 60 min after application of iodinated contrast agent Iomeron into albumen (A). Delineation of xenograft based on µCT is not possible; organs cannot be distinguished. In comparison MRI image (T2w, TurboRARE) of an EDD16 chick embryo (B). Xenografts are indicated with an arrow.

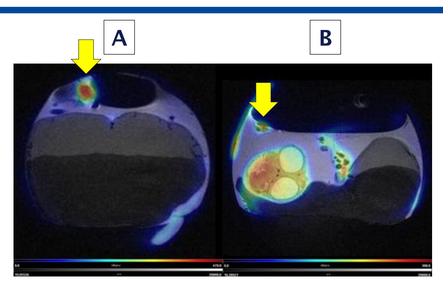
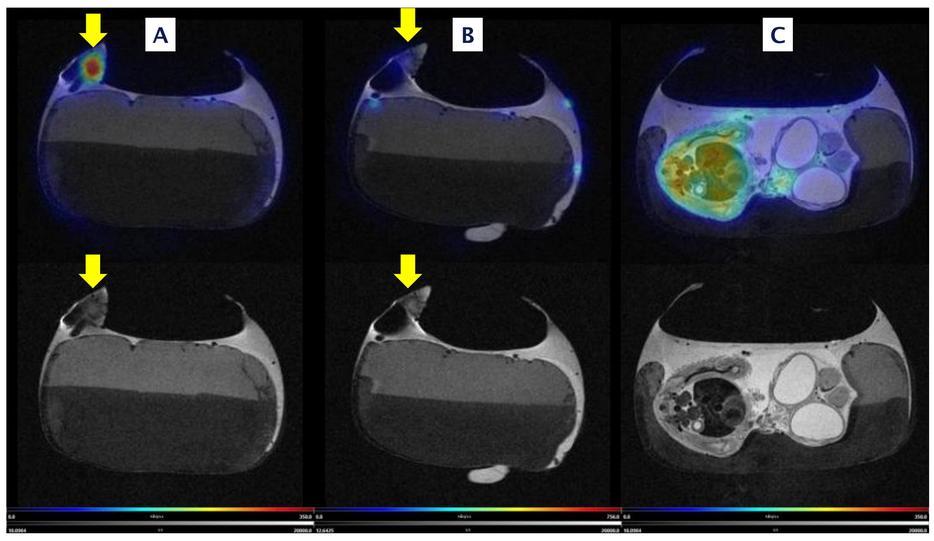
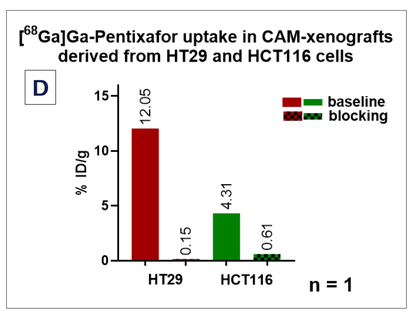


Figure 4: [^{18}F]FDG uptake in CAM-xenografts
MR image fused with [^{18}F]FDG PET. CAM-xenograft grown from HT29 (A) or HCT116 cells (B) is indicated with an arrow. The µPET static measurement was performed for 30 min starting at 60 min *p.i.* of 4.7 MBq (A) or 48 min *p.i.* of 8.1 MBq (B) [^{18}F]FDG at EDD14 (A) or EDD13 (B).





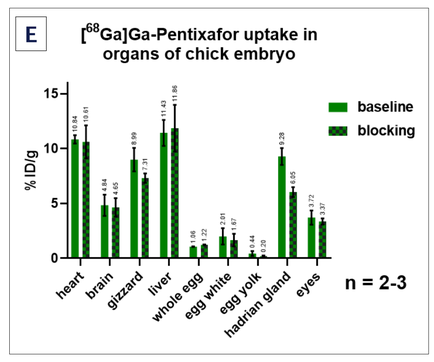


Figure 5: [^{68}Ga]Ga-Pentixafor uptake in CAM-xenografts and organs of chick embryo
MR image only (lower row) or fused with [^{68}Ga]Ga-Pentixafor PET (upper row) with (B) or without (A,C) blocking using CXCR4 Antagonist I (AMD3100). CAM-xenograft grown from HT29 cells is indicated with an arrow (A,B). C: Representative image of [^{68}Ga]Ga-Pentixafor uptake in chick embryo organs. Axial view (A,B,C). The µPET static measurement was performed for 30 min starting at 60 min *p.i.* of 3.5 MBq (A,C) or 7.6 MBq (B) [^{68}Ga]Ga-Pentixafor at EDD15 (A,C) or EDD16 (B). [^{68}Ga]Ga-Pentixafor uptake in HT29 and HCT116 xenografts 60 min. *p.i.* (D, (n=1)) and in organs of chick embryo 60 min *p.i.* (E, (n=2-3)).

Conclusion

These results show the potential of the *in ovo* model for investigating radiotracer accumulation in xenografts derived from CRC cell lines. The model may lead to reduction of animal experiments in the future. As a step towards novel precision medicine concept, we aim to implant patient-derived organoids.

References

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