

The chorioallantoic membrane (CAM) assay as a relevant *in vivo* model to study the effect of thymol on colorectal tumor progression.

Eva Sedlackova¹, Maria Makovicka¹, Peter Makovicky¹, Michaela Blazickova¹, Maria Bartosova²

¹Cancer Research Institute, Biomedical Research Center SAS

²Institute of Virology, Biomedical Research Center SAS

eva.sedlackova@savba.sk

It is obvious that preclinical *in vivo* studies are essential for discovering new therapeutic agents, however, rodent models are time-consuming, expensive, and require approval from the Animal Ethical Committee. Recently, the avian embryo chorioallantoic membrane (CAM) model was well described in human medicine as a cost-effective, easy-to-perform preclinical oncological model for observing pro- and antiangiogenic response, nanotoxicology, tumor biology, and metastasis.

One of the aims of this work was to demonstrate the alternative *in vivo* technique in which the highly vascular and accessible chorioallantoic membrane of Japanese quail was used. The second goal of this work was to monitor the tumor growth from the colon cancer cell line (HCT 116). A new approach to the treatment and prevention of colon cancer may be based on the use of natural substances and because of that, we treated the HCT116 cell line with thymol and applied this on CAM. Subsequently, we observed the changes in tumor growth and in angiogenesis after the application of thymol.

Our study demonstrates that the colon cancer cell line (HCT 116) can form solid, vascularized tumors on the CAM. After the application of thymol the size of the tumor decreased. The thymol also had an effect on the vascular network of the CAM. Tumor invasion could be demonstrated by both histological and optical sectioning. We can conclude that thymol has great potential in the prevention and treatment of colorectal cancer.

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