

**VBR 06****DEMONSTRATION OF POLYCLONALITY IN HUMAN CARCINOMA WITH A NEW METHOD**Th.Riemenschneider, W.Heitland, H.Hörth, G.Gaebel

The polyclonality of individual human tumor is one of the essential causes of their incalculable response to therapy. The demonstration of different clones in one tumor produce considerable technical problems. The Clonogenic Assay (Ham - burger, A. and Salmon, S. Science 197, 461, 1977) offers definite conditions and clonal growth of the tumor stem cells.

From Feb. 82 to Sept. 85, 153 non-selected human colo-rectal adenocarcinoma were put in the assay system. The cell- and colony-bearing overlayers from 1. and 14. day after culture were stained with Papanicolaou; a quantitative evaluation of number and size of the colonies, a morphological and immunological examination with CEA-PAP stain of original tumor and embedded colonies was achieved.

Colony growth of the carcinomas was detected in 96 specimen (62.7%); poor growth (<10 colonies) in 48, intermediate (10-30 colo.) in 29 and good (>30 colo.) growth in 19 tumors (12.6%); only the well growing tumor were evaluated. The histogram of the colony sizes in each tumor showed only one peak in 12, but two (n=5) or three (n=3) different peaks in 7 (>1/3) of the tumors. The morphological study shows for the different sized colonies different cell-size and -sharp, the CEA staining intensity was respectively different for the 10/12 CEA-producing tumors.

The quantitative analysis of the colony size in the Clonogenic Assay may be an appropriate, relatively simple procedure to directly demonstrate different cell clones in human carcinoma.

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**VBR 07****TIME RESOLVED LASER FLUORESCENCE MICROSCOPY OF HEMATOPORPHYRIN-DERIVATE (HPD) FOR DETECTION OF SUPERFICIAL CANCER**  
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**Introduction:** Due to its specific properties-accumulation in tumor tissue, typical emission spectra. and photosensitivity - HpD is used for Photoradiationtherapy (PRT) on endoscopically remote cancer. The weak HpD-fluorescence and the immediate photobleaching caused by high light intensities are enhancing the problems in fixing a point for PRT, which means a high accumulation in tumor tissue accompanied by an ineffective, low concentration in the surrounding tumorfree tissue.

**Methods:** Transparent access chambers were adapted to the dorsal skin fold of Syrian Golden Hamsters and cells of the amelanotic melanoma (AMel3) implanted into the center of the chamber. After i.v. application the HpD-distribution was observed over a period of 8 days. The 400x400µm observation area was irradiated by ultrashort laserpulses (irradiation power: 1mW/cm<sup>2</sup>). The resulting HpD fluorescence was detected by photomultiplier. Additionally we made efforts by time resolution of the fluorescence decay time to verify two different states of binding of the porphyrin with probably different therapeutic effects.

**Results:** The HpD fluorescence showed a maximum between 6 and 9 hrs after i.v. injection. The relation tumor/tumor-free tissue was approximately 3-8 at this time. The third day of examination showed nearly identical values for both tissues. Between day 6 and day 8 we found an increase of HpD contents in tumor vs. adjacent tumorfree tissue.

Two different states of molecular binding were identified by their fluorescence decay time of 1.7 and 11ns which are supposed to be dimers or aggregates, and monomers.

**Conclusion:** The combination of this animal model and the laser steered detection device enables the detection of superficial tumors as well as the determination of the optimum point for photoradiationtherapy.

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**VBR 08****IMMUNOHISTOCHEMICAL DETECTION OF HERPES-VIRUS AND PAPILOMA-VIRUS ANTIGENS IN ANAL WARTS AND CARCINOMAS.**P. Schmidt and F. Borchard

In addition to papilloma viruses, herpes viruses are especially thought to cause anal warts and carcinomas. In order to investigate this problem PAP immunohistochemistry with antisera against HSV1, HSV2, and against HPV cross-reacting with all subtypes (Dako) was applied to these lesions. The material consisted of 22 cases, comprising 13 condylomata accuminata, 3 anal carcinomas and 6 control tissues.

Immunoreactivity for HPV was detected in 7 of the 13 condylomata accuminata, but in none of the investigated carcinomas. Of the control cases, only one anal fibrous polyp showed immunoreactivity for HPV. The number of infected nuclei varied greatly between 30-40 % of the cells on the one hand and single nuclei on the other hand. The cells mainly displayed the features of koilocytes and lay in the suprabasal layer. HSV1 was only present in a few cells of one condylomatous lesion whereas HSV2 could not be detected. Thus it seems that herpes viruses do not play a major role in the genesis of anal warts or cancers as judged by immunohistochemistry. Our results underline the role of HPV in the genesis of benign anal warts while a possible causal relationship to carcinogenesis could not be shown with this method.

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**VBR 09****TUMORPROMOTER MODULATE SKIN TUMORGENESIS INDUCED BY PAPILOMAVIRUS IN MASTOMYS NATALENSIS**K.Wayss, E.Amtmann, G.Fürstenberger, G.Sauer and M.VoIm

In the skin of *Mastomys natalensis* tumors appear spontaneously when the animals are older than approximately one year. *Mastomys natalensis* papillomavirus (MnPV) was identified as the causative agent. The relative amount of extrachromosomal papillomavirus genomes in the skin of *Mastomys* increased with ageing of the animals. Nevertheless the skin continues to be histologically normal. First studies in skin carcinogenesis by the two-stage protocol showed besides benign and malignant tumors in the groups treated with 7,12-dimethylbenz(a)anthracene (DMBA) or the combination of DMBA and 12-O-tetradecanoylphorbol-13-acetate (TPA) also tumors in the skin of *Mastomys* only treated with the promoter TPA. In recent studies TPA strongly stimulated the amount of MnPV DNA in the skin. In contrast the topical administration of DMBA has no effect on the amount of MnPV genomes. After chronic application of DMBA keratoacanthomas, squamous cell carcinomas and a few basalomas were induced. These tumors contained only minute amounts of MnPV DNA and no viral mRNA transcripts could be detected. By chronic treatment with TPA or the second stage promoter 12-O-retinoyl-phorbol-13-acetate (RPA) fibroepitheliomas and keratoacanthomas but no carcinomas were induced. These tumors arised several weeks earlier and in a higher rate than in the untreated controls. These promoter induced tumors contained large amounts of MnPV DNA and produced virus particles. The stimulating effect of TPA and RPA on tumor induction could only be demonstrated when young animals (8-10 weeks old) were chronically treated. In contrast TPA or RPA had no stimulating effect in 30 weeks old animal. This suggests that during ageing the promoting effect of TPA and RPA seems to occur spontaneously.

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