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ESTABLISHMENT OF A TISSUE SLICE CULTURE MODEL FOR PROSTATE CANCER

Introduction:
The interaction between the epithelial, stromal, and vascular compartments of the prostate is essential for normal prostate development and function and may play a role in the development and progression of prostate cancer (PC). In vitro cell culture systems lack important interactions and consequently poorly mimic the in vivo situation. To establish a more relevant model system that better reflects the complexities of the tumor environment we are developing an ex vivo tissue slice culture model directly from fresh tumor tissues.

2. Fluorescent Viability End Point Evaluation

Fig. 2: End point fluorescent analysis of PC205 slices after culturing: After 1, 4, and 10 days of culture non-fixed tissue slices (thickness 300 µm) obtained from the human PC xenografts PC205 and dead cells in epithelial and stromal compartments of the tissue slice were visualized with mitochondrial and DNA selective fluorescent dyes (Yan der Kuijp et al., 2006, BMC Cancer, 6:92), respectively penetrance (magnificaton water (TMRE, 0.5 µM); Indirubin TSBG) and propidium iodide (0.1%) and analyzed by confocal microscopy (Zeiss LSM710; photos 20x magnification).

3. Immunohistological End Point Evaluation

Fig. 3: End point immunohistological analyses of PC205 slices after culturing: After 0, 1, 4, and 10 days of culturing tissue slices with different thicknesses (200, 250, and 300 µm) obtained from the human PC xenografts PC205 were fixed in formalin 4% when after embeded in paraffin. Formalin-fixed, paraffin-embedded (PFFE) sections (5 µm) were prepared and Ki67 was measured by IHC: Ki67 staining, photos 10x magnification. Ki67-positive cells were labeled using the monoclonal mouse anti-human Ki-67 antigen clone MIB-1 (Dako Cytomation/Roche) and visualized by using Rabbit-Mouse Peroxidase/ABC (Dako Real Envision detection System), photos 10x magnification and Ki67 receptor (AR) expression and cellular localization was labeled using monoclonal AR antibody (clone PV434; 1:45) visualized by using UltraView Universal Diaminobenzidine peroxidase-quik, photos 10x magnification.

4. Metabolic Activity as Control Read-out Marker

Fig. 4: Assessment of viability by measuring metabolic markers: At several time points the metabolic activity of PC210 tissue slices was assessed by measuring: a) pH (pH electrode, Metrohm); b) glucose (GluCell System, Cesco Biotechnology); and c) lactate dehydrogenase (LDH, Cytotox 96 Non-Radioactive Cytotoxicity Assay, Promega) in the culture medium. Influence of medium refreshment at different time points was tested on tissue slice viability during of culture: vv: not refreshed; vv: refreshed at noted time point; % of half of the medium volume was refreshed at noted time point.

Conclusions & Discussion:
- PC xenograft tissue slices (250-300 µm) remained viable for at least 5 days.
- hierarchy and morphology were comparable to slice at time point zero (uncultured)
- epithelial PC cells still proliferated and expressed AR
- Metabolic activity markers (pH, glucose, LDH) can be used as read-out system markers to follow slice viability over time.
- Optimize culture conditions and study other possible control read-out markers
- Equalize findings and standardize conditions and markers for slices prepared from human prostate (cancer) tissues.