An Organ Slice Model to Evaluate Carcinoid Tumorogenesis in Liver

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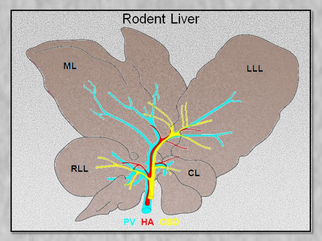
Background

Midgut carcinoid cancers typically metastasize to the liver. However, the underlying cellular and molecular events that regulate the cancer cell interactions with the liver tissue microenvironment are poorly understood. In combination with live-cell imaging methods and multi-photon microscopy, we developed an organ slice culture system that more closely resembles the three-dimensional, multicellular tumor microenvironment than does a dispersed cell culture system. This model could be used to monitor the development of tumor like structures and assess cancer cell dynamics *in situ.* We found that tumor-like structures are formed by both cell proliferation and aggregation.

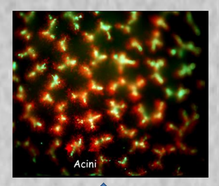
Parallel experiments demonstrated that cell aggregation could be recapitulated in cultured cells when exposed to conditioned media or liver tissue pieces.
We provide evidence that this *ex vivo* organ slice/xenograft model is a promising tool with considerable potential as a means to probe the early events mediating metastasic tumor growth n the liver.

Methods

Cell Culture: Carcinoid cell lines were grown in 75 mm flask and maintained in DMEM with 10% FBS at 37°C at 95%/5% O_2/CO_2. 0.2 µg/ml puromycin was added to maintain the selection pressure.



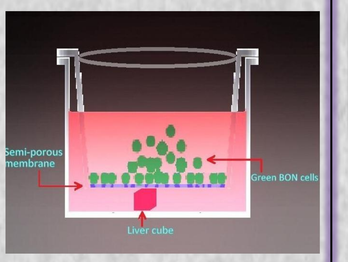
Organotypic slicing: 10^6mg/ml BON cells stably transfected with GFP were introduced to liver by portal vein injection. A long lasting vital dye was also introduced into the liver to mark the vasculature. Liver was removed, cut into ~5 mm cubes, embedded in 3% LTMP agarose and sliced into 200 µm sections with vibratome. Slices were maintained in culture on nylon inserts and with DMEM at 37°C at 95%/5% O_2/CO_2 for up to 2 weeks.



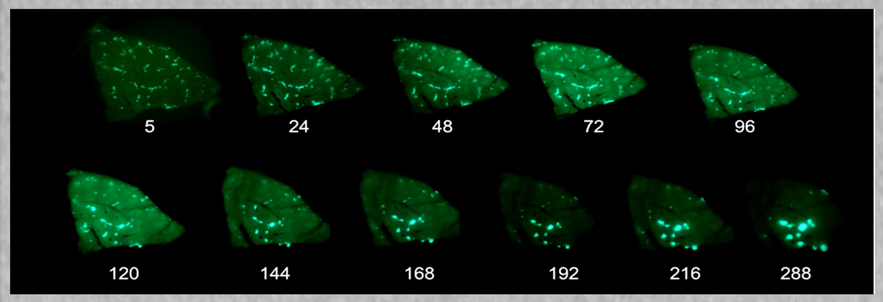
Immunostaining: The organotypic slices were fixed using 4% paraformaldehyde. After day 1, day 3 and day9, slices were cut into 20 µm sections and immunofluorescence was performed using specific antibodies. Proliferating cells in the tumor-like structures were stained using rabbit polyclonal antibody against Ki67.

Microscopy: Live cell and fixed tissue was analysed using a Leica SP5 broadband confocal/multi-photon microscope equipped with Chameleon XR MPE and Luden environmental control chamber. Wide-field imaging was performed using Olympus MVX10 fluorescence macroscope (slices) or Nikon T-2000 microscope coupled to with TILL Polychrome V imaging system (cells).

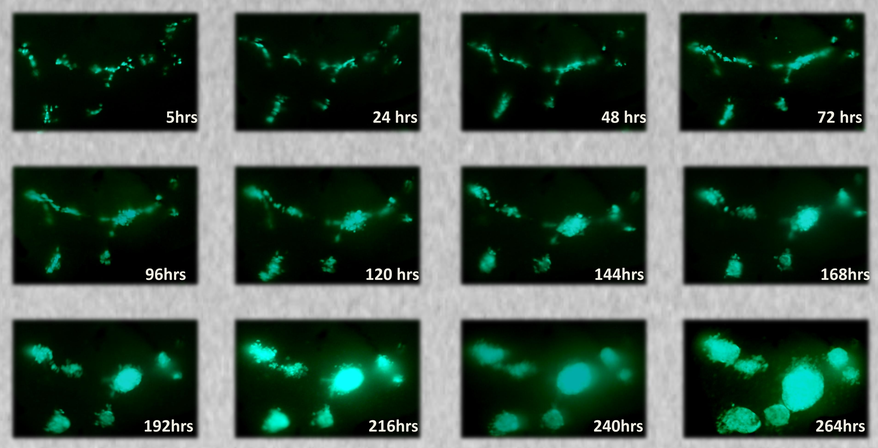
Cell aggregation assay: $0.1X10^6$ cells in serum free media were seeded on fluroblok inserts (0.8 µm pore size) in 24 well plates. The bottom chamber consisted of liver cubes (~5 mm^3) in serum free media or conditioned media. The cells were then maintained at 37°C at 95%/5% O2/CO2 for 24 hours. Imaging of the fluoroblok membrane was done using Olympus MVX10 fluorescence macroscope.



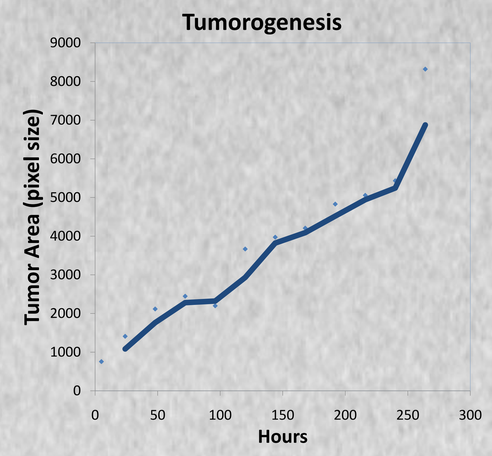
Results



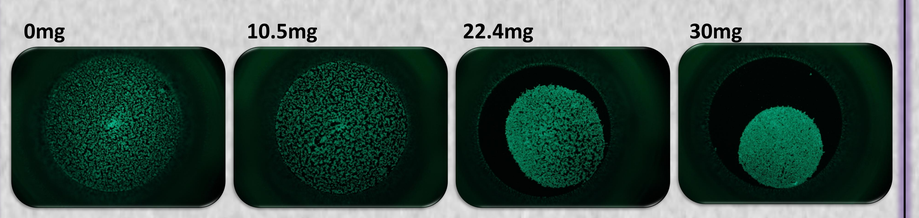
Organotypic liver slices in culture showing development of "tumors" from seeded GFP labeled BON cells



Timecourse of BON carcinoid "tumorlet" proliferation in cultured precision cut liver slices at low magnification.

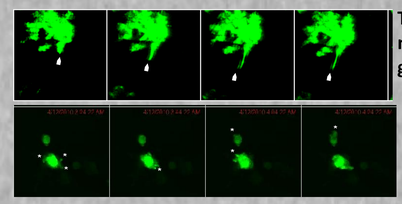


Graph showing increase in tumorlet area within organotypic slices in culture over time



Representative images from a cell aggregation assay experiment qualitatively showing dose dependence of aggregation on amount of liver tissue (wet weight) following continuous exposure for 24hours.

Results continued…



Top panel: Carcinoid cells in a mesenchymal mode of motility. The white symbol marks the growing extension.

The bottom panel: Carcinoid cells displaying ameboid type motility. The white symbols mark the membrane blebs

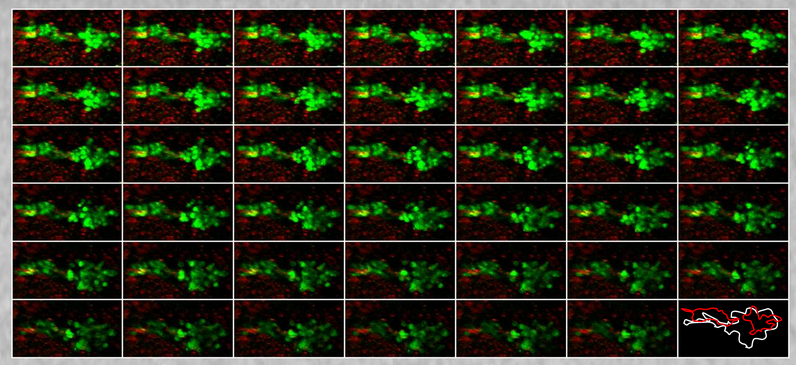
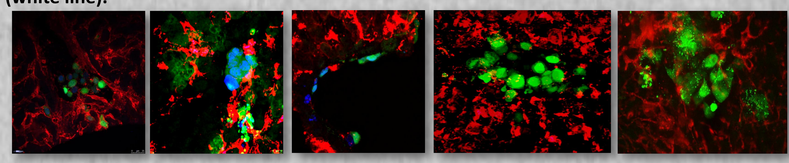
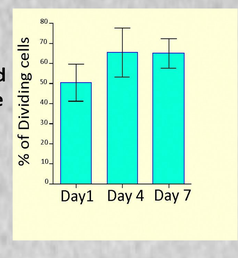


Image montage of two tumor cell groups in a vessel. Cells in the right group migrate into surrounding parenchyma. Subsequently (white symbol) cells in the left group migrate toward first group. Last panel shows cell redistribution from 1st frame (red line) to last (white line).



Immunostaining of Liver slices in culture. GFP labeled BON cells stained with antibody against Ki67 after day 1 (a), day 4 (b) and day 9 (c). (d) Confocal imaging of intact tumor after day 7. (e) Confocal imaging of intact tumor after 15 days in culture showing cells that have started to die.

Histograms showing the percentage of Ki67 stained cells remains fairly uniform at various stages of the experiment .



Summary

- We present a cost effective, tractable *ex-vivo* model system to study behavior of carcinoid cell-lines in a liver tissue microenvironment.
- This model system was shown to be amenable to a variety of optical techniques including live cell imaging and demonstrated that tumor cell dynamics were altered compared to dispersed 2D culture systems.
- The carcinoid cells exhibited multiple modes of motility within liver slices and could switch between a "path-finding" ameboid type of motility to a "path-generating" mesenchymal type within liver slices.
- The formation of the tumor-like structure appeared to be a result of two concurrent processes— cell division and cell aggregation, the latter likely stimulated by an unidentified soluble factor released by the liver.
- Current studies on this project are focused on mechanistic details of the cancer cell interaction with the tumor microenvironment.

Acknowledgement

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