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An Organ Slice Model to Evaluate Carcinoid Tumorogenesis in Liver

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Background: Midgut carcinoid cancers typically metastasize to the liver. However, 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. Using this system we assessed cancer cell dynamics *in situ* and demonstrated that tumor-like structures are formed as a result of two concurrent processes — cell proliferation and aggregation.

Methods: Human carcinoid cancer cells, stably transfected with GFP were introduced to intact mouse liver via hepatic portal vein injection. A long-lasting vital dye was also introduced to mark the vasculature. The liver was sectioned into 200 µm thick slices and maintained in culture for up to two weeks. Cancer cell dynamics were assessed by immunofluorescence methods or by time-lapse imaging.

Results: The formation of tumor-like structures was observed by the 4^{th} to 6^{th} day in culture and it was revealed that more than half of the carcinoid cells were still dividing in the liver slices. Moreover, we found that tumor cells exhibited a variety of characteristic movements and rapidly inter-converted between elongated and rounded (blebbing) modes of locomotion. A rapid form of ameboid movement, observed only after several days in culture, was correlated to tumor cell aggregation in slices. These observations were consistent with *in vitro* experiments that demonstrated that liver tissue pieces or conditioned medium promoted carcinoid cancer cell aggregation in culture in a dose-dependent manner.

Conclusion: Proliferation and aggregation induced by an unidentified tissue-specific soluble factor contributed to the

formation of tumor-like structures in mouse liver slices. This *ex vivo* model provides a tractable and cost efficient system for studying tumor formation in mouse liver.