



WINN FELINE FOUNDATION

For the Health and Well-being of All Cats

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FELINE LIVER ORGANIDS FOR THE STUDY OF LIVER DISEASE IN CATS

PROJECT STUDIES: Feline liver organoids for the study of liver disease in cats

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Final report summary, W15-037

To this date, little is known about the causes and disease mechanisms of many feline liver diseases, such as fatty liver disease or bile duct inflammation. This hampers the development of specific curative treatments. In order to study these diseases without the use of animal experimentation, an appropriate cell culture model is required that mimics the real organ in a relevant manner. A recent advance in the field is the use of adult stem cells to study disease effects in vitro. Adult stem cells from the liver can be isolated from a small piece of liver and then cultured in the lab as so-called liver organoids ('a structure resembling an organ'). These organoids cultures have been successfully created from mouse, dog and human liver. They can be kept in culture for many months. In addition, these liver organoids have a high proliferative capacity (they multiply quickly and can be expanded to high cell numbers in the lab). With special culture protocols, they can be transformed into more mature liver cells, recapitulating many liver functions in vitro. In study W15-037 the investigators aimed to isolate stem cells from the cat liver and to establish the first feline liver organoid culture system. It was possible to successfully start organoid cultures from 5 different cat liver samples, without the use of experimental animals. They were able to start the culture from fresh biopsy material, from frozen liver samples and from fine needle aspirates of cat liver (a procedure that is minimally invasive and routine practice in feline hepatology).

A second study aim was to establish the optimal culture conditions for feline liver organoids. Different types of culture media were tested and finally a culture medium was created that is specific for cat liver organoids and is superior to the culture media as published for mouse, dog and human liver organoids. After establishing the optimal culture conditions the investigators fully characterized the cultures by gene-expression profiling, immunocytochemistry (staining procedure) and proliferation assays. This showed that the organoids were indeed true adult stem cells of the liver. Feline liver organoids multiplied very fast and could be kept in culture for more than 6 months. Importantly, their chromosome numbers did not change upon long term culture, indicating they do not become tumor cells and remain genetically stable, similar to findings in mouse, dog and human liver organoids. Plasticity of the organoid cell-fate was assessed by differentiating the organoids into more mature liver cells. They confirmed differentiation capacity by gene expression analysis and found that the cells also acquired cytochrome P450 activity, an important drug metabolizing enzyme found only in liver cells.

A third study aim was to investigate feline liver organoids as potential research tool to model feline hepatic lipodosis (fatty liver disease). Since this disease also occurs in humans, they asked whether liver organoids from different species would accumulate fat to a similar extent. Interestingly, they indeed found that organoids from mouse, human, dog and cat liver can all accumulate fat, but that feline organoids store much

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more fat than human organoids. The investigators then wanted to test if they could interfere in this process using specific drugs or nutrients. They could make the fat accumulation worse or better by providing specific substances to the feline liver organoids and also noted a significant effect on the viability of the cells.

In conclusion, the investigators have developed a very robust culture system for feline liver cells that was not available to liver researchers before. Only a very small liver sample is enough to culture organoids from and a fine needle aspirate will provide more than enough cells. This opens up many opportunities to isolate and culture patient-specific liver stem cells from individual cats. Feline liver organoids are a highly relevant cell culture for disease modeling research into feline liver disease. More specifically, they show that feline liver organoids can be used as research tool to study feline hepatic lipidosis and that they can test new drugs or nutrients that may be potential new therapeutic targets. This shows that these investigators are now finally able to start investigating fatty liver disease in cats on a cellular level.

Summary prepared by Dr. Bart Spee and Dr. Hedwig Kruitwagen © 2016

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