Project Title: How exercise prevents obesity-related hepatocellular carcinoma: insights for chemoprevention of liver cancer
Grant Awarded: $200,000 over 2 years: $100,000 in 2017-18
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Obesity and diabetes-related fatty liver disease contributes to rapidly increasing rates of primary liver cancer (hepatocellular carcinoma, HCC). Using obese, diabetic (foz/foz) mice with fatty liver disease, we have shown this accelerates onset of HCC following a single low dose of carcinogen early in life. During 2017, we completed research using mice provided with an in-cage exercise wheel to combat obesity: this prevented early-onset liver cancer. The goal of the present research was to identify the protective mechanisms so they could be harnessed using drugs to lower liver cancer risk in predisposed persons.

We identified a key pathway activated by obesity and dampened by exercise as the c-Jun N-terminal kinase-1 (JNK1). This research has been provisionally accepted by the Journal of Hepatology, the top international journal in liver disease, pending additional supportive data. We have now completed further experiments to buttress this paper, including experiments on stored liver cancer cells (derived from earlier research) to test two specific molecular links between JNK1 activation and cell cycle regulation. By selective inhibition and knockdown approaches, we proved that p53 does indeed up-regulate the cell cycle inhibitor p27 (a point challenged by one of the reviewers), and that JNK up-regulates the cyclin kinase, cyclin E1. These findings strongly support the proposed role of exercise acting through cell cycle regulation to combat accelerated proliferation of altered liver cells that leads to cancer.

Another issue was whether exercise exerted its protective effects directly on obese mice, or indirectly by reducing obesity. This mandated careful study of the role of excessive nutrient intake, by subjecting another group of foz/foz mice to dietary restriction by pair-feeding with wildtype mice. We needed to purchase specific cage dividers and to conduct these additional experiments over 6 months, delaying our progress. However, the new data clearly demonstrate that the reduction of obesity by pair feeding does not protect against accelerated hepatocarcinogenesis in foz/foz mice. This means that the protective effect of exercise observed earlier must be exerted directly (such as the proposed effects on cell cycle regulation) rather than via weight reduction. We are in process of resubmission of the manuscript for J Hepatol and are excited that this research, supported in large part by ACT Cancer Council will be published prominently.

In other experiments, we tested whether “switching off” JNK1 accounts for the protective effects of exercise against HCC development. We created (by cross-breeding) a line of foz/foz mice deficient in JNK1 protein. Such Jnk1-/-.foz/foz mice appear to develop less severe forms of fatty liver disease (definitive analysis of the disease phenotype is currently underway). We anticipated that onset of liver cancer after carcinogen (diethylnitrosamine, DEN) injection at day 12 of life would be slow - despite equivalent obesity as ordinary (JNK1 intact) foz/foz mice. As predicted, none of the JNK1-deficient obese mice developed liver cancer at 6 months, versus 60-100% (depending on experiment) of obese, diabetic mice. We have also tested the effectiveness of what was purported to be a selective and safe inhibitor of JNK1 (CC-003, Celgene). We administered CC-003 to DEN-injected foz/foz mice for 21 weeks in an attempt to prevent early onset liver cancer (i.e., at 24 weeks after DEN injection). Unfortunately, despite promising preliminary data, at the completion of the experiment similar proportions of CC-003 treated mice as vehicle-treated foz/foz mice had developed HCC. Whether this was due to inadequate dosing or to activation of alternative molecular pathways to liver cancer that are negated by exercise will require further study. The model systems we have established by sponsorship of this project place us in a highly competitive position to progress this important research.