Project Report

By Qiuwei Pan; 28th, August, 2014

Title: Unveiling the Effects and Mechanisms of Immunosuppressants on Hepatocellular Carcinoma

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Supervisor: Prof. Nancy Kwan MAN, Department of Surgery, University of Hong Kong

Background

Hepatocellular carcinoma (HCC) is the most common but very aggressive liver cancer. Liver transplantation (LT) is a potential curative treatment for small proportion of patients. However, tumor recurrence is a major challenge for the ultimate success. Immunosuppressants used after transplantation to reduce the risk of graft rejection, are known to impact recurrence.

Fig. 1. Research laboratory at Department of Surgery, University of Hong Kong

This training was focused on the study of immunosuppressants, in particular mycophenolate mofetil (MMF)/mycophenolic acid (MPA), on HCC in experimental models. The Department of Surgery at University of Hong Kong harbors a start-of-the-art equipped research laboratory with long-standing expertise in liver transplantation and liver cancer (**Fig. 1**). Prof. Man is leading an interactive research team with diverse expertise that provided an excellent training environment (**Fig. 2**)

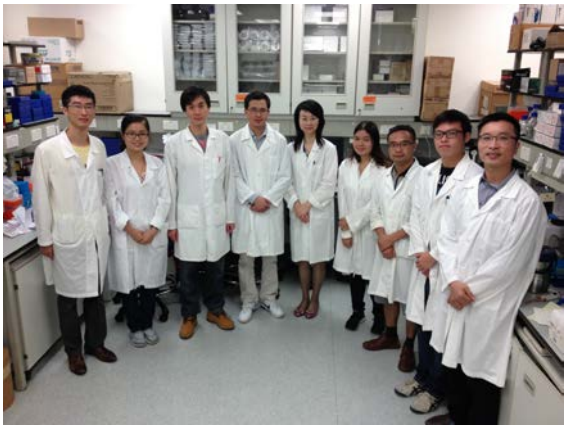

Fig. 2. An interactive research group at the laboratory of Prof. Man with diverse expertise that provided an excellent training environment

Major achievements

1. A comprehensive understanding of the role of different immunosuppressants in HCC recurrence

It was a great opportunity to work under supervision of Prof. Man that her long-standing expertise in liver transplantation and liver cancer has helped to understand the field of immunosuppressants and HCC recurrence, although my previous experience was largely based on viral hepatitis. This has resulted in a nice review[1] article entitled "Rationale of personalized immunosuppressive medication for hepatocellular carcinoma patients after liver transplantation", which was selected as cover article by Liver Transplantation (**Fig. 3**)

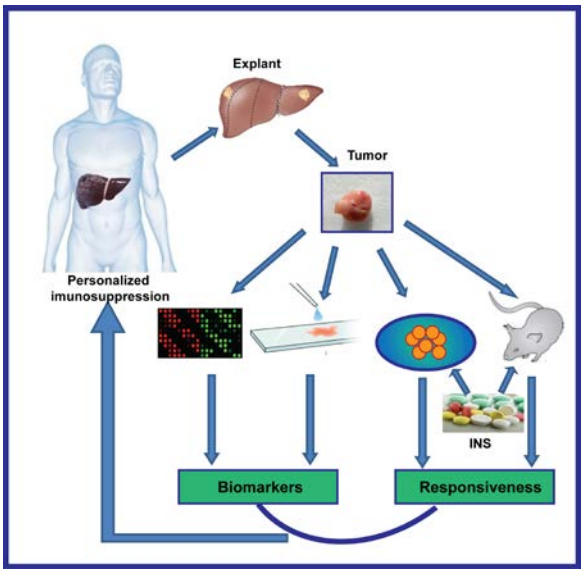

Fig. 3. Concept and approaches of achieving personalized immunosuppressive medication for patients with hepatocellular carcinoma (HCC) after liver transplantation. Different classes of immunosuppressants (INS) may exert diverse effects on hepatocellular carcinoma (HCC) recurrence after liver transplantation. The response to particular INS could also vary between patients. Thus, it is rationale to develop personalized immunosuppression protocol. Using surgically resected HCC tumor at the time of transplantation, potential biomarkers to predict the responsiveness to particular INS could be explored by conventional (e.g. qRT-PCR or immnuohistochemical staining) or high-throughput (e.g. transcriptome or proteome profiling) approaches. Furthermore, resected tumor tissues can also be used to establish *ex vivo* cell culture or xenograft tumor model for per patient to functionally evaluate the responsiveness. (selected as cover by Liver Transplantation)

2. In depth study of the role of MPA on HCC

Preliminary data from early study at Erasmus Medical Center has indicated the potential anti-HCC effect of MPA. During this training, this project has been extensively investigated. METHODS: Five HCC cell lines and nude mice with partial immunodeficiency were used (**Fig. 4**). The association of treatment with MMF, the pro-drug of MPA, with HCC recurrence was retrospectively analyzed in a LT cohort. RESULTS: With clinically achievable concentrations, MPA effectively inhibited HCC cell proliferation and single cell colony formation. It triggered cell apoptosis and arrest of HCC cells in the G0/G1 phase. Supplementation of exogenous guanosine nucleotide partially restored the inhibitory effects of MPA on HCC cells. Ectopic over-expression of a mutated IMPDH2, lacking binding site of MPA but retaining its enzymatic activity resulted in complete resistance of HCC cells to MPA. In nude mice

subcutaneously engrafted with HCC cells, MPA significantly delayed tumor formation. Immunohistochemical staining of harvested tumor tissues confirmed the cell cycling arresting and apoptosis triggering effects of MPA in mice. Most importantly, the use of MMF was associated with significant less HCC recurrence and improved survival after liver transplantation. CONCLUSIONS: By targeting IMPDH2, MPA can specifically counteract HCC growth in vitro and in mouse models. In liver transplant patients, the use of MMF is associated with reduced HCC recurrence and improved survival. These results warrant prospective clinical trials for further investigation.

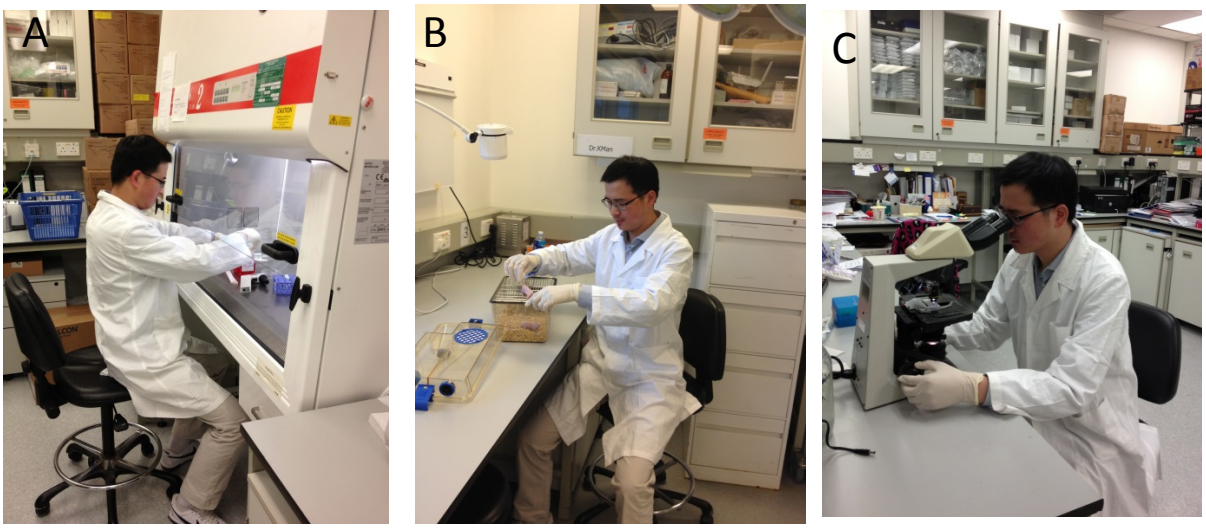

Fig. 4. Experimental approaches. (A) Molecular and cell culture techniques. (B) Mouse models of HCC. (C) Immunohistochemical staining and microscopic evaluation

These data have been presented at a seminar of Department of Surgery, University of Hong Kong (**Fig. 5**), and accepted by the 2014 ILTS annual congress as a poster[2], as well as a manuscript is under preparation.

Fig. 5. Final presentation at Department of Surgery, University of Hong Kong

Future perspective

During this training, Prof. Man has provided the opportunity to work within an interactive team that various techniques can be learned. More importantly, Prof. Man has allowed me (as a young investigator) to take the lead of the project and having opportunity to supervise junior researcher. This has helped for my future career development as a young principal investigator at Erasmus Medical Center. Currently, I have recruited new PhD students to establish my research group working on HCC in the context of (but not restricted to) liver transplantation.

Obviously, such a relatively short-term training is certainly impossible to master all the important techniques available at Prof. Man's lab. Therefore, we now have established strategic long-term collaboration, in respect to technical training, animal model, patient material and information. Finally, I would like to express my sincere gratitude to the support of Prof. Man and ILTS.

Reference

1. Kan Chen, Kwan Man, Herold J. Metselaar, Harry L. A. Janssen, Maikel P. Peppelenbosch and Qiuwei Pan. Rationale of Personalized Immunosuppressive Medication for Hepatocellular Carcinoma Patients After Liver Transplantation. LIVER TRANSPLANTATION 20:261–269, 2014. Cover Article
2. Kan Chen, Kwan Man, Herold J. Metselaar, Harry L. A. Janssen, Jaap Kwekkeboom, Dave Sprengers, Marco J. Bruno, Maikel P. Peppelenbosch and Qiuwei Pan. IMPDH2-targeted Constraint of Cell Growth in Hepatocellular Carcinoma by Mycophenolic Acid. 2014 ILTS annual congress, poster presentation