

## Impact Objectives

- Understand the impact of tumour heterogeneity, and how it affects cancer treatment
- Develop potential therapeutic strategies for heterogenous tumours, specifically in the context of colorectal cancer

# Tumour heterogeneity: from theory to practice

*Assistant Professor Wei-Lun Hwang and Dr Lan-Ting, Emily, Yuan are part of a project that investigates the heterogeneity of tumours and its impact on treatment strategies. Here they discuss Hwang's contributions to research into cancer stem cells, and the current state of colorectal cancer treatment, which they hope they can improve*



Assistant Professor  
Wei-Lun Hwang



Dr Lan-Ting,  
Emily, Yuan

**Can you provide some background about cancer stem cell theory and how it has contributed to your research?**

**W-LH:** My research career started with a cancer stem cell (CSC) study in colorectal carcinoma. The CSC theory has been challenged for years. The first study outlining the theory was carried out with acute myeloid leukaemia, and from 2003-2006 CSC populations were reported in many solid tumours. At that time, we knew that CSCs were involved in the development of malignant progression, including cancer metastasis. By exploiting the established CSC cultivation platform I developed as a master's student in the Institute of Microbiology and Immunology of National Yang-Ming University, Taiwan, our research group unravelled the significance of the epithelial-to-mesenchymal transition (EMT) transcription factor, Snail, in CSCs during my doctoral research. This study is one of the building blocks for research into EMT-induced stemness. Hereafter, CSCs have served as a unique cell model to study the biological and clinical relevance of stem-like

cells in cancers in my lab. I would like to dedicate our CSC researches to the memory of Professor Hsei-Wei Wang of the Institute of Microbiology and Immunology at National Yang-Ming University, who passed away during the period of this research. Our studies could not have been completed without his long-lasting devotion to colon cancer research.

**What is the goal of your research project? Which varieties of cancer are you focusing on?**

**W-LH:** The primary goal of my research is to study how tumour heterogeneity regulates tumour-host interaction, as it is involved in disease progression and therapeutic optimisation. We focus mainly on colorectal cancer and have established Cetuximab-resistant colorectal CSCs (K-RAS wild type and mutant) and Cetuximab-resistant colon cancer cells (K-RAS wild type) as models to elucidate the impact of the secretome of distinct tumour subclones on host cells.

**Can you briefly outline the methods that you are employing in your research?**

**W-LH:** We established the Cetuximab-resistant cells by long-term drug selection and collected secreted proteins and vesicles by concentration and differential centrifugation, respectively, in order to investigate their contents and corresponding impacts on host cells. Upon identification of valuable targets, animal

studies are initiated and patients are recruited for further validation.

**What is the current status of colorectal cancer treatment, and what needs to be improved?**

**L-TY:** Colorectal cancer screening programmes have been ongoing for several years. As they have been implemented, colorectal cancer mortality has been decreasing steadily because more people with colorectal cancer at early stages have been found. However, when patients with colorectal cancer are discovered at a later stage, their five-year survival decreases dramatically. It is our mission, therefore, to discover colorectal cancer at an early stage.

**How do you see your work progressing in the coming years? What would you like to work on next?**

**W-LH:** We believe our work will provide a proof-of concept idea and feasible strategies to combat pathological cell communication in drug-resistant colorectal cancer patients. We would like to extend our findings to clinical applications by initiating clinical trials, and fulfilling the ultimate goal of translational medicine from bench to bedside to business. Hopefully, the tumour-stromal cell interaction would be monitored and serve as parameters for personalised medicine.

# Looking at tumour-host interactions in colorectal cancer

Researchers at **Taipei Medical University**, Taiwan, are exploring the molecular mechanisms of tumour-host interactions during disease progression. With a focus on colorectal cancer, the team hopes to find ways of combating pathological cell communication in drug-resistant colorectal cancer patients

Tumour heterogeneity is a term that describes the differences between the same types of tumours in different patients, as well as the differences between cancer cells within a tumour. Such differences are brought about by interactions between the tumour and the supportive tissue – or stroma – surrounding it, as well as the molecular mesh termed the extracellular matrix, and stress such as hypoxia and drug treatment. All of these factors can imbue tumours with distinct features and different sensitivities to conventional therapy. As such, what might prove to be an effective course of treatment for one patient could be ineffective in another. Tumour heterogeneity means therapeutic heterogeneity, and this is obviously a problem for medics trying to treat cancer – not to mention their patients.

To address this issue, a group of researchers based at Taipei Medical University in Taiwan is studying the molecular mechanisms of tumour-host interactions during disease progression. The interaction between tumour and host is now known to be involved in reprogramming cell plasticity, local inflammation, host cell recruitment and constructing microenvironments that enable tumour progression. As such, it is increasingly being seen as a target for future therapeutics. Led by Dr Wei-Lun Hwang, the Taipei team wants to understand the impacts of the pathological cell communication that results from tumour heterogeneity.

## DRUG RESISTANCE AND STEM-LIKE CANCER CELLS

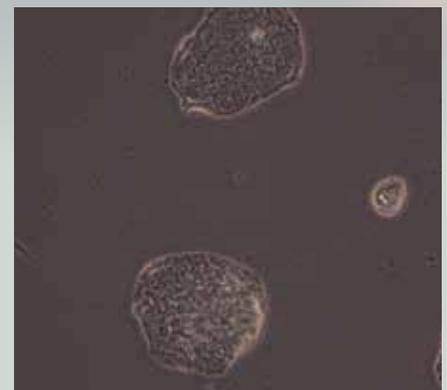
Obviously when considering the



The Cetuximab resistant colon cancer cells

heterogeneity of a tumour, an important characteristic to watch out for is the development of drug resistance. Drug resistant cells can result from either primary or acquired mutations and, as the name suggests, they are unaffected by conventional therapeutic schemes. The example that Hwang and his team have been focusing on is resistance to Cetuximab, an epidermal growth factor receptor (EGFR) inhibitor used for the treatment of metastatic – that is, advanced – colorectal cancer. The initial, or primary, mutation status of certain genes must be considered when treating patients – specifically, mutant KRAS codon 12 and 13 alleles are known to imbue a tumour with resistance to Cetuximab.

Acquired resistance to this drug, on the other hand, only occurs following treatment, which can generate a mutation known as S492R on a cell's EGFR that prevents it binding to the drug. Another way is by generating an alternative activation signal,



The Cetuximab sensitive colon cancer cells

as Hwang explains: 'The alternative signal that is acquired – the Snail-LTB axis – in Cetuximab-resistant cells functions as a surrogate pathway to engender drug resistance'.

The other key aspect of tumour heterogeneity that Hwang is focusing on is cancer stem cells (CSCs), so-named because they share many of the properties of actual stem cells – which, in a tumour, is not a positive thing. 'CSCs are a subpopulation of cells with stem cell properties and tumour-initiating capacity in tumours,' explains Hwang. 'They exhibit stem cell traits, meaning they divide asymmetrically and differentiate into daughter progenitors or daughter differentiated cells for orchestrating hierarchy within tumours, and divide symmetrically to maintain CSC pools.'

Several studies have shown that between one and 10 CSCs are able to generate a tumour in mouse models. This is in sharp

## Cancer stem cells are a subpopulation of cells with stem cell properties and tumour-initiating capacity in tumours

contrast to the tumourigenic capacity of non-CSCs, which need thousands to millions of cells for tumour generation. In addition, CSCs have been implicated in cancer metastasis, disease relapse and, crucially, therapeutic resistance.

### ESTABLISHING CELLS

To see how specific cancer clones impact cancer malignancy, specifically that of colorectal cancer, Hwang and his team have established Cetuximab-resistant colorectal CSCs and Cetuximab-resistant colon cancer cells. They are using these models to understand the impact that proteins and vesicles released by tumour subclones have on host cells.

Isolating CSCs, and the expansion of drug-resistant cell lines, is not easy; in fact, Hwang says that undertaking these processes has provided some of the biggest challenges his team has faced. They isolated and characterised CSCs from colorectal carcinoma back in 2006, and following around a year of validation the cells were approved and have since been utilised to research many different types of cancers. 'Unlike other solid tumours, there are plenty of markers and approaches to purify colorectal CSCs from both cell lines and clinical specimens,' explains Hwang. 'In an attempt to enrich all potential stem-like populations in tumours, we exploited the serum-free spheroid cultivation approach as it was originally used to enrich neuron stem cells.' Crucially, cells cultured by this approach retain their original molecular features, and therefore resemble *in vivo* tumours.

Then there is the expansion of drug-resistant cells: 'To mimic the acquired resistance in tumours, cancer cells were treated with drug for at least three months for clonal expansion *in vitro*,' Hwang explains. 'As the establishment of drug-resistant cells is essential for our projects, it took three rounds of clonal selection from the very beginning to obtain the experimental cell lines as initial materials.' As the project is all about communication between tumour cells and regular host cells, key components

of Hwang's research look at exosomes and microvesicles. These are nanoparticles, secreted by almost all cells, which are used predominately to communicate between host cells and cancer cells found in tumours. There are lots of extracellular vesicles found in blood and other bodily fluids, suggesting they are useful intercellular communication substances. 'Exosomes and microvesicles are likely to exert a distal regulation in hosts away from the localised, primary tumours, and they hold potential as diagnostic factors,' says Hwang. 'Therefore, the function of secreted vesicles in drug resistance is intriguing.'

### TRANSLATING TO COLORECTAL CANCER

The work that Hwang and the rest of the team are doing is particularly notable given that colorectal cancer, their primary focus, is so lethal, and also difficult to treat. Colorectal cancers routinely rank in the top causes of cancer-related death for both men and women, and they tend to require a combination of surgery and chemotherapy if they are to be eradicated from a patient's body. Clearly the confounding factor of tumour heterogeneity contributes negatively to patient outcomes, which is why the kind of fundamental research being undertaken by these researchers is so essential. Dr Lan-Ting, Emily, Yuan, a key member of Hwang's team, is a clinician with an interest in colorectal cancer screening, and she is involved with the clinical side of this research. The goal is to initiate clinical trials that incorporate some of the research group's ideas about tumour heterogeneity, to see if they will improve colorectal cancer outcomes.

Ultimately, by interrogating the impacts of pathological cell communications that are a result of tumour heterogeneity, the team hopes to develop potential therapeutic strategies that could transform current approaches. Characterising exosomes and microvesicles that are secreted from drug-resistance cells will one day lead to identifying potential diagnostic factors and have a significant impact on treating cancers.

## Project Insights

### FUNDING

Taipei Medical University (TMU104-AE1-B11) • Yuan's General Hospital (106YGH-TMU-02) • Ministry of Science and Technology (105-2320-B-038-009-MY2 and 106-2628-B-038-001-MY3)

### CONTACT

**Wei-Lun Hwang**  
Principal Investigator

**T:** +886 228235870

**E:** wlhwang930@tmu.edu.tw

**W:** <https://tmu.pure.elsevier.com/en/persons/wei-lun-hwang>

### PROJECT COORDINATOR BIOS

**Assistant Professor Wei-Lun Hwang** completed a master's and PhD at the Institute of Microbiology and Immunology and Institute of Biochemistry and Molecular Biology in National Yang-Ming University, Taiwan, respectively. After conducting his postdoctoral research at the University's Institute of Clinical Medicine, he took up his current position at Taiwan Medical University.

**Dr Lan-Ting, Emily, Yuan** received her medical degree from George Washington University, USA, and subsequently received her internal medicine residency training at LAC+USC Medical Center, USA. She moved back to Taiwan and completed her gastroenterology fellowship training at Yuan's General Hospital, where she took up her current position.

中華民國科技部  
Ministry of Science and Technology, R.O.C.

