Student: Maria Nonis
Title: Analysis of the hypoxic pathway in kidney cancer
Supervisors: Associate Professor Gabi Dachs and Christina Wohlrab
Sponsor: Centre for Translational Cancer Research

Introduction: The incidence of kidney cancer is rising in New Zealand. Kidney cancer is often detected at an advanced stage as an incidental finding. Once it has spread beyond the kidney it is a lethal disease with few treatment options. Treatment usually involves partial or full removal of the affected kidney. The hypoxic pathway is a key pathway upregulated in aggressive kidney cancer, promoting tumour progression and reducing patient survival. Vitamin C is a key regulator of the hypoxic pathway, causing degradation of the key hypoxic transcription factor, hypoxia inducible factor (HIF). Vitamin C must be transported into cells via one of the two vitamin C transporters, SVCT1 and SVCT2, in order to regulate the hypoxic pathway. In hypoxic conditions HIF1 and HIF2 act on DNA in the nucleus causing a large group of genes to be transcribed into proteins. Proteins under HIF control aid in tumour progression, cancer cell survival and resistance to therapy, and include the following: GLUT1, a glucose transporter providing glucose for tumour growth; CA-9, a pH regulator allowing tumour cells to maintain a neutral pH while pumping acidic H+ ions out of the cell and allowing tumour survival; and Cyclin-D1, promoting cell growth and therefore tumour growth.

Aim: Investigate the location and level of vitamin C transporters and HIF-pathway proteins in clinical samples from patients with kidney cancer

Impact: Vitamin C use in cancer is an ongoing debate due to a lack of robust clinical evidence. Despite this, many cancer patients opt for high dose vitamin C treatment. This data will add to the debate regarding vitamin C in kidney cancer and aid in the design of clinical trials in cancer patients.

Method: Immunohistochemistry (IHC) was used to stain hypoxic pathway proteins on 105 clinical samples from kidney cancer patients. Sectioned cancer tissue samples were provided by the Cancer Society Tissue Bank, following ethical approval by the University of Otago Human Ethics committee. Vitamin C transporters (SVCT1 and SVCT2), HIF1α (subunit of HIF1), HIF2α (subunit of HIF2), and multiple downstream targets (GLUT1, CA-9 and Cyclin-D1) were stained. IHC involves detecting a target protein using antibodies and then adding a substrate to produce a detectable coloured product. Microscope images were taken for each tumour sample for each protein stained. Following this, images were analysed to determine the number of tissue samples staining positive for each protein.

Results: In normal kidney cortex samples, both vitamin C transporters (SVCT1 and SVCT2) had strong membrane staining. In tumours samples there was less clear localisation of SVCT1 and SVCT2 to the membrane, staining was instead seen in the cytoplasm with some nuclear staining for SVCT1 (Figure 1). Of the patient tumour samples, 96% stained positive for SVCT1 and 98% stained positive for SVCT2.

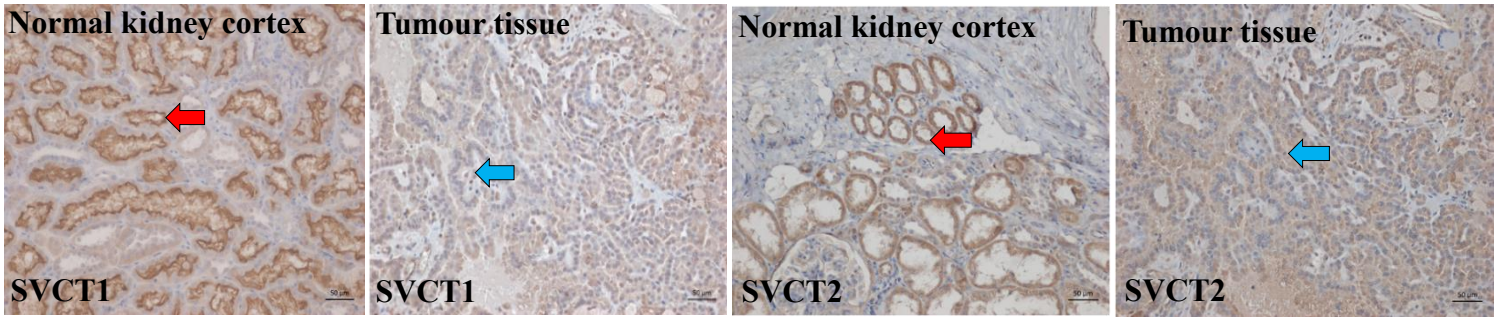

Figure 1. *Images of normal kidney and kidney cancer tissue sections stained using immunohistochemistry. Images are taken at 20x magnification. Positive staining is shown in brown (DAB chromogen) against the blue/purple background staining, showing cell features (H&E). Red arrows show membrane staining; blue arrows show cytoplasmic or nuclear staining.*

HIF1α displayed nuclear staining, as expected, with 51% of patient tumour samples staining positive for HIF1α (Figure 2). HIF2α staining was seen in the cytoplasm with 90% of tumour samples staining positive. Of the downstream targets in the hypoxic pathway GLUT1, CA-9 and Cyclin-D1 were investigated. GLUT1 stained positive in both the nucleus and the cytoplasm, with 91% of patient tumour samples staining positive. CA-9 staining was expected to be predominantly found in the membrane, but was seen in the nucleus, with 98% of the patient samples staining positive. As expected, Cylin-D1 was located in the nucleus with 83% of samples staining positive.

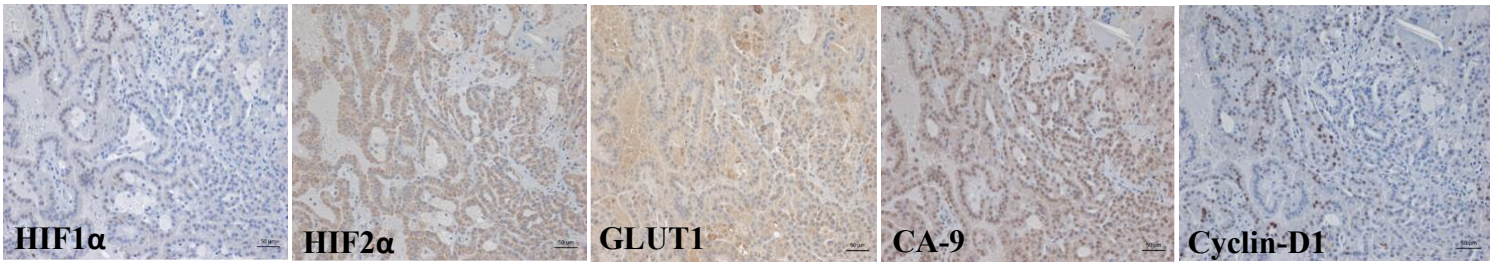

Figure 2. *Microscope images of kidney cancer tissue sections stained using immunohistochemistry. All tumour tissue images shown here are from a single kidney cancer patient as a representative of results seen for other tumour samples (n=105). Images are taken at 20x magnification. Positive staining is shown in brown (DAB chromogen) against the blue/purple background staining showing cell features (H&E).*

Conclusion: Our data shows for the first time the location and extent of staining of the vitamin C transporters and members of the hypoxic pathway in kidney cancer. The vitamin C transporters appeared to lose membrane localisation in tumours compared to normal tissue, and this may change the ability of tumour cells to accumulate vitamin C. This may have clinical implications, as vitamin C may be able to dampen the hypoxic pathway, as was previously shown in cell culture and in other types of cancer. The role of vitamin C in kidney cancer is currently unknown. Besides the location of CA-9, all other hypoxic factors stained as expected. Determining the location and intensity of staining of key proteins in the hypoxic pathway will aid in understanding the importance of this pathway in kidney cancer. This data may lead to further research into vitamin C in kidney cancer and impact the use of vitamin C in kidney cancer patients.