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Project: Analysing gene expression data to understand ascorbate transport in tumours of colorectal cancer patients

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Introduction:

Colorectal cancer is the third most common cancer in the world, in which New Zealand has one of the highest incidence rates. Current treatments for colorectal cancer include combinations of surgery, radiation therapy, chemotherapy and other targeted therapies. Interestingly, an increasing number of cancer patients are choosing to undergo alterative treatment options, with many opting for high-dose ascorbate (Vitamin C) treatments. The role of ascorbate in cancer remains controversial. However, recent studies from our laboratory on human tumour samples from patients with endometrial (Kuiper, 2010) and colorectal cancer (Kuiper, 2014) have shown intriguing associations between low tumour ascorbate levels with a more aggressive and hypoxia-related tumour phenotype, as well as shorter disease-free survival. Further research also showed that ascorbate levels in normal human colon tissue was not associated with ascorbate levels in human colorectal tumour samples from the same patient. Factors which may contribute to ascorbate accumulation in tumours are vascularity, tumour tissue density, and the level of ascorbate transporters (SVCT-1, SVCT-2, GLUT-1). Sodium-dependent vitamin C transporters (SVCT's) are the main ascorbate carriers, concentrating ascorbate in tissue at levels many times greater than in plasma. The glucose transporter (GLUT-1) facilitates the uptake of dehydroascorbate to the tissues, requiring subsequent intracellular reduction to convert it to ascorbate. Many aspects of tumour pathology are governed by the hypoxic transcription factor HIF-1. HIF-1 levels and activity are regulated by a family of enzymes called HIF hydroxylases. These hydroxylases require ascorbate as a co-factor to function efficiently. The result of this requirement is that HIF-1 accumulates in cells with insufficient ascorbate, and likely explains the relationship in cancer patients where reduced tumour ascorbate correlates with increased HIF-1 levels and poorer survival. It is important therefore to improve our understanding of why some tumours accumulate ascorbate whereas others do not.

Aim:

The aim of this project was to determine whether expression levels of ascorbate transporters in tumours from a large international cohort of colorectal cancer patients are associated with hypoxia-related gene expression and clinicopathological factors.

Method:

The Cancer Genome Atlas, an online repository, was used to obtain tumour expression data from a large cohort of colorectal cancer patients. Expression levels of ascorbate transporters (SVCT1, SVCT2, GLUT1) were correlated with HIF genes (HIF1A, EPAS1), HIF-controlled genes (VEGFA, BNIP3, CA9), and HIF-hydroxylases (EGLN1, HIF1AN) in colorectal tumours from 382 patients. Additionally, disease free survival (DFS), overall survival (OS), gender, stage and grade were investigated using Kaplan-Meier curves and two-tailed independent t-tests. For

survival analysis, colorectal tumours expressing high or low levels of ascorbate transporters were categorised as Z-scores above and below zero, respectively. Statistical analyses were carried out using in R Studio v3.2.2.

Results:

We proposed that increased transporter expression would result in greater ascorbate accumulation in the tumour and therefore activation of HIF hydroxylases that would inhibit the HIF-1 transcription factor, and result in reduced hypoxic gene expression. Our data showed that SVCT1 mRNA expression was significantly associated with the expression of three of the HIF-related genes (BNIP3, CA9, HIF1A) ($p < 0.01$). As SVCT-1 expression increased in the colorectal tumours, there was no significant change in EGLN1 and HIF1AN hydroxylase expression ($p > 0.05$), but despite this, HIF-1A expression significantly decreased ($p < 0.01$, $R2 = -0.19$). However, we saw conflicting results within the HIF controlled genes, with only CA9 significantly decreased, as expected ($p <0.01$, $R2 = -0.15$). The expression of SVCT2 mRNA showed a significant relationship with five HIF-related genes (BNIP3, HIF1A, EPAS1, EGLN1, HIF1AN) ($p < 0.01$). Although SVCT2 showed some correlations with the genes of interest, for the most part these relationships were in the opposite direction of what was expected. With increasing expression of the SVCT2, unexpectedly HIF-1A and HIF controlled gene expression significantly increased, but there was an expected increase in the HIF hydroxylases (EGLN1: $p < 0.01$, $R2 = 0.16$) (HIF1AN: $p<0.01$, $R2 = 0.30$). Additionally our results suggest that, at the transcript level, there was no association with the expression of the ascorbate transporters and the stage and grade of the tumours, or to gender and survival outcomes (DFS and OS) of the patients ($p > 0.05$).

Conclusion:

This study investigated ascorbate transporter mRNA expression levels in relation to the expression of HIF hydroxylases, HIF genes and HIF-controlled genes in 382 human colorectal tumours. Overall, we observed that when SVCT-1 ascorbate transporter expression increased, there was a significant and expected decrease in some hypoxic gene expression. This was not the case for SVCT-2.This study has provided valuable scientific data for the ongoing debate surrounding the use of ascorbate as part of cancer treatment. The role of ascorbate transporters in tumours from colorectal cancer patients is complex and requires further analysis.