FINAL PROJECT REPORT

Date: 31 January 2010

Name:

Margreet C M Vissers

Project Title:

A new role for Vitamin C: control of the hypoxic response in cancer cells

Please copy the "Outcome(s)" statement, entered on your application form, in the space below.

The hypoxic response in tumours is due to increased oxygen consumption and poor vascularisation, and HIF-1 is thought to contribute to angiogenic signaling, increased dependence on glycolysis, and alterations in apoptosis and cell proliferation, all of which act to promote tumour survival. A considerable effort is being made to investigate the effect of HIF-1 on the tumour microenvironment, with the aim of modulating this activity to influence cancer outcome. Our studies will provide information on the role of ascorbate in modulating these processes and could provide a link between Vitamin C and the responsiveness of tumour cells to cancer chemotherapy. This may begin to provide some understanding of an important role for Vitamin C in cancer.

Will your work contribute to this outcome(s) in the manner you envisaged? If not, what has changed?

The results of this project have been exciting and important, and have validated our initial hypothesis. The project has generated interesting data on the ability of Vitamin C to impact on the activity of the hypoxic response in tumour cells. We have been able to determine an important function for Vitamin C in the gene expression controlled by hypoxia-inducible factor-1 and the consequent ability of cancer cells to promote angiogenesis. Our studies have been able to refine our understanding of the importance of Vitamin C in determining the survival and tumour promoting activity of cancer cells.

The contribution of this research to our understanding of the importance of intracellular Vitamin C is significant, and the publications that result from this study will highlight the ability of Vitamin C to inhibit cancer cell growth and increase tumour susceptibility to chemotherapy.

Please copy the "Specific Objective(s)" statement, entered on your application form, in the space below.

Specific objectives

The objectives of this project are to determine the effect of intracellular ascorbate on HIF-1α regulation and gene expression in tumour cells and on subsequent tumour cell survival. Our aims are:

1. To probe the role of intracellular ascorbate on the hypoxic response in cancer cells with and without ascorbate supplementation.
2. Using transiently transfected cells to monitor the expression of GFP-tagged reporter genes, and to measure levels of VEGF and GLUT-1 mRNA and protein.
3. To develop a Microarray assay to investigate the effect of intracellular ascorbate on HIF-1α-dependent gene expression more generally

Briefly describe how successful you were in achieving the stated objective(s). If the objective(s) was not achieved, explain why that is the case and describe what you did manage to achieve.

Specific objective 1: To probe the role of intracellular ascorbate on the hypoxic response in cancer cells with and without ascorbate supplementation.

This aspect of the study has generated an interesting new finding, showing that Vitamin C is highly influential on the gene expression induced by the hypoxic response and down-regulates the expression of pro-survival proteins. This information is being combined with further data to show that this does indeed affect the response to chemotherapy and these results will be published in a peer-reviewed journal.

Specific Objective 2. Using transiently transfected cells to monitor the expression of GFP-tagged reporter genes, and to measure levels of VEGF and GLUT-1 mRNA and protein.

We developed the technology to measure hypoxia-induced gene expression with GFP-tagged reporter genes and have shown that this expression is down-regulated when cells contain Vitamin C. We have measured a number of hypoxia-inducible genes that are important for tumour growth and survival; in particular, VEGF and GLUT-1 which determine angiogenesis and the glycolytic response. We have also developed an assay to measure BNIP3, an important pro-survival protein and have shown that this is readily influenced by Vitamin C.

Specific Objective 3. To develop a Microarray assay to investigate the effect of intracellular ascorbate on HIF-1α-dependent gene expression more generally.

To date we have not used a Microarray system to measure global gene expression as more recent technology tends to favour the use of specific pathway analysis to determine effects on gene expression. This change in technology caused us to re-think the Microarray approach and we are currently exploring the use of specialised pathway analysis by using specialised kits that target the hypoxic response more specifically. This is likely to provide useful information.

Please confirm delivery of the outputs listed on your application form. If these outputs were not to be delivered, please explain why.

To our knowledge, this project has delivered according to the outputs given at the time of application. This is an area of novel research, and the results generated are significant, not only in the advancement of knowledge, but in their likelihood of determining future research directions.

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