

Identification of colon cancer stage specific N glycoproteins that may serve as novel prognostic or diagnostic biomarkers

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Colorectal cancer (CRC) is one of the leading five cancers afflicting both men and women in South Africa. Glycosylation of aberrantly expressed proteins is a key post-translational modification that plays an important role in the development and progression of CRC. The nucleoside antibiotic Tunicamycin inhibits glycosylation leading to reduced proliferation, migration, and invasion of colon cancer cells. The aim of this study is firstly to profile tumor stage specific glycoprotein expression in cell lines representing CRC tumour stages 1, 2 and 3, respectively. Next, changes in the glycoprotein profiles following Tunicamycin treatment will be determined in each cell line. *In vitro* cell culture was used to establish the cell line specific IC₅₀ values for Tunicamycin. Total protein was isolated from the cells before and after drug treatment; and the N-glycoprotein fractions were isolated and enriched from these extracts using *conA* lectins. The IC₅₀ of Tunicamycin was determined to be 49 μ M for early tumour stage SW480 cells (stage 1), 52 μ M for mid-stage HT29 cells (stage 2) and 100 μ M for late stage DLD1 cells (stage 3). SDS-PAGE analysis of total protein extracts and enriched N-glycoproteins from untreated cells showed differential patterns of protein expression in each cell line; and tumour stage specific patterns of N-glycoprotein expression. Since the DLD-1 cells had the highest IC₅₀ values, these late-stage CRC cells were the most resistant to Tunicamycin. The cellline specific N-glycoprotein expression will be profiled using mass spectrometry, to aid in the identification of glycoprotein stage-specific tumour biomarkers.