ABSTRACT

Longevity Assurance (LASS) genes also known as Ceramide Synthases (CerS) belong to a family of six related genes. CerS gene products have been shown to produce ceramide, hence their name CerS. Ceramide is a bio-effector molecule, belonging to the family of sphingolipids (SLs), which are important components of cell membranes. Ceramide has been implicated in cancer and apoptosis. Cancer still remains the second leading cause of death, globally and in South Africa. The proper regulation of the balance between cell growth and cell death is essential for cellular homeostasis. Failure to properly regulate this balance may lead to pathologic conditions such as cancer development. CerSes have been implicated in cancer biology, especially apoptosis, through the action of ceramide. The precise roles of CerSes in different cancers is not yet fully understood, especially the role of CerS4 and CerS5 in colon and endometrial cancers.

The broad aim of this study was to investigate the role of CerS4 and CerS5 in apoptosis and, thus in cancers of the endometrium and colon, which are among the top five prevalent cancers globally. Bioinformatics tools (STRING, BioGRID and IntAct databases) were used to determine the CerS4 and CerS5 potential protein-interacting partners, to assess their possible roles in cancer biology. Sequence alignment tools (Multi-Align, Clustal Omega and COBALT) were then used to examine if there was sequence similarity between CerS4 and or CerS5 and their possible protein interacting partners. Total RNA was extracted from cancerous and non-cancerous cells, or calibrator and test samples. Quantitative relative expression of CerS4 and CerS5 was then determined in all cell lines, normalised to β-actin. Apoptosis was induced in cultured colon cells using 5-fluorouracil, while anastrozole was used to induce apoptosis in

endometrial cells. Fluorescence activated cell sorting (FACS) was used to analyse and quantify apoptosis. Quantitative Real-Time Polymerase Chain Reaction (qRT-PCR) was again used to determine change in expression level of CerS4 and CerS5 after apoptosis induction.

Bio-informatics analysis revealed at least 30 proteins that could possibly interact with CerS4 and 12 that may interact with CerS5, and these are involved in apoptosis, cell-cycle regulation, ubiquitination and interestingly in bone and cartilage formation, as well as the central nervous system. Surprisingly, no sequence similarity was revealed between CerS4 and/or CerS5 and the possible interacting partners, when all aligned together. Using quantitative real-time PCR, both LASS4 and 5 were shown to be up-regulated in colon and endometrial cancer cells. Apoptosis induction resulted in down-regulation of LASS 4 and LASS 5 in colon and endometrial cancers. These findings implicate the involvement of these genes in cancer and apoptosis. Whether these genes play a pro- or anti-apoptotic roles in the cancers of the colon and endometrium is not conclusive at this stage. It may also be possible that these genes could exert opposing roles in the same or different tissues. Targeting this family of genes and understanding their precise individual roles in different types of cancer, is a promising therapeutic tool to new anti-cancer drug discovery or improving on the existing ones.