Characterization of a novel cell death related gene, DWNN, in human cervical cancer.

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Background: DWNN (Domain with No Name) was first identified by promoter-trap mutagenesis in Chinese hamster ovary cells, and a knockout of this gene in these cells renders them resistant to staurosporine-induced apoptosis. Human DWNN gene is located on chromosome 16p21, with 18 exons and is 36 kb long. It is alternatively spliced at exon 16 and makes two major transcripts, 1.1 and 6.1 kb, encoding 13 kDa and 200 kDa proteins respectively. Apoptosis is referred to as programmed cell death, whereby a cell deliberately commits suicide, and thus regulates cell numbers during development and maintenance of cellular homeostasis. Its dysregulation leads to the development of various diseases, including cancer. Cervical cancer is rated the second most common malignant tumour globally, and is aetiologically linked to human papillomavirus (HPV). It mostly affects black and colored South African women.

Aim: The purpose of the study is to elucidate 1) the possible role of DWNN in cervical cancer and apoptosis 2) to establish expression patterns and to determine expression levels of DWNN at protein and mRNA levels in cervical cancer.

Methods: To determine the expression patterns of the three mRNA transcripts, colorimetric and fluorescent *in situ* hybridization were performed in tissue sections for normal and cervical cancer. Anti-human DWNN antibodies were used to localize DWNN protein and this was accomplished by immunocytochemistry. Image analysis was done to determine the intensity of labelling of the DWNN protein. Apoptosis was correlated with the levels of DWNN expression using DNA *in situ* labelling technique, TUNEL. Proliferation assay using ki67 marker was done to evaluate proliferative status of the tumour samples. Anti-apoptotic protein Bcl-2 was localized to correlate its expression to DWNN. The DWNN expression levels were also confirmed by quantitative RT-PCR using Roche Lightcycler

Results: There were elevated levels of the three mRNA transcripts in cervical cancer as compared to the normal tissues. The transcripts were localized in the nuclei of invaded stroma; moderately differentiated islands of tumour, dysplastic epithelium and some infiltrating lymphocytes. DWNN protein was highly expressed in the dysplastic epithelium; dysplastic endocervical glands, moderately and well differentiated islands of tumours, dysplastic endocervical glands and the invaded stroma. Image analysis indicated elevated expression levels in the islands of tumours. There was high apoptosis in the invaded stroma and moderately differentiated islands of tumour and this was significantly correlated with DWNN localization. Proliferation was found to be indirectly proportional to DWNN expression. There were low levels of anti-apoptotic protein Bcl-2 in the areas where DWNN expression levels were high.

Discussion: There were up-regulated levels of DWNN in cervical cancers in contrast to normal tissues. This suggests DWNN to be proapoptotic as there were elevated levels of apoptosis in the same sites where there were high levels of

DWNN expression and Bcl-2 was down-regulated in the same sites. DWNN expression significantly correlated with apoptotic index and was indirectly proportional to ki67 in human cervical cancers. Quantitative RT-PCR also confirmed the up-regulation levels of DWNN in cervical cancer.

Conclusion: DWNN might be proapoptotic in cervical cancer. Further characterization of this gene could lead to its manipulation as a diagnostic markers and a potential therapeutic target for cancer treatment