

Honours Project

Expression of p16INK by Flow Cytometry and Human Papillomavirus Genotype in Cervical Dysplasia

Cytological screening of pap smears is the primary method of detecting dysplastic cervical cells and cervical cancer. Pap screening programs have and continue to be very successful in reducing the cervical cancer rate by detecting early neoplastic change. Because the precision and accuracy of the screening process depends on the experience and interpretative ability of the individual scientists reviewing the smears, the detection of subtle, early dysplastic changes could be described as somewhat subjective. The proposed study will determine if flow cytometry could be used in conjunction with conventional cytology to improve the overall diagnostic efficiency of identifying samples with early stages of cervical dysplasia.

Dysplastic cervical epithelial cells specifically express the protein p16INK on their surface. This is the result of the Human Papilloma viral protein E7 binding to host retinoblastoma tumour suppressor protein (pRB). Conventional studies of the expression of p16INK involve immuno-histochemical staining of biopsies or Pap smears and assessment by light microscopy. In the proposed study, cells obtained from clinical Thin Prep (liquid based Pap) samples will be stained with p16INK conjugated to fluorescein isothiocyanate (FITC) and analysed by flow cytometry. Flow cytometry enables large populations of cells to be analysed with high sensitivity and specificity, very rapidly. Results obtained by flow cytometric analysis will be compared with the routine cytology results for each sample to determine if this assay would be a sensitive adjunct to routine cytology.

It is also recognised that certain Human Papillomavirus (HPV) genotypes cause cervical cancer. HPV types 16 and 18 cause approximately 70% of cervical cancers worldwide. The study will also attempt to determine if the presence and level of expression of p16INK staining correlates with the presence of particular HPV genotypes in the samples. Certain oncogenic HPV genotypes produce E7 proteins that bind to pRB with greater affinity, which may lead to enhanced expression/production of p16INK. HPV DNA will be amplified by PCR and the HPV genotype determined by DNA sequencing.

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