**Immunohistochemical Expression of p16, MCM2, Topo IIα and MCM2/Topo IIα in Cervical Squamous Intraepithelial Lesions**

A Heras1, W Bakeman1, A Sanchez1, G King1, E Luevano2 and G Ghirardi3

1 BIO SB, Inc., Santa Barbara, CA, USA, 2 Hospital CIMA, Chihuahua, Mexico and 3 Hospital Córdoba, Córdoba, Argentina

**Background:** The protein p16INK4a (p16) is a cell-cycle regulator; its expression is tightly controlled in normal cells, and it’s shown to help in the detection of high-risk HPV infections. p16 has been considered a useful marker in identifying squamous dysplasias with high sensitivity and specificity for HSIL’s and some diagnostic value in LSIL’s. Minichromosome maintenance protein 2 (MCM2) is essential for eukaryotic DNA replication and drives the formation of pre-replicative complexes, which is the key first step during G1 phase. Therefore, altered MCM2 expression may be a hallmark of cell-cycle deregulation, which could be the most essential mechanism in the development and progression of human cancers. This protein is overexpressed in cervical dysplasia as a result of HPV infection. The overexpression of MCM2 provides the link between oncogenic HPV infection and the molecular event of cervical dysplasia. DNA Topoisomerase IIα (Topo IIα) is a nucleic enzyme that affects the topological structure of DNA by interacting with the double-helix DNA, thus playing an important role in DNA replication, transcription, recombination, condensation, and segregation. The objective of this study was to evaluate the Immunohistochemical (IHC) expression of p16, MCM2, Topo IIα, and MCM2/Topo IIα cocktail in different grades of malignancy of cervical squamous intraepithelial lesions.

**Design:** The expression p16 (mouse monoclonal clone 16P04), MCM2 (rabbit monoclonal clone MCM2/24), Topo IIα (rabbit monoclonal clone Topo2a/D6) and a cocktail of MCM2/Topo IIα in 119 surgically resected FFPE tissues (43 normal, 40 LSIL and 36 HSIL’s) was analyzed using IHC. Results were reported as follows: 0 when immunostaining was found only in the parabasal and basal cells, 1+ when 1/3 of the epithelium showed immunoreactivity, 2+ when 2/3 of the epithelial layer produced immunostainings and 3+ when positive reactions were seen throughout the cervical epithelium, including superficial cells. An independent sample T-test was used to compare the expression of p16, MCM2, Topo IIα, and MCM2/Topo IIα in normal, LSIL and HSIL samples. A paired sample T-test was used to investigate statistically significant differences of expression among p16, MCM2, Topo IIα, and MCM2/Topo IIα for normal, LSIL and HSIL’s.

**Results:** p16, MCM2, Topo IIα, and MCM2/Topo IIα showed a statistically significant difference of expression among normal, LSIL and HSIL (p <0.001), with increased immunoeexpression correlating with higher degree of malignancy for all markers. MCM2 and MCM2/Topo IIα cocktail were always expressed in higher quantities and generated stronger signals than p16 and Topo IIα in LSIL (p<0.001). The average immunopositivity for p16, MCM2, Topo IIα, and MCM2/Topo IIα in LSIL was 1.28, 1.71, 0.45 and 1.76, and in HSIL was 2.5, 2.4, 1.05 and 2.5, respectively. There was a 100% correlation in the expression and localization of IHC signals for p16, MCM2 and MCM2/Topo IIα in both LSIL and HSIL’s.

**Conclusions:** The results of the current study show that expression patterns of MCM2 and MCM2/TOPO IIα correlate with p16, especially in HSIL. Their IHC expression is closely associated with progression of cervical squamous intraepithelial lesions, and they may be useful markers for assessing the staging of SIL’s. MCM2/TOPO IIα increases the diagnostic sensitivity for both LSIL and HSIL’s.
CASE 122

IHC of p16 of an HSIL

IHC of Topo IIα of an HSIL

IHC of MCM2 of an HSIL

IHC of MCM2/Topo IIα of an HSIL

CASE 150

IHC of p16 of an LSIL

IHC of Topo IIα of an LSIL

IHC of MCM2 of an LSIL

IHC of MCM2/Topo IIα of an LSIL
CASE 149

IHC of p16 of an HSIL

IHC of Topo IIα of an HSIL

IHC of MCM2 of an HSIL

IHC of MCM2/Topo IIα of an HSIL