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Epigenetic methylation status of P16, MGMT and SPOCK2 in diffuse Large B cell lymphoma

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Introduction: Epigenetic methylation has been implicated in the pathogenesis of diffuse large B cell lymphoma (DLBCL). This study investigated the methylation status of *p16*, *MGMT* and *SPOCK2*. Aberrantly methylated *p16* and *MGMT* have been linked to DLBCL, but not *SPOCK2*. *p16* inhibits cyclin-dependent kinase, which results in retinoblastoma phosphorylation and blockage of cell cycle at G1 phase. *MGMT* removes alkyl adduct at O^6-guanine, thus preventing lethal cross-links. *SPOCK2*, an extracellular chondroitin and heparin sulfate proteoglycans, abolishes the inhibition of membrane-type 1-matrix metalloproteinase which might enhance the angiogenesis. The absence of *SPOCK2* methylation was therefore hypothesized in the majority of cases in this study. **Methods:** Extracted DNA from 88 formalin-fixed paraffin-embedded (FFPE) tissues of DLBCL were subjected to bisulfite conversion followed by methylation-specific PCR (MSP) analysis for *p16*, *MGMT* and *SPOCK2* methylation. *p16* methylation was also quantified in 16 samples through pyrosequencing assay. **Results:** *p16* methylation was observed in 65/88 (74%) samples by MSP. Pyrosequencing detected *p16* methylation in all 16 samples ranging from 18% to 81%. *MGMT* methylation was detected in all 88 (100%) cases. Methylated *SPOCK2* was found in 83 (94.3%) samples. There was a significant association between *p16* methylation status with patients above 50 years of age (p= 0.04). **Conclusions:** These preliminary discoveries may serve as a good platform in order to gain a comprehensive overview on the epigenetics contribution in the pathogenesis of DLBCL. Pyrosequencing is a robust tool in detecting and quantifying methylation.

KEYWORDS: DLBCL, epigenetics, MSP, pyrosequencing