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 \textit{p16}, \textit{MGMT} and \textit{SPOCK2}. Aberrantly methylated \textit{p16} and \textit{MGMT} have been linked to DLBCL, but not \textit{SPOCK2}. \textit{p16} inhibits cyclin-dependent kinase, which results in retinoblastoma phosphorylation and blockage of cell cycle at G1 phase. \textit{MGMT} removes alkyl adduct at O\textsuperscript{6}-guanine, thus preventing lethal cross-links. \textit{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 \textit{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 \textit{p16}, \textit{MGMT} and \textit{SPOCK2} methylation. \textit{p16} methylation was also quantified in 16 samples through pyrosequencing assay. Results: \textit{p16} methylation was observed in 65/88 (74\%) samples by MSP. Pyrosequencing detected \textit{p16} methylation in all 16 samples ranging from 18\% to 81\%. \textit{MGMT} methylation was detected in all 88 (100\%) cases. Methylated \textit{SPOCK2} was found in 83 (94.3\%) samples. There was a significant association between \textit{p16} methylation status with patients above 50 years of age (\textit{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.

\textbf{KEYWORDS:} DLBCL, epigenetics, MSP, pyrosequencing