

**FC-160****Methylation of p14ARF and p16INK4a – A possible role in the pathogenesis of liposarcoma****J. Sopta**¹, R. Davidovic², R. Kovacevic¹, D. Ristic³, J. Bokun³, A. Djordjevic⁴, N. Lujic⁴, G. Djuricic⁵¹ *Institute of Pathology, Medical Faculty, University of Belgrade, Belgrade, Serbia*² *Institute of Nuclear Sciences Vinca, Belgrade, Serbia*³ *Institute of Oncology and Radiology of Serbia, Belgrade, Serbia*⁴ *Institute of Orthopaedic Surgical Diseases Banjica, Belgrade, Serbia*⁵ *University Children's Hospital, Belgrade, Serbia*

Introduction: Liposarcoma represents the most abundant group of soft tissue sarcomas, but their pathogenesis is not quite clear. Molecular studies showed that p53-p14 and Rb-p16 pathways may play important roles in it. Due to histopathological differences between liposarcoma subtypes it is likely that the genetic and epigenetic alterations are subtype specific.

Material and Method: The study included 33 liposarcoma (23 low and 10 high grade) samples that were diagnosed in Institute of Pathology, Medical Faculty, University in Belgrade. Immunohistochemistry was done for p53, Ki-67, p16 and Cyclin D1. Direct sequencing was performed for mutational analysis of the p53 gene. Methylation status of p16INK4a and KLF6 promoters has been analyzed using the methyl-specific PCR method.

Results: Immunohistochemical analysis showed increased expression of p53 in correlation with tumor grade: 4% in atypical lipomatous tumor (ALT), 30% in myxoid liposarcoma (MLS), 100% in pleomorphic subtype (PLS). Expression of p16 protein was very similar, with overexpression in 28/33 samples (84.5 %). All of the pleomorphic samples had increased p16 expression, while ALT and MLS showed p16 immunoreactivity in 3/6 and 16/18 cases. The Cyclin D1 expression was increased in 19/33 (61.3 %) samples: WDLS 2/6 (40.0 %), MLS 11/17 (62.5 %) and PLS 6/10 (66.7 %). There was significant difference in Ki-67 expression between p53 positive and negative tumors, and correlation between p16 and Ki-67 expression was established. Regardless of histological subtypes of the samples analysis showed that 18.2% MLS and 33.3% PLS contained mutated p53 gene. Overall, mutation frequency for p53 was 23.5%. Analysis showed that 16.7% samples had methylated p16 promoter. In contrast to recurrent tumors (37.5%), none of the primary tumors had methylated p16INK4a gene promoter. Hypermethylation of the p14ARF promoter showed 72.2% of samples, without statistical significance between primary and recurrent lesions.

Conclusions: The results from the current study suggest increased expressions of p16 and cyclin D1 are early changes in the pathogenesis of liposarcoma. Also we postulate the significant impact of the p14ARF gene methylation on the pathogenesis and for progression. Despite the limited number of samples, our study points to necessity of further investigate on of p53-p14 and Rb-p16 pathways in liposarcoma.