BOP1 protein and morphometric parameters of the nucleoli as a new tool for the determination of renal clear cell carcinoma grading.

P-27-037

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Clear cell renal cell carcinoma (ccRCC) is the most common subtype of kidney cancer. Its malignancy is determined based on the size and shape of the nucleoli in the immunohistochemical image and relies on the subjective analysis of an experienced pathomorphologist. The objective algorithms for differentiating evaluation of ccRCC tumor grade are lacking. Here, we aimed to identify nucleolar proteins whose expression correlates with nucleolus morphology, to find objective biomarkers of ccRCC tumor grade. To verify the hypothesis, LC-MS/MS proteomic analysis of nucleoli isolated from normal cells (RPTEC/TERT1) and ccRCC cells (786-O and Caki-1) was performed. The proteins with the most differential expression between cell lines were selected to evaluate correlations between their expression and nucleolar morphology using RCC cell lines of different tumor aggressiveness: 786-O (primary tumor) and Caki-1 (skin metastasis). Cells seeded on coverslips were fixed after 48 hours and stained using: i) Diff-Quick for morphometric measurements (area, perimeters, Feret diameter, number of nucleoli); ii) immunocytochemistry (ICC) to verify the fluorescence intensity of the tested protein. Imaging was performed on a ZEISS LSM800 confocal microscope using ZEN 3.7 software. Morphometric measurements were performed using ImageJ software. Among 233 proteins with impaired expression in ccRCC, GLTCSR2, DDX21 and BOP1 were selected for further analysis. ICC intensity of all three proteins was increased in RCC cell lines when compared with RPTEC cells. BOP1 fluorescence intensity correlated with tumor aggressiveness. Moreover, we also observed statistically significant correlation between the number of nucleoli in the nucleus and the arithmetic mean fluorescence intensity of the BOP1 protein. In conclusion, BOP1 protein expression is a promising potential biomarker of ccRCC tumor aggressiveness.

Financed by National Science Center, Poland grant 2019/35/B/NZ5/00695.