Evaluation of RKIP expression and *KRAS*/*BRAF* mutational profile of anal tumors

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Anal cancer is a rare type of digestive tract disease, which has had a crescent incidence in several parts of the world. The major risk factor is human papillomavirus (HPV) infection. However, there are studies showing anal cancer patients HPV-/p16- with a worse outcome than the HPV+/p16+ patients. This suggests that molecular profile drives anal cancer progression. Therefore, the aim of this study was to evaluate the RKIP expression, as well as the mutational status of *KRAS* and *BRAF*, in a series of anal cancer lesions. Fifty-five patients' resected of tumors of the anal canal were evaluated (12 high-grade squamous intra-epithelial lesion - HSIL, 16 adenocarcinomas, and 37 squamous-cell carcinoma - SCC). *KRAS* (codons 12 and 13) and *BRAF* (codon 600) sequencing was performed in 53 samples, whereas RKIP immunohistochemistry was performed in 48 samples. Survival curves and log rank statistical analysis were performed according to histological type, and RKIP expression was correlated to the clinic-pathological features, being considered statistically significant when $P<0.05$ in chi-square or Fisher's exact test. There was a trend of worse overall survival of adenocarcinoma patients ($P=0.067$). High RKIP expression was correlated to age (33.3% in younger *vs*. 0% in elderly patients) and histology type (37.5% in HSIL *vs*. 0% in adenocarcinoma). Importantly, low RKIP expression was correlated to HPV infection status (94.7% in HPV+ *vs*. 60% in HPV-), indicating the possible interference of HPV infection in the expression of this tumor suppressor gene. We found a low percentage of patients showing *KRAS*/*BRAF* mutation: one SCC patient (1.9%) exhibited *KRAS* p.G13D mutation, and one adenocarcinoma patient (1.9%) presented *BRAF* p.V600E mutation. Besides rare in anal tumors, there are a percentage of patients that might be impaired of targeted therapies based on *KRAS* and *BRAF* mutational status. Given this low mutation percentage in SCCs, adenocarcinomas and HSIL, there may exist other molecular alterations that drive to anal cancer development. Additionally, there is an indication of the possible role of HPV infection in the down-modulation of RKIP, contributing to the oncogenesis of anal cancer, which should be better elucidated.

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