

## **Histopathological characterization of esophageal tumors and potential involvement of high-risk HPV infections in carcinogenesis**

**Reynders C**, Ancion M, Lerho T, Luyckx M, Hendrick E, Bruyère D, Pilard C, Roncarati P, Delvenne P, Hubert P and Herfs M

*Laboratory of Experimental Pathology, GIGA-Cancer, Department of Pathology, University of Liege, Belgium*

celia.reynders@uliege.be

In the esophagus, two main cancer subtypes can be diagnosed: adenocarcinoma and squamous cell carcinoma. Interestingly, these two cancers are found in both uterine cervix and anal canal where the etiological role of HPV is largely described in the literature. In contrast, the link "HPV-cancer" in esophageal cancers is still very controversial. The main goal of this project is to histopathologically characterize the esophageal malignancies and to determine the potential role of HPV infection in the development of a subset of these latter. In collaboration with both the local and European biobanks, we collected a large cohort of adenocarcinoma (n=128) and squamous cell carcinoma (n=174). We analyzed the HPV status as well as the viral transcriptomic activity of each specimen. This procedure allowed us to classify all samples into three categories: HPV-negative, HPV DNA+/RNA- and HPV DNA+/RNA+. These three sub-groups were finally compared with each other according to various characteristics using immunohistochemistry. The clinical data of the patients were then collected and an analysis of the survival of these patients was performed. Among 128 esophageal adenocarcinoma samples already analyzed, 59 samples displayed a positive DNA signal (59/128, 46%). Regarding the viral transcriptional activity, 32 tissue specimens were HPV16 or 18 RNA positive (32/128, 25%). When the three categories were compared with each other, no significant difference was observed regarding the proliferative index, p53 status, p16<sup>ink4a</sup> and Keratin 7 positivity as well as PD1+ and CD8+ cell density. Regarding the clinical data of patients, HPV status doesn't seem to affect overall survival either. Regarding the cohort of esophageal squamous cell carcinoma, only 21 samples were positive for DNA and RNA HPV (21/174, 12%). The category (HPV DNA+/RNA+) seems to strongly express p16<sup>ink4a</sup>, show more frequently basaloid tumour differentiation and have a non-aberrant p53 status compared to the HPV-negative category. RNAscope techniques as well as in situ hybridization confirm a "total" infection of the tumors (in 100% of the cancer cells) contrary to what we have detected in adenocarcinoma samples. Regarding the clinical data of the patients, HPV status doesn't significantly affect the survival. Overall, 46% of esophageal adenocarcinoma samples have been shown to be positive for HPV infection but a transcriptionally active infection was encountered in a relatively modest proportion of samples. In addition, HPV status doesn't seem to affect neither the histopathological features of adenocarcinoma nor the patient survival. As for squamous cell carcinoma, a small subset seems to be etiologically linked to HPV16. The survival of patients with squamous cell carcinoma could potentially be impacted by the HPV status.