**SESSION 1:** Common and specific mechanisms of viral oncogenicity

**TITLE:** HPV16 up-regulates the expression of RNA-binding protein Sam68 in head and neck cancers

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**BACKGROUND AND AIM:** Human papillomavirus (HPV) has emerged as the etiological agent of a sub-group of head and neck squamous cell carcinoma (HNSCC), especially oropharyngeal cancers (OPSCC). HPV-related HNSCC have distinct molecular and clinical characteristics compared to HPV-negative HNSCC. Different studies investigated the role of splicing factors and RNA-binding proteins (RBP), such as Sam68, in the production of HPV E6\*I isoform, which is overexpressed during cancer progression. The aim of the study was to analyse the expression profile of these factors in HPV-related and unrelated-HNSCC and their possible interplay with HPV oncoproteins.

**MATERIALS AND METHODS:** The study included 30 patients diagnosed with HNSCC, including oropharyngeal SCC (n=14), oral cavity SCC (n=9) and SCC at other sites (n=8). HPV DNA was searched by broad spectrum PCR and quantified by droplet digital PCR (ddPCR). The expression of the splicing factors HNRNPA1, HNRNPA2B1, SRSF1, SRSF2, SRSF3, BRM and SAM68 as well as of HPV16 E6 and E6\*I isoform was measured by real-time quantitative PCR (RT-qPCR). SCC-derived PCA5 cell line were transduced with LXSN E6 or E6/E7 expressing vector and the effect on Sam68 levels was evaluated by RT-qPCR and western blot analysis. In addition, differential expression of splicing factors in HPV-negative and HPV-positive HNSCC has been validated in 531 HNSCC from TCGA dataset.

**RESULTS:** HPV sequences were detected in 47% of HNSCC with HPV16 being the most frequent genotype. HPV16 viral load ranged from <1 to 27 copies/genome equivalent and the HPV16 E6\*I mRNA levels were higher than E6. The expression of SRSF3, BRM and SAM68 was significantly higher in HPV-positive compared to HPV-negative HNSCC (p <0.05). In particular, SAM68 levels were shown to correlate with E6\*I expression (R=1, p=0.02). Western blot and RT-qPCR analyses revealed up-regulation of SAM68 at mRNA and protein levels in LXSN HPV16 E6 transduced PCA5 cells. Accordingly, the SRSF3, BRM and SAM68 were found significantly up-regulated in HPV-related HNSCC, but not in HPV-negative cases, from TCGA dataset.

**CONCLUSIONS:** The results showed that expression of the splicing factors SRSF3, BRM and SAM68 is significantly higher in HPV-positive HNSCC. The heterologous expression of E6 oncoprotein in the HPV-negative PCA5 cell line has been shown to induce up-regulation of Sam68. These data highlight the complex interplay between viral oncoproteins and cellular splicing factors in the carcinogenic process and suggest innovative therapeutic approaches based on Sam68 inhibitors.

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