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ePoster Session

Viral infections: from the lab to the clinic

Deep-sequencing analysis of E6 and E7 single nucleotide variants and genome methylation of high-risk human papillomaviruses

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Background: Persistent infection by high-risk HPV (HR-HPV) is a necessary cause, but not sufficient, for the development and progression of cervical cancer. Different putative molecular markers associated with the neoplastic progression of cervical lesions, such as methylation or presence of single nucleotide variants (SNVs) of gene sequences of HR-HPV, are currently under study.

Objective: Aim of this study was the development of high-throughput deep-sequencing techniques to study the presence of SNVs in the *E6* and *E7* genes of HR-HPV types and the methylation status of the *E2*, *L1* and *L2* genes of HPV16 and HPV18 in clinical specimens from patients with HR-HPV infection.

Methods: A dataset including all available HR-HPV genome sequences was retrieved from databases and used to design new sets of multiplex primers for SNV and methylation analysis on a 454 FLX+ platform (Roche). For SNVs analysis, a set of 31 primers was designed to amplify a 800 bp region including the *E6* and *E7* oncogenes of all HR-HPV types. For methylation analysis, DNA was converted with sodium bisulfite and the gene regions of interest were amplified in four overlapping 800 bp amplicons. The setup and analytical validation of the techniques was carried out on HeLa and CaSki cell lines, WHO proficiency testing samples, and a group of clinical specimens positive for HR-HPV by Hybrid Capture 2 (Qiagen) testing and genotyped by INNO-LiPA HPV Genotyping Extra (Innogenetics).

Results: Analysis of HR-HPV E6 and E7 by 454 deep-sequencing in control and clinical samples demonstrated that the method could correctly identify all HR-HPV types both in single and in multiple infections. In addition, SNV analysis of HR-HPV E6 and E7 in a group of cervical samples identified some SNVs that had not been previously reported. SNVs occurred in 100% of reads, confirming the high stability of HPV genome which does not seem to accumulate mutations in the host. For HPV16 and HPV18 methylation analysis, an adjusted preprocessing was carried out to avoid the elimination of bisulfite-treated sequences, rich in A and T homopolymers. In cervical samples, methylated CpG islands were correlated with pathological grade of the disease.

Conclusion: This study has enabled the development of deep-sequencing methods based of 454 FLX+ technology for methylation and SNV analysis in gene sequences of HR-HPVs. These methods are currently under clinical investigation as potential biomarkers of tumor progression in a perspective study in patients with HR-HPV-related cervical lesions.