In this study, a new approach for gene expression analysis termed comparative expressed sequence hybridization (CESH) was established and further developed. Until now, CESH with cDNA library probes was performed to metaphase chromosomes as a target for hybridization. This allowed the assessment of the relative expression of genes in different tissue types. This procedure was greatly improved by substituting metaphase chromosomes by BAC arrays as a target for hybridization (array-CESH), which increased the resolution for the detection of altered gene expression levels. The CESH approach allows to assess the level of protein-coding as well as non-coding transcripts.

Recently, the role of non-coding RNAs (ncRNAs) has gained increasing attention. Prominent members of ncRNA are small RNAs. These small RNAs control a wide range of developmental and physiological pathways in mammalian cells, and are implicated in various cancers and other diseases. Therefore, analyzing ncRNA, new insights into the mechanisms of cancer development and etiology can be obtained.

The CESH approach was applied to study head and neck squamous cell carcinoma (HNSCC). Comparing HNSCC to the healthy oral mucosa by CESH to metaphase chromosomes, commonly highly expressed chromosome regions were 1q22-q23, 3q26.3-qter, 4q31.1-q32, 11q12-q13.2, 14q32, 18q12, 19q13.2-q13.3 and 22q13.1-q13.2, while commonly low expressed regions were 8p22-p23, 16p12 and 16q23-q24. Moreover, in order to identify new candidate genes that may play a role in HNSCC, the same tumor cases were tested by array-CESH. Numerous BAC clones showed changes in the sequence expression profile in HNSCC compared to the healthy mucosa. We selected BAC clones with altered transcript levels and tested the expression profile of the genes located in the respective genomic insert. Two new genes, leucin-rich repeats and guanylate kinase domain containing (LRGUK) and xylosyltransferase1 (XYLT1), were identified and validate by RQ-PCR. Both genes are showing low expression in the investigated tumor entities. The detailed mechanism of these two genes in HNSCC pathogenesis remains to be elucidated in further studies.
Sequences on the BAC clones consist of protein-coding as well as non-coding RNA genes. To test the expression level of the non-coding sequences, specifically micro-RNAs, in HNSCC we analyzed the expression level of selected micro-RNAs located on the BAC clones, which showed alterations of transcript levels in the CESH profile. Tested micro-RNAs were *mir-214*, *mir-650*, *mir-617* and *mir-618*. Reduced expression in HNSCC was observed for *mir-214*, while *mir-650*, *mir-617* and *mir-618* showed high expression in tumors compared to mucosa. The target molecules of these micro-RNAs, which are likely to play a role in pathogenesis of HNSCC, remain to be elucidated.

In conclusion, using CESH, common patterns of altered sequence expression in different HNSCC samples were obtained and novel candidate genes of pathogenic relevance were identified. This approach is a powerful tool for the analysis of ncRNAs, and was successfully used to identify novel micro-RNAs, which play a role in HNSCC.