miRNA-Analysis of mesopharynx and hypopharynx tumors

PhD Thesis

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INTRODUCTION

In addition to the widely-known etiological factors including various chemical carcinogens and poor oral hygiene, the human papilloma viruses (HPV) are major risk factors in the development of head and neck squamous cell carcinomas (HNSCC). Oral HPV positivity and the number of viral-induced oropharyngeal tumors have significantly higher frequency in males. In the oropharyngeal region the highest HPV positivity can be detected in the palatine tonsils. Te HPV positivity ratio of these tumors can amount to 70% compared to the other sub regions. Te mucosal surface of lacunas and crypts constitute a strong conjunction between the epithelial mucosa and the lymphatic tissue underneath and is ofen revealed in the background of tonsillar predilection of HPV initiated carcinogenesis. Clinical investigations have shown revealed remarkable diferences between tumors induced by HPV and chemical carcinogenesis: HPV induced tumors particularly emerge at younger ages, and in many cases, the frst clinical sign of the malignancy is the lymph node metastasis. Te size of primary tumor is frequently small and thereby, it is difcult to explore in the early stage, however, later it ofen increases in tumor size compared with those HPV negative tumors in the same stage. HPV positive cancers respond much better to oncological and surgical therapy, thus prognosis is generally, far more favorable. Considering the diferences from aspects of prognosis, behavior and development, a separate TNM classifcation was released in 2017 for HPV positive oropharyngeal tumor.

Human Papilloma Virus

Human Papilloma Virus HPV belongs to the family of Papillomaviridae and the HPV Reference Center has classifed more than 120 types (Papillomavirus Episteme – NIH). Te viral genome in this family is a circular, double-stranded DNA (deoxyribonucleic acid). Regarding the most conserve sequence of the viral genome, which is the L1 open reading frame (ORF) region, the HPV types belong to seven taxonomical genera (alpha-, beta-, gamma-, mupa- and nupa- etaand thetapapillomaviridae). Protein products translated from L1 and L2 ORFs constitute the viral capside, while E6 and E7 ORFs encode oncoproteins that modulate the viral transformation process. HPV infects the basal cells of the multilayer epithelium. Te modifcation of the epithelial cell diferentiation, through regulation of the cell cycle, results in the generation of the continuous reactivation of DNA replication and leads to the replication of the genome of the HPV virus. Cutaneous and mucosal invasive types of the HPV can be distinguished, accordingly. Based on oncological risk groups mucosal types can be assigned according to low, intermediate and high risk HPV. Te identifcation of multiple viral infections is frequently observed and the resulting virulence might be infuenced by the diferent genotypes. However

interference between diferent HPV types and the impact on their virulence are events that have not been demonstrated yet. Due to the fact, that clinical observations about the behavior of the HPV positive oropharyngeal cancer difer so much from HPV negative HNSCC, a new TNM classifcation was needed to be introduced with a specifc staging guideline. Tis statement is underlined by the Te Cancer Genome Atlas (TCGA) publication on comparative molecular characteristics of the HPV positive and negative HNSCCs. Te diference between the HPV associated and non-viral groups was clearly confrmed. Te TCGA study was not able to determine sufcient number of oncogenic mutation HNSCC that can drive the mechanisms of carcinogenesis; beside of this fact many epigenetical alterations were proved to characterize this cancer type. Linking to this concept, a recent publication of Masuda et al. stated that HNSCC is strongly linked to changes in epigenetic regulatory. miRNAs as translator molecules between the environmental exposures and genomic regulation might refect the carcinogenic process through their expression profle changes.

Micro-RNA

micro RNAs (miRNA) are small, 19–25 mer regulator molecules. Interestingly, miRNAs can behave either as oncogenes (oncomirs) or tumor suppressors according to which target mRNA they regulate. Te comparison of cancer and healthy cells originating from the same tissue presents diferences in the expression patterns of miRNA. Te analysis of the miRNA expression profle proved to be an appropriate method towards diferentiating between normal and tumorous tissues, and selecting subgroups specifcally in relation to tissue regarding several tumorous diseases. Previous reviews have observed a discrepancy of miRNA fndings across studies focusing on cancers of the head and neck, and there has been no consensus on specifc miRNAs that are associated to HPV positivity and HNSCC. In our study, we aimed to analyze the miRNA profle of oropharyngeal squamous cell carcinomas through a cancer feld model in tumors and surrounding healthy tissues. We collected mapping biopsy series of tissue samples from the tumors and from the peritumoral mucosa 1-2-3 cm away from the macroscopic tumor margin.

Cancer Field

Chemical and viral carcinogens constitute a genetically and epigenetically modifed feld in the intact cells of the mucosal surface as the frst step of carcinogenesis. Te expression, “cancer feld“ or “tissue organization feld” is described in the literature. Later, followed by precursor changes, a primary tumor, and recurrence of a tumor or a second primary tumor can develop

within this feld. Te behavior of the oral cavity, oropharyngeal, hypopharyngeal and laryngeal squamous cell carcinoma can be represented via this model of tumor development.

PHASE I.

Materials and Methods I.

Sample collection

Samples of meso- and hypopharynx carcinomas were collected from patients diagnosed and operated on at the Department of Otorhinolaryngology, Head and Neck Surgery of the Clinical Center at University of Pécs. All removed tissue blocks were verified by histopatolocigal examination. During tissue sample collection we followed a standard mapping biopsy strategy and removed small fractions (approx. 0,5-1 mg) of mucosal tissues from the tumor edge (0), 1cm (1) and 2 cm (2) distance from the macroscopic tumor margin and from intact mucosal tissue being at least 3 cm (3) distance from the tumor margin. During the study 52 tissue samples, originating from 13 patients, were collected. Five patients suffered from hypo- and eight from mesopharnyx carcinoma. On clinicopathological classification tumors were listed into TNM II, III and IV stage squamous cell carcinomas.

RNA isolation

Tissue samples from mapping biopsy were immediately snap-frozen to –80˚C following collection until molecular laboratory processing. Sixty µg of tissue sample were homogenized in 150 µl lysis puffer (HighPure miRNA Isolation Kit, catalogue number: 05080576001, Roche, Mannheim, Germany) by using MagNa Lyzer Green Beads (Roche) cheramic beads tubes in MagNA Lyzer (Roche) shaking homogenizer. Total RNA was isolated with downstream application of the High Pure miRNA isolation kit (Roche), following the manufacturer's instructions. RNA quality was checked by nano-drop absorption photometry and only the RNA fractions with (260/280 nm A>1,9) were used for reverse transcription.

cDNA synthesis and RT PCR

Five µg/µl of the RNA templates was used for cDNA synthesis with the Universal cDNA synthesis kit (Quiagen, Woburn, MA, USA), applying the random hexamer priming, included in the kit. cDNA samples were evaluated for miRNA expression in Roche LC480 system (Roche). For miRNA expression analysis specific primers were chosen from the universal miCURY LNA primer set (Exiqon, Vedbaek, Denmark) according to: hsa-miR-21, -27a, -34a,

-143, -146a, -148a, -155, -221. Universal miCURY LNA 5S rRNA and U6 snRNA were used as controls for relative quantification. PCR mastermix contained 2 µl specific primer mix, 8 µl cDNS-template and 10 µl LC480 SYBR Green I Master mix in a total volume of 20 µl. Amplification was carried out on a 8×12 plate according to the following design: 6 tumor specific miRNA and two internal controls, that were examined on ten nucleic acid samples with unknown concentrations, on one set of positive samples with known concentrations and against one set of negative controls.

Statistical analysis

Relative quantification results were calculated by Exor4.0 software of LC480 (Roche), using the ΔΔ-CP method. Calculated relative quantification rates grouped according to tumor and peritumoral tissues were used for further statistical analysis by two tailed two sample T-test, as well as binary logistic regressionusing the software SPSS 21.0.

Results I.

During miRNA expression analysis of meso- and hypopharyngeal mapping biopsies from tumor and peritumoral tissues, we found perceptible differences in characterizing the miRNA expression of mucosa surrounding the tumor tissue. Mucosal tissues located 1 cm away from surgical excision line show only a little difference in expression rate pattern from the tumor itself, while those at 2 and 3 cm away from the tumor differ prominently. The samples taken from 2 cm and 3 cm distance from the tumor center shared many similarities in miRNA expression patterns. In the different line segments of peritumoral mucosa specific miRNA expression pattern has been found. miR-21, -27a and -146a showed a decreasing relative expression rate scaling from the tumor tissues, where their expression is high, towards the peritumoral tissues where their expression is gradually lower according to the distance. miR-34a and -143 was found to have similar but inverse expression pattern being the lowest in the tumor and higher in the tumor surrounding tissues. We could not observe such expression changes according to miR-148a, -155 and 221.

PHASE II.

Materials and Methods II.

Sample collection.

Tissue samples (10–60 mg) were collected from patients undergoing surgical intervention and treatment at the Department of Otorhinolaryngology, Head and Neck Surgery, Clinical Center, University of Pécs. During our study period between 2017 and 2018, 48 patients were diagnosed and received multimodal therapy for oropharynx, hypopharynx and larynx HNSCCs. From this patient cohort 25 patients were selected for miRNA analysis based upon the presence of stage T2-3 squamous cell carcinoma of the oropharynx. Tumor classifcation and clinicopathological evaluation were carried out by a pathology expert according to the "8th Edition TNM Classifcation for Head and Neck Cancer, American Joint Committee on Cancer (AJCC), 2017". Clinicopathological staging, HPV P16 immunohistochemistry and HPV genotyping were performed in all HNSCC cases. Our sample collection strategy followed a strict scheme towards efectively mapping the biopsy originating from the cancer area. We obtained our frst sample from the non-necrotic tumor tissue, and next, we collected peritumoral macroscopically normal tissue samples at 1 cm, 2 cm and 3 cm distance from the edge or perimeter of the tumor. Following collection, tissue samples were snap frozen at −80 °C and stored until processing.

RNA isolation and reverse transcription

Cell free total RNA was isolated from 60μg fresh frozen tissue samples using the Aurum Total RNA mini kit (Cat. no. 7326820; Bio-Rad, Madison, USA). Isolated RNA quality was checked using the Termo Scientifc NanoDropTM 2000 (Termo Fisher Scientifc, Grand Island, NY). 5ng total RNA from each sample was reverse transcribed using the miRCURY LNA Universal RT microRNA PCR Kit (Cat. no. 339340; Qiagen).

Droplet digital PCR analysis

Regarding quantitative PCR analysis, we procured the Qiagen miRCury LNA miRNA PCR assays (Cat. no. Qiagen, Hilden, Denmark), in accordance with the following miRNA targets: hsa-miR-21-5p, hsa-miR-143, hsa-miR-155 and hsa-miR-221-5p. Droplets were immediately analyzed following the PCR reaction in the QX200 Droplet Reader. Fluorescence data were converted into concentrations according to the Poisson distribution statistical analysis used by the QuantaSof® Analysis Pro sofware, version 1.0.596 (Bio-Rad, CA, USA).

HPV P16 Immunohistochemical staining

The p16 positive staining was concluded based on the identifcation of strong nuclear and cytoplasmic staining in at least 70% of malignant cells. No staining, faint nuclear and cytoplasmic staining with background marking, small foci of staining or staining in non-malignant cells were considered negative results. Te positive control was a cervical squamous cell carcinoma previously proven to be p16 positive, as part of the standard clinical laboratory procedure.

DNA extraction and HPV genotyping

60 μg of fresh frozen tumor tissue sample was used for the total DNA extraction using the HighPure template preparation kit (Cat. no. 11796828001; Roche, Molecular Diagnostics, Mannheim, Germany). DNA concentration and quality was examined using NanoDrop spectrophotometry. Following PCR amplifcation, genotyping is performed using a single nylon strip coated with HPV type-specifc and human beta-globin-specifc oligonucleotide probes. Testing was performed in accordance with the recommended manufacturer's instructions.

Statistical analysis

Concentration data acquired from QuantaSof sofware (BioRad) were next exported and analyzed for quantitative diferences between the tissue samples according to histopathology, distance from the tumor, and, the location and HPV status were analyzed using IBM SPSS Statistics 21.0 for ANOVA with statistical signifcance level $p \leq 0.05$.

Image reconstruction for illustration of sample collection sites

CT DICOM data including arterial and venous phase contrast-enhanced high resolution series (slice thickness=0.75mm, matrix=512×512, pixel size=0.445mm) of the patient above were converted to nifi format using MRIConvert 2.1.0 (Lewis Center for Neuroimaging, University of Oregon, USA) and loaded in 3DSlicer 4.1. Based on contrast enhancement, anatomy and asymmetry, tumor mass was delineated in axial plane slices by a radiologist using 3DSlicer segment editor draw tool. Tumor contours were revised and modifed if necessary by a board-certifed neuroradiologist with over 10 years of clinical experience. Using the segment editor module of 3DSlicer 4.1 sofware 3D segmentation of the tumor was created according to the delineation. Using the same module, the 3D segmentation of the air inside the pharynx was created as well. Using the margin and hollow tools a 1mm thick boundary was created from the segmentation of the air in order to model the mucous membrane. Using the margin tool on the

3D segmentation of the tumor 10mm20mm and 30mm ofset volumes were created according to the sample collection site distances. Using a logical operator tool, intersections were created using the segmentations of the mucous membrane and the ofset volumes applied to the tumor

Results II.

miRNA profle analysis associated with HPV positivity

In the study period our clinical site diagnosed 48 patients with HNSCC 34 men and 14 women. Afer clinicopathological classifcation of the tumors we enrolled to this study only the patients with stage T2-3 HNSCCs with oropharyngeal location, based on the "8th Edition TNM Classifcation for Head and Neck Cancer, American Joint Committee on Cancer (AJCC), 2017". Out of the 25 mesopharyngeal tumors, eight cases proved to be HPV positive (28%) with p16 immunohistochemical staining. Genotyping HPV positive tumor tissues showed higher expression levels of all investigated miRNA compared to the HPV negative. Signifcant over expressions in the HPV positive tumor tissue biopsies were found with ANOVA for miR-21-5p (F=5.53, p=0.022), miR-143 (F=5.627 p=0.021), and miR-221-5p (F=5.065 p=0.028). Te statistical analysis did not confrm miR-155 to be consequently correlated to HPV infection in the tissue biopsy series (F=0.70, p=0,793).

HPV positive tumor tissues showed higher expression levels of all investigated miRNA compared to the HPV negative. Signifcant over expressions in the HPV positive tumor tissue biopsies were found with ANOVA for miR-21-5p (F=5.53, p=0.022), miR-143 (F=5.627 p=0.021), and miR-221-5p (F=5.065 p=0.028). Te statistical analysis did not confrm miR-155 to be consequently correlated to HPV infection in the tissue biopsy series (F=0.70, p=0,793). Based on our results, we were not able to identity a single miRNA marker specifc for HPV positivity, but patterns of the miRNA expression profles correlated with the presence of HPV. miRNA expressions in correlation with the distance from the tumor margin on the peritumoral mucosa showed patchy pattern alterations of miR-21 and miR-155 in HPV positive cases, while gradual change of all investigated miRNAs was seen in HPV negative tumors.

miRNA profles correlating with tissue organization feld (TOF)

In HPV negative tumors and peritumoral tissues miRNA expression changes followed gradual pattern which depended on the measured distance from the tumor propagation itself. On the peritumoral normal mucosal feld, the gradual concentration decrease of two miRNAs (miR-21-5p, miR-221-5p) was found to be statistically signifcant on the ANOVA test. miR-143 and -

155 also showed a similar miRNA distribution pattern, however, it did not reach the limit of statistical signifcance. In the tumors and their neighboring intact mucosa, miR-21-5p showed gradually decreasing values correlating with distance from the tumor propagation. Average miR-21-5p values do not difer signifcantly among the tumor samples and in the 1 cm peritumoral normal mucosa. We found a signifcant decrease in miR-21-5p expression levels in tissues at 2 and 3 cm distance from the tumor margin. Te concentration of miR-221-5p was found to be high in tumor tissues while it decreased rapidly in the hystopathologically normal peritumoral mucosa.

3 Dimensional model

Te patient's HR CT DICOM fles were uploaded to 3D slicer version 4.1. Te 3D model was generated based on a HPV negative right side exulcerated tonsillar tumor (stage T3) with the anatomical extension into the vallecula and deeply infltrating the lateral pharyngeal wall. In this 3D model we were able to visualize the tumor propagation into the surrounding mucosal tissues where the biopsy samples were taken from and evaluated for miRNA expression levels. On the images the clinical tumor mass is painted deep orange, peritumoral mucosa at 1 cm is inked red, 2 cm is yellow and 3 cm is green.

DISCUSSION

We could confrm HPV 16 as the most frequent genotype in all our clinical samples (7 cases out of 48) and we also detected HPV 52 besides HPV 16 in a single case. HPV 52 belongs to the high cancer inducing risk category (HR) HPV group and it was found to be the third most prevalent genotype- afer HPV 42 and HPV 16- during the routine cervical HPV genotyping at our laboratory site. In the head and neck region the oropharynx presents the highest ratio of HPV associated HNSCC. Te mucosal surface of lacunas and crypts constitute a strong conjunction between the epithelial mucosa and the lymphatic tissue underneath and is ofen revealed in the background of tonsillar predilection of HPV initiated carcinogenesis.

miR-21

We found miR-21-5p expression to reach higher levels in HPV positive tumors and 1 cm distant peritumoral area. In study evidences published so far, miR-21 showed signifcantly lower expression in HPV positive head and neck tumors, compared to HPV negative ones. However, these studies lacked the clinical and histopathological homogeneity of their samples. Sample

enrollment was heterogenic for the clinical stage. Furthermore, tumor negative samples were collected close to the tumor itself; consequently, these studies neglected to take in consideration the tumor organization feld.

miR-143

miR-143 is a tumor suppressor miRNA, which has been identifed as having a lower expression level in HPV associated head and neck tumors. It inhibits the proteins E2F (E2 transcription factor) and survivin and their protein translation, which contributes to tumor proliferation and neoangiogenesis. It is associated with a better prognosis, hence its higher expression was observed in HPV positive oropharyngeal tumors. We have also found a high miR-143 expression in HPV positive tumors compared to the negatives, but the diference was more characteristic for the peritumoral mucosa, which showed signifcantly higher concentrations of miR-143.

miR-221

As well as miR-21-5p, we recognized miR-221-5p with signifcantly higher expression levels in the HPV positive cancers compared to the negatives, which may also be explained by the fact that HPV positive tumors were large in size. On our mapping biopsy evaluation the highest miRNA values were measured for the HPV positive tumor tissues and miR-221-5p expression. In conclusion, our fndings confrm that miR-221 is involved in HPV positive HNSCC tumor growth.

miR-155

miR-155 is a type of miRNA, identifed in squamous cell cancers of the larynx, it promotes tumor invasion by blocking the SOC (suppressor of cytokine signaling proteins) and STAT (signal transducer and activator of transcription) transcription factors. Te changes in biomarker values in miR-155, measured at diferent biopsy distances, could not prove the role of miR-155 in the peritumoral feld in our study.

The CT based 3D model

In the CT based 3D model we tried to visualize the tumor mass and the peritumoral tissues considering our mapping biopsy strategy and the tissue miRNA expression results. Tis model can be utilized in preoperative surgical planning to decide the optimal tumor resection line. In case the surgical line afects the functionality (swallowing, breathing and speaking) we prefer

the organ preservation among therapeutical modalities (chemoirradiation). Te 3D sofware might be efective in assisting preoperative planning, especially in current clinical settings. Our ultimate goal was to optimize the diagnostic steps in HNSCC management, to estimate and minimalize the chances of tumor recurrence and to achieve better survival with higher life quality of HNSCC patients.

Summary, theses, new clinical findings

I. To molecularly characterize the cancer field or tissue organization field, we were the first who established a mapping biopsy model, which subsequently became suitable for the study of this epigenetically altered field.

II. It was the first time when microRNA expression patterns were analyzed and used for isolating meso- and hypopharynx cancer and detecting the altered tissue organization field surrounding them. The mi-R21, -143 and -155 markers showed higher expression in hypopharynx squamous cell carcinoms, whereas miR-221 was significantly higher in mesopharynx tumors. Oncomirs (miR-21, miR-221) were mostly expressed in the tumor and in the immediate peritumoral tissues (1 cm from the tumor), whereas tumor suppressor-like miRNAs (miR- 34a, miR-143) were found further away from the tumor.

III. Different microRNA patterns were discovered in HPV positive and negative oropharyngeal squamous cell carcinomas. In respect of all kind of miRNAs observed, HPV positive tumor tissues showed higher expression compared to HPV negative ones. Significant overexpression was detected for miR-21-5p, miR-143 and miR-221-5p in HPV positive tumor tissues.

IV. In the mucosa around the oropharyngeal tumor, miR-21 and miR-155 showed a scattered pattern in HPV positive cases, while we could observe a gradual decrease in the expression of all miRNAs tested in HPV negative tumours, with significant changes in miR-21 and miR-221. The above may confirm a different tumour development pattern in HPV positive and negative tumors.

V. Our molecular and radiological based 3D model was the first to visualize tumor propagation and altered tissue organization around the tumor, taking into account our mapping biopsy strategy and tissue miRNA expression results. The model may be useful for preoperative surgical planning.

Publications related to the topic of the thesis

Éva Orosz, Katalin Gombos, Nerina Petrevszky, David Csonka, Istvan Haber, Balint Kaszas, Arnold Toth, Krisztian Molnar, Krisztina Kalacs, Zalan Piski, Imre Gerlinger, Andras Burian, Szabolcs Bellyei, István Szanyi, Visualization of mucosal field in HPV positive and negative oropharyngeal squamous cell carcinomas: combined genomic and radiology based 3D model. Scientific Reports, Sci Rep., 2020, Jan 8;10 (1) doi: 10.1038/s41598-019-56429-4. (IF 4,4)

Éva Orosz, Katalin Gombos, Nerina Petrevszky, Bálint Kaszás, Krisztina Kalács, Gréta Bodzai, Imre Gerlinger, István Szanyi, miRNA expression pattern differences of HPV negative and HPV positive advanced stage mesopharynx cancers. Fül-Orr-Gégegyógyászat, 2019, 65 (2): 63-68.

Orosz Éva, Gombos Katalin, Tamás Riedling, Priscilla Afiakurue, Kiss István, Pytel József, Gerlinger Imre, Szanyi István, Comparative miRNA expression profile analysis of squamous cell carcinoma and peritumoral mucosa from the meso- and hypopharynx. Cancer Genomics and Proteomics. 2017 JulAug;14 (4): 285-292. (IF 2,5)

Orosz Éva dr., Gombos Katalin dr., Révész Péter dr., Kiss István dr., Pytel József dr., Gerlinger Imre dr., Szanyi István dr., MicroRNA expression profiles in squamous cell carcinomas of the meso- and hypopharynx. Orvosi Hetilap, 2014, 155, 27: 1063–1070. (IF 0,3)

Lectures related to the topic of the thesis

Orosz Éva, Kiss István, Pytel József, Gerlinger Imre, Szanyi István, Gombos Katalin, Mesopharynx, hypopharynx daganatok vizsgálata mikro-RNS markerek segítségével.
Fiatal Onkológusok III. Konferenciája, Budapest, 2014. 09.12.

Orosz Éva, Szanyi István, Révész Péter, Gombos Katalin, Meso- és hypopharynx lokalizációjú fej-nyaki laphám karcinómák együttes miRNS-mRNS expressziós mintázatainak értékelése.
Magyar Onkológusok Társaságának XXX. Kongresszusa, Pécs, 2013.11.14-16.

Orosz Éva, Gombos Katalin, Kiss István, Gerlinger Imre, Szanyi István, Mesopharynx, hypopharynx daganatok és a peritumorális ép szövetek mikro-RNS expressziós mintázatának analízise.
A Magyar Fül-, Orr-, Gége és Fej-, Nyaksebész Orvosok Egyesületének 43. Kongresszusa, Tapolca, 2014.10.15-18.

Orosz Éva, Gombos Katalin, Szanyi István, Molekuláris epidemiológiai biomarkerek jelentősége, lehetőségei a fej-nyaki daganatokban.
MFOE Tudományos Ülés, Budapest, 2015.

Orosz Éva, Afiakurue Priscilla, Gerlinger Imre, Gombos Katalin, Szanyi István, Magas kockázatú HPV összefüggései meso-, hypopharynx és szájüregi daganatok megjelenésével és micro-RNS mintázatával.
Magyar Fül-, Orr-, Gége és Fej-, Nyaksebész Orvosok Egyesülete 44. Kongresszusa és az MFOE Audiológiai Szekciójának 53. Vándorgyűlése, 2016.10. 6-9.

Orosz Éva, Nerina Petrevszky, Gombos Katalin, Szanyi István: MicroRNA expression profiles in squamous cell carcinomas of the meso- and hypopharynx.
4th Congress of European ORL-HNS, Barcelona, 2017. 10. 7-11.

Orosz Éva, Gombos Katalin, Petrevszky Nerina, Kaszás Bálint, Háber István, Csonka Dávid, Szanyi István: Szöveti miR-21, miR-143, miR-155 és miR-221 expresszió, mint a tumor lokalizáció és HPV pozitivitást jelző biomarkerek laphámsejtes fej-nyak karcinómákban.
Magyar Fül-, Orr-, Gége és Fej-, Nyaksebész Orvosok Egyesülete 45. Kongresszusa és az MFOE Audiológiai Szekciójának 55. Vándorgyűlése, Szeged, 2018.09.13-16.