MEETING ON EXPERIMENTAL AND TRANSLATIONAL RESEARCH IN HEAD AND NECK CANCER

23.2.-24.2.2024
Klinikum rechts der Isar, Technical University Munich

ABSTRACT BOOK

TALKS

HPV-RELATED HEAD AND NECK CANCER

Vortrag-001

Differentiation of head and neck squamous cell carcinoma cells

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Head and neck squamous cell carcinoma (HNSCC) is the 6th most common malignancy in the world causing high mortality. To improve patient outcome, researchers need to find new treatment approaches. Human papillomavirus (HPV)-negative HNSCCs contain areas of differentiated cornified tissue, which is not characteristic for HPV+ cases. Dissecting the mechanisms underlying the differentiation of HNSCC cells may identify targets for anti-tumor therapy. We created a HNSCC differentiation model using HPV+ and HPV- patient-derived tumor cells and observed a loss of cell malignancy in vitro and in vivo upon differentiation treatment. In differentiated HPV- HNSCC cells, multi-omics analysis showed a higher accessibility of small promoter regions despite overall genome closure, activation of wound healing-associated cell signaling, and increased expression of stress keratin 17 (KRT17) and cornification markers. In contrast, RNA-seq revealed that HPV+ HNSCC cells displayed increased myocyte-like differentiation under differentiating conditions. Immunofluorescence staining of HPV- HNSCC tissue showed that KRT17 transitioned from a stem-cell marker in normal mucosa to a marker of early differentiation in tumor tissue and dysplastic mucosa. Therefore, KRT17 may be a suitable biomarker for HPV- HNSCC early detection. Moreover, cornified HNSCC tissue was frequently located adjacent to necrotic and immune-infiltrated areas suggesting an involvement of pro-inflammatory stimuli. In HPV- primary tumor spheroids, inflammatory mediators induced cell adhesion and cornification resembling normal mucosa maturation, proposing a possible treatment strategy. Our study thus indicates that the targeted differentiation of HNSCC cells may serve as a future therapy approach.

Vortrag-002

Early detection of HPV-driven cancer: Current status of the Hamburg HPV Oropharyngeal Cancer Screening Study (PHORECAST)

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Differing from HPV-driven cervical cancer, screening and early diagnosis of HPV-driven oropharyngeal cancer (HPV-OPC) is hampered by a lack of detectable precursor lesions. Serum antibodies against HPV16 early proteins are detectable several years before diagnosis and especially cell-free HPV DNA in liquid biopsies is emerging as a potential short-termed pre-diagnostic marker. PHORECAST aims to assess the possibility for HPV-OPC screening and early detection through these and further markers in the Hamburg City Health Study (HCHS), a single center, prospective epidemiologic cohort study that started enrolling 45,000 participants (45-74 years) in 2016.

In a proof of concept study, the sera of the first 4424 participants (blood draw 2016/17) were analyzed for antibodies against four HPV16 early proteins (E1, E2, E6, E7) using multiplex serology. Twelve participants (0.3%) were seropositive for E6 and at least one other early protein and therefore considered at highest risk for HPV-OPC development. Ten of these could be invited to six-monthly head and neck follow-up (FU) exams starting in 2019. So far, five were diagnosed with a stage I HPV-OPC (1x pT1 pN0 c0; 4x pT2 pN1 cM0, all with a single lymph node involved) and treated according to international guidelines.

According to these results, the applied method enables the detection of asymptomatic HPV-OPC at an early stage. We will further extend and improve the approach through 1) an increased number of additional 10,000 participants screened, 2) expanding the screening population to participants who are solely E6 positive, and 3) incorporating emerging liquid biopsy markers, such as plasma cell-free HPV DNA, HPV DNA in oral gargle samples and exosomes in plasma and saliva to optimize the short-term positive predictive value.

MOLECULAR ALTERATIONS

Vortrag-003

Targeting Rb/E2F and BRD4 potentiate YB-1 dependent oncolytic virotherapy in HNSCC

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For the treatment of head and neck squamous cell carcinoma (HNSCC) multimodal approaches are commonly applied and new therapeutic options are of great interest. Virotherapy is an approach using modified viruses, which selectively replicate in and kill cancer cells. Clinical studies revealed limitations of oncolytic viruses as monotherapy. However, combinational approaches with chemotherapeutics unveiled enhanced efficacy. The oncolytic E1A-13S-deleted adenovirus XVir-N-31 displays YB-1 dependence leading to cancer cell selectivity. CDK4/6 inhibitors targeting Rb/E2F were shown to enhance therapeutic efficiency of XVir-N-31 as well as inducing local and systemic immunological antitumor effects. Bromodomain containing protein BRD4, a positive regulator stimulating RNA polymerase II-dependent transcription, is another promising therapeutic target in HNSCC. Hence, a combination therapy of CDK4/6 inhibitor Ribociclib (LEE), BRD4 inhibitor JQ-1 and XVir-N-31 in HNSCC cell lines was evaluated. DNA replication and gene expression of XVir-N-31 was measured by (RT-)qPCR, viral particle formation by immunostaining of viral hexon protein and cell lysis by SRB assay. The results show that each combination increases the efficacy of XVir-N-31, with high synergistic effects in the triple therapy. Viral DNA replication, particle formation and cell killing were strongly enhanced by combinational approach. Furthermore, additional molecular analyses on this subject revealed an

influence on transcriptional regulation on different levels. The combination of CDK4/6 and BRD4 inhibition with XVir-N-31 is an attractive strategy to achieve substantial cancer cell killing and probably immunological antitumor effects making it highly suitable for clinical testing.

POSTER

POSTER SESSION 1 (PAVILLION)

Session1-001

Exploring the Implications of Y Chromosome Loss and Low Expression in Head and Neck Squamous Cell Carcinoma (HNSCC)

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Loss of chromosome Y (LOY) and extreme downregulation of chromosome Y gene expression (EDY) are common features in various cancers among male patients, including HNSCC. About 25% of tumors from male patients with HNSCC exhibit LOY. However, the biological and potential therapeutic significance of LOY in HNSCC is still under investigation.

In this study, an integrative multi-omics analysis was conducted, which included data from bulk and single cell RNA sequencing (scRNA-seq) from The Cancer Genome Atlas (TCGA) and Gene Expression Omnibus (GEO) databases. The research results revealed a positive and significant association between chromosome Y gene expression and HPV16-positive oropharyngeal SCC (OPSCC), higher pathological grading, and smaller tumor size. Focusing on HPV-negative HNSCC, tumors with EDY exhibited a higher proportion of genomic alterations, an increased tumor mutational burden, and demonstrated distinct pathway activities. Notably, non-EDY tumors exhibited increased activity in processes associated with E2F or MYC target genes and regulation of the G2/M cell cycle. Meanwhile, EDY tumors displayed elevated expression of inflammation- and complement-related genes but had decreased tumor infiltrating lymphocyte counts. To identify possible vulnerabilities related to EDY, a drug sensitivity analysis was conducted using the OncoPredict algorithm and indicated that HNSCC with EDY may be more sensitive to drugs targeting pathways associated with cell cycle regulation, genome integrity, and mitosis. ScRNA-seq analysis was also performed, which showed varying levels of EDY in malignant epithelial cells, and in accordance with bulk RNA-seq confirmed higher expression of chromosome Y genes in HPV-positive HNSCC and a greater level of differentiation in cancer cells possessing EDY.

In summary, our study provides a detailed examination of the expression of chromosome Y genes in HNSCC, revealing their clinical and molecular implications. Further studies are underway to investigate additional aspects of chromosome Y biology in HNSCC and other solid tumors, with the ultimate goal of identifying innovative therapeutic strategies.

Session1-002

Impact of chromosome X-related gene regulatory networks in the pathogenesis and therapy of head and neck cancer

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Background: Sex-related differences in cancer development are rooted in the complex interplay of hormones and sex chromosomes. Head and neck squamous cell carcinoma (HNSCC) exhibits a pronounced sex bias, particularly in HPV16-positive oropharyngeal SCC, and a better understanding of underlying principles is critical to uncover susceptibility patterns and accurately predicting prognostic outcomes in HNSCC.

Aims: This study explores the influence and prognostic significance of chromosome X gene expression in HNSCC patients through bioinformatics analysis.

Methods: Consensus and unsupervised hierarchical clustering analyses were performed to define distinct subgroups within TCGA-HNSC by assessing expression patterns of chromosome X genes. Differences in histopathologic and clinical characteristics among these subgroups were analyzed by cross-tabulation analysis. A prognostic risk model and survival-related candidate genes were established using LASSO penalized Cox regression. The performance of the chromosome X-related risk model was confirmed by multivariate Cox regression analysis in independent HNSCC cohorts.

Results: Both consensus and unsupervised clustering revealed two groups: Cluster A characterized by high and Cluster B characterized by low chromosome X gene expression. Cross-tabulation showed significant differences in HPV16, anatomical subsites, pathological grading and lymph node metastasis between the clusters. A prognostic risk prediction model was established using TCGA-HNSC as a training cohort and confirmed in independent HNSCC validation cohorts.

Summary: Our study reveals a high variability in chromosome X gene expression in HNSCC and shows a strong association with clinical variables and survival outcomes. These findings underscore the need to integrate sex-specific considerations when formulating approaches for cancer prevention, assessing prognostic risk, and tailoring targeted therapies for individuals diagnosed with HNSCC.

Session1-003

Influence of plasma-derived exosomes from HNSCC patients on macrophage differentiation

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Head and neck squamous cell carcinoma (HNSCC) patients often have poor prognosis and bad response to therapy. One problem is the highly immunosuppressive character of HNSCC.

Plasma-derived exosomes from HNSCC patients contain molecules, which contribute to the immunosuppressive tumor microenvironment (TME), e.g. by reprogramming tumor-infiltrating immune cells towards an immunosuppressive phenotype. One important part present of the TME are tumor-associated macrophages, which further contribute to tumor progression and immune evasion. Here, we investigate the influence of plasma-derived exosomes of HNSCC patients on macrophages.

Exosomes were isolated from plasma of HNSCC patients and healthy donors by size-exclusion chromatography. Monocytes from buffy coats were used to generate primary macrophage cultures, which were incubated with plasma-derived exosomes to investigate their effects on the proteome, analyzed by mass spectrometry. To examine the effect on M1 or M2 polarization of macrophages, cell surface markers were investigated by spectral flow cytometry and cytokine production was determined by ELISA.

Incubation with HNSCC-exosomes changed the proteome of macrophages in a time-dependent manner. Additionally, direct protein transfer from exosomes to macrophages after co-incubation was noticed. The presence of exosomes furthermore inhibited differentiation from monocytes to macrophages and resulted in a mixed, but not fully differentiated phenotype of macrophages.

Plasma-derived exosomes from HNSCC patients alter immunosuppressive properties of macrophages. We show that uptake of exosomes can alter the proteome of macrophages and inhibit proper macrophage differentiation and therefore contribute to an immunosuppressive phenotype. This may be useful for future clinical therapeutic strategies on modulation of tumor-associated macrophages through targeting exosomes in the TME.

Session1-004

A B cell-related prognostic risk model links sex hormone signaling with a favorable immunophenotype in head and neck squamous cell carcinoma

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B cells have received significant attention for their potential role in cancer progression and therapy response. Main objectives of this study were to classify patients with head and neck squamous cell carcinoma (HNSCC) based on the presence of B cells, evaluate its impact on survival, and explore underlying molecular mechanisms. Our goal was to create a risk model linked to B cells that would assist in treatment decisions and identify patient subsets that may benefit from immune checkpoint blockade (ICB).

Using immunohistochemistry, we quantified CD20-positive B cells in FFPE samples of an HNSCC cohort (n=80) and verified a positive correlation with an ESR1-related risk model. An immunophenotype linked to elevated CD20-positive B cell counts was found through inferred immune cell subsets and confirmed with bulk RNA-seq data from TCGA-HNSC (n=518). LASSO-penalized Cox regression was used to establish a prognostic risk model involving B cells and B cell-related immune cells for TCGA-HNSC, which was validated in HNSCC cohorts and other tumors (TCGA). Molecular differences between the two risk groups were investigated using bioinformatic approaches. Low-risk patients showed elevated ESR1-related pathway activity, indicating the influence of sex hormones on the tumor microenvironment (TME). Immune-related genes were enriched among the differentially expressed genes (n=110), with down-regulation in high-risk tumors. Toll-like receptor 10 emerged as a promising target for further research. High-risk tumors displayed

elevated oxidative phosphorylation, increased genomic instability, and decreased presence of mature tertiary lymphoid structures, which are essential for local anti-tumor immunity and predictive for ICB response.

In conclusion, this study underscores the vital importance of B cell presence in HNSC. It also highlights the effect of sex hormone signaling on a favorable TME and introduces a new risk model for patient classification and potential ICB utilization.

Session1-005

Marginal Zone B Cells in Tumors: Potential Players of Antigen Presentation and T-cell Activation

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Background: Marginal zone (MZ) B cells, originally residing outside the marginal sinus of the spleen, possess a unique capability to recognize ectopic antigens and trigger humoral immune responses. Our research has uncovered the presence of MZB cells in the context of head and neck squamous cell carcinoma (HNSCC), both within tumor entities and peripheral blood, leading us to explore their role in tumor immunology.

Methods: We harnessed two single-cell RNA sequencing (scRNA-seq) datasets, encompassing 44 untreated HNSCC patients with paired peripheral blood mononuclear cells (PBMCs) and tumors, as well as 6 PBMCs and 5 tonsil samples from healthy volunteers. Additionally, we incorporated a cellular indexing of transcriptomes and epitopes (CITE-seq) dataset with well-defined MZB cell clusters for reference. Unbiased cell clustering and annotation were performed, revealing two distinct MZB populations. Gene set enrichment analysis and cell communication analysis were employed, and the deconvoluted cellular matrix was applied to The Cancer Genome Atlas (TCGA) HNSCC dataset for survival analysis.

Results: Our investigation identified two MZB clusters characterized by robust intercellular communication and anti-viral pathways. Notably, cell-cell interactions featured abundant MHC-I and MHC-II antigen presentation between MZB clusters and various T cell subpopulations, including effector memory T cells and tissue-resident memory T cells. MZB-2 exhibit a higher degree of functional maturity and engage in more pronounced interactions with T cells. The Kaplan-Meier estimator underscored the prognostic significance of the MZB-2 cluster for HNSCC patients.

Conclusion: Our study unveiled the existence of two distinct subsets of MZB cells within HNSCC patients, with a pivotal role in antigen presentation and the activation of T cells. Harnessing the potential of these immunogenic B cells represents a promising therapeutic avenue that could benefit HNSCC patients in the future.

Session1-006

Pro-coagulant platelets misguide immune cell responses in the tumor microvasculature

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In addition to tumor cells, the tumor environment synthesizes immune checkpoint (IC) molecules. Here, we report that particularly pro-coagulant platelets bear large amounts of such immunomodulatory factors and that the presence of these activated cellular blood components in malignant tumors relates to pro-tumorigenic immune cell activity and impaired survival. Mechanistically, tumor-released nucleic acids attract platelets into the aberrant tumor microvasculature where they undergo pro-coagulant activation, thus delivering specific stimulatory and inhibitory IC molecules. This concomitantly promotes pro-tumorigenic myeloid leukocyte responses and compromises anti-tumorigenic lymphocyte activity, ultimately supporting tumor growth.

Interference with platelet-leukocyte interactions prevented immune cell misguidance and suppressed tumor progression, nearly as effective as systemic IC inhibition.

Hence, our data uncover a self-sustaining mechanism of solid malignacies in utilizing platelets to misdirect immune cell responses. Targeting this irregular multicellular interplay might represent a novel immunotherapeutic strategy for oncological disorders including head and neck squamous cell carcinoma (HNSCC) without side effects of systemic IC inhibition.

Session1-007

Tissue-resident memory CD8 T cells as a potential target for anti-PD-1 immune checkpoint inhibition in head and neck squamous cell carcinoma

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Introduction: Response rate to anti-PD-1 immune checkpoint inhibition (ICI) is moderate in head and neck squamous cell carcinoma (HNSCC) and it remains unclear which cells exactly cause tumor inhibition. In this context CD103+ tissue-resident memory CD8 T cells (Trm) might play a relevant role. The aim of this work is to characterize Trm in HNSCC and to investigate their impact on the outcome of anti-PD-1 ICI in HNSCC.

Methods: Trm were analyzed by flow cytometry in tissue samples from 19 HNSCC patients. Immunofluorescence (IF) was performed to quantify CD8+ CD103+ Trm in tumor tissue from a retrospective cohort of 25 HNSCC patients who had received anti-PD-1 ICI. This cohort was divided into the groups CD8+ CD103+ Trm high and low based on the median number of cells detected. Survival curves of these groups were generated according to Kaplan-Meier and were compared by the log-rank test.

Results: Trm could be detected by flow cytometry in tumor tissue of all HNSCC patients (proportion among CD3+ CD8+ T lymphocytes mean±SD: 40.3% ± 22.8%). PD-1 expression was significantly higher in Trm compared to CD103neg cytotoxic T lymphocytes ($p<0.0001$). With co-expression of TIM-3 and TIGIT, further checkpoint molecules were detected in CD103+ PD-1+ Trm. No significant differences in overall survival and progression-free survival could be found between CD8+ CD103+ Trm high and low tumors during anti-PD-1 ICI.

Conclusions: Trm express PD-1 at high levels in HNSCC and are therefore a potential target for anti-PD-1 ICI. However, our analysis did not show any impact of the number of intratumoral CD8+ CD103+ cells on the outcome of PD-1 ICI in HNSCC.

Session1-008

Co-expression patterns of cancer associated fibroblast markers reveal distinct subgroups and predict patient survival in oropharyngeal squamous cell carcinoma

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Background: The incidence of oropharyngeal squamous cell carcinoma (OPSCC) is rapidly increasing in high income countries due to its association with persistent high-risk human papilloma virus (HPV) infection. Recent scientific advances have highlighted the importance of the tumor microenvironment in OPSCC. In this study, including 216 OPSCC patients, we analyze the composition of four established markers of cancer associated fibroblasts (CAFs) in the context of intratumoral CD8 T-cell infiltration.

Methods: Immunohistochemical staining for fibroblast activation protein (FAP), platelet-derived growth factor receptor beta (PDGFRb), periostin, alpha smooth muscle actin (α-SMA) and CD8 were analyzed digitally and their association with survival, tumor- and patient characteristics was assessed.

Results: Co-expression of CAF markers was frequent but not associated with HPV status. FAP^{high} and $PDGFRb^{high}$ expression were associated with increased CD8 T-cell infiltration. Low expression of PDGFRb improved patient survival in female patients but not in male patients. We identified $PDGFRb^{low}$ $periostin^{low}$ $\alpha\text{-}SMA^{low}$ status as an independent predictor of improved survival (hazard ratio 0.377, $p=0.006$).

Conclusions: These findings elucidate the co-expression of four established CAF markers in OPSCC and underscore their association with T-cell infiltration and patient survival. Future analyses of CAF subgroups in OPSCC may enable the development of individualized therapies.

Session1-009

3D In Vitro tumor metastasis model : A tool for preclinical drug testing

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Introduction

Metastasis to the head & neck region usually indicates a late stage of advanced malignancy. Successful management to metastatic tumours is still a challenge. With conventional in-vitro 2D culture methods, it is challenging to recapitulate the Cell-cell and cell-ECM interactions in physiologically relevant microenvironment. Conversely, several experimental studies highlighted the capabilities of in-vitro 3D cultures to represent the tumor tissues in structure and function. The emergent 3D ECM-loaded cancer spheroid model has been widely used in research recently. Matrigel as a natural matrix material has been used for this model to support the cell-cell and cell matrix interaction, moreover to enhance the integrity and circularity of the spheroid model.

In our study, we established a 3D ECM-loaded cancer spheroid model that is suitable for drug testing.

Materials and Methods

Cell culture and generation of ECM-loaded 3D spheroids

Human breast adenocarcinoma cell line (MDA-MB-231) were cultured in an ultralow attachment 96 well u shaped plate at a density of 10,000/well. Matrigel (Corning,Germany) was diluted in the cell culture media into two different concentrations(1% and 3%). Cells cultured to the ultralow attachement plates without Matrigel served as a control.

Live/Dead fluorescent staining of tumor spheroids

On day 7 of cell culture, the spheroids were stained by Calcein-AM/EthD-III (Live/Dead fluorescent Cell Staining Kit II, PromoKine, PromoCell GmbH, Germany) according to the manufacturer instructions. After staining the spheroids were imaged using the inverted fluorescent microscope (Zeiss AxioObserver Z1,Germany).

Drug testing on cell viability

On day 3, Alendronate 100µM was applied to the tumor spheroids with 1% Matrigel dilution for 4 days. The cell viability was tested using the cell titer glo assay 3D (Promega,Germany). In addition, Live/dead fluorescent staining on treated spheroids was performed on day 4 of treatment.

Results:

Characterization of Tumor spheroids

ECM loaded spheroids with Matrigel were generated faster than the control. The circularity and Cell-cell junction in spheroids with Matrigel were much better than without Matrigel. The spheroids generated were uniform in size around 500 µm. Final concentration of Matrigel used was 0,1 mg/ml.

Effect of Alendronate on viability of Tumor spheres:

The anticancer activity of alendronate were tested by Cell Titer glo 3D showed that 100µM of Alendronate caused a reduction of cell viability on tumor spheroids.

Conclusion:

The ECM loaded spheroids is a promising model for drug testing. The percentage of the Matrigel was controlled in our experiments as it affects the permeability to the inside of the model.

POSTER SESSION 2 (CONFERENCE ROOM 1)

Session2-010

Saliva-derived exosomes as biomarker for head and neck cancer – does saliva provide better diagnostic potential than plasma?

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Head and neck squamous cell carcinoma (HNSCC) is a highly aggressive disease, and early non-invasive detection is crucial due to the high prevalence of advanced disease and low survival rates. Plasma-derived exosomes from HNSCC patients correlate with clinical parameters. Here, we investigated the cargo of saliva-

derived exosomes. By comparing exosomal miRNA profiles from saliva and plasma, we identified liquid biomarker candidates for HNSCC.

Exosomes were isolated from saliva and plasma by differential ultracentrifugation. Exosomal surface levels of immune checkpoints and tumor antigens were measured by on-bead flow cytometry. Adenosine production by exosomes was examined by mass spectrometry. Exosomal miRNA profiles, determined by nCounter technology from paired saliva/plasma samples, were analyzed regarding their diagnostic and prognostic potential and integrated into network analysis.

Saliva-derived exosomes from HNSCC patients carried high levels of CD44v3, PDL1 and CD39, and strongly produced immunosuppressive adenosine. Compared to plasma, saliva was rich in tumor-derived CD44v3+ and poor in hematopoietic CD45+ exosomes. 29 tumor-exclusive miRNAs were identified within exosomes from both biofluids and associated with tumorigenic pathways (*TP53*, *TGFB1*, *CDH1*). The top 10 candidates with the strongest co-expression within each biofluid emerged as diagnostic panels for HNSCC detection and potentially prognostic panels for disease-free survival. Exosomal miRNAs exhibited differential representation in HPV positive and negative, as well as low and high stage disease.

Saliva from HNSCC patients contains tumor-derived exosomes and thus resembles a liquid biopsy source. However, we propose separate exosome-based, tumor-exclusive miRNA panels from saliva and plasma, which are valuable for future biomarker studies and, upon validation in a larger cohort, could be implemented in clinical practice for diagnosis of HNSCC.

Session2-011

Defining immunological biomarkers for head-and-neck-cancer: First Erlanger results of the prospective ImmunBioKHT trial

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In the grading and treatment of tumor diseases, immunological biomarkers become increasingly important. These markers include the expression of immune checkpoint molecules such as PD-L1 but also the presence of certain immune cell types. Until today, such biomarkers play only a minor role for therapeutic decisions in head-and-neck cancer (HNSCC) and predictive biomarkers from peripheral blood remain scarce. In order to define predictive and prognostic biomarkers for HNSCC, the prospective and multicentric ImmunBioKHT trial (NCT05375266) was initiated.

For the determination of a longitudinal immune status from peripheral blood, a flow cytometry-based assay is applied. Thus, blood is taken before and 7 days after the tumor surgery, as well as after the adjuvant or definitive radio-chemotherapy (RCT). The serum and plasma of the patients is used for further analyses of soluble immune modulators. Further, potential markers on the metabolome and microbiome are obtained from saliva, stool and tumor swab samples. From the excised tumor tissue a detailed histology and tumor grading, as well as the determination of common immunological markers is performed.

In this first interims analysis, we present the data of the immune monitoring from the peripheral blood of 150 patients. We found that the tumor surgery is already inducing significant modulations of the peripheral immune system. In detail, the cell counts of monocytes and granulocytes, as well as the activation status of various immune cell types was regulated. Further modulations occurred after RCT, such as an expected significant decrease of cells of the adaptive immune system, but also a modulation of the activation markers of T cells and monocytes. Also, we already quantified 35 immunomodulatory biomolecules from patients' serum via multiplex ELISA.

The here presented interims analysis shows that the standard therapy of HNSCC leads to significant modifications of the immune system. Those modulations need to be connected to the biological tumor activity in the future in order to define predictive and prognostic immunological biomarkers for HNSCC.

Session2-012

Molecular biomarker identification in non-invasive material for OSCC diagnostics

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Oral squamous cell carcinoma (OSCC), is a group of related neoplasms that develops in any parts of the oral cavity, e.g. tongue, lips, floor of the mouth and tonsils. Most oral malignancies are diagnosed in the late stage, resulting in a worse prognosis rate for oral cancer patients. Therefore, early diagnosis is necessary to prevent disease complications and a precise, simple diagnosis that is transferable to everyday clinical practice is of great importance in this field. In the light of emerging diagnostic methods, molecular cancer biomarkers are measurable proteins and nucleic acids that can be detected in patient samples and help to identify cancer. Until December 2023 our study includes a total of n=116 patients. These are divided into four different groups. A control group with healthy people, a high-risk group consisting of patients with high tobacco/alcohol consumption, patients with acute cancer (HPV positive and negative) and another control group with cancer patients after treatment. We collect blood serum from all patients, as well as oral samples (swabs and mouthwashes) for RNA analysis. To analyze the protein consumption of the serum, we have established a method based on liquid chromatography and mass spectrometry. We have already been able to detect up to 250 proteins in total. Comparison of tumor and control group shows different occurrence of some proteins. In addition to the protein analysis, RNA expression measurements are carried out on the same patients using oral samples. The opportunities of molecular biomarkers in non-invasive sample material could be important, not only for diagnosis, but also for control after tumor therapy.

Session2-013

Liquid biopsy in NUT carcinoma in the parotid gland – a case report

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Liquid biopsies (LBs) open new possibilities in precision cancer therapy using circulating tumor DNA (ctDNA). Fragments of ctDNA are released into the blood or saliva from tumor cells. Therefore, they can reveal considerable information on a tumor's molecular profile with promising diagnostic and prognostic value. Nuclear testis protein (NUT) carcinoma is a rare, aggressive squamous cell carcinoma that mainly occurs in the head, neck, and chest region. Underlying gene fusions of NUT carcinoma render patients as candidates for LB diagnostics based on ctDNA using droplet digital polymerase chain reaction (ddPCR).

We report on a 41-year-old male patient diagnosed with NUT carcinoma of the parotid gland. We designed a patient-specific ddPCR assay based on the fusion evidence of NUTM1::BRD4 as detected through sequencing a formalin-fixed paraffin-embedded (FFPE) tumor biopsy collected during total parotidectomy. Both EDTA blood and saliva in the form of an oral rinse were collected from the patient before parotidectomy and at four-time points after surgery. All LB samples were prepared for ddPCR-based analysis of the NUTM1::BRD4 fusion within ctDNA.

We detected fusion evidence of NUTM1::BRD4 in the saliva sample collected directly before parotidectomy.We were unable to detect the fusion in the corresponding blood sample in a non-metastatic state. No evidence of the NUTM1::BRD4 fusion was detected in the LB samples collected at later time. Our negative LB-based results supplement the findings of the concurrent cancer staging at which the disease progression was classified as 'stable.'

We aim to collect additional blood and saliva samples from this patient for subsequent analysis of the NUTM1::BRD4 fusion. We aim to detect recurrence and a beginning treatment failure before imaging and further clinical examinations. Considering both blood and saliva samples, it will also be interesting to determine whether it is possible to discriminate between local and distant recurrence.

Session2-014

The evolution of premalignant lesions in the upper aerodigestive tract

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Early relapse and development of metastatic disease are some of the primary reasons for the poor prognosis of patients with HNSCC. HNSCC is a heterogeneous disease which may develop in large

premalignant fields of genetically altered cells. Yet knowing which individuals will progress and develop clinically significant cancers within their lifetimes remains one of the most important challenges to reducing HNSCC morbidity and mortality. Here, we performed a focused analysis of the genome and immune microenvironment from multiple, matched normal squamous tissue, premalignant lesions and primary and recurrent tumours from seven patients with p16-negative HNSCC, as well as analysis of brush biopsies.

We performed targeted panel Next Generation Sequencing (161 genes) to analyse somatic variants from sequentially collected, matched FFPE tissue (normal, premalignant, HNSCC) from 2 patients. These samples plus samples from 5 additional patients were analysed with the Nanostring PanCancer Immune Panel. In addition, we performed shallow whole genome sequencing (0.5x coverage) on samples from one patient. Copy number analysis of DNA obtained from prospectively collected minimally invasive brush biopsies was included as well.

The most frequently mutated gene was *TP53*. Other mutated genes included *NOTCH1*, *NOTCH3* and *CDKN2A*. A significant number of alterations were shared between dysplasia and carcinoma. Pathways related to interferon alpha and gamma response were upregulated even in low-grade dysplastic lesions with increasing expression in higher grade dysplasia and carcinoma. *SPINK5*, a known tumour suppressor gene in HNSCC, was already downregulated in low-grade dysplastic lesions, indicating an early deactivation in the evolution of the disease.

Genomic alterations as well as aberrant immune gene expression can be observed early on in the evolution of tumours of the upper aerodigestive tract, highlighting the potential for targeting early mechanisms of disease progression.

Session2-015

Multimodal treatment of HNSCC with RT and ATR inhibitor VE-822 alters the tumor cell phenotype

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Introduction: The tumor biology of Head and Neck Squamous Cell Carcinoma (HNSCC) differs between tobacco / alcohol-induced and human papillomavirus (HPV)-associated tumors. HPV-negative tumors show greater radioresistance influencing e.g. the radiotherapy (RT) efficacy, leading to a less favorable prognosis and overall survival. Current ongoing clinical trials investigate if addition of inhibitors of the DNA damage repair (DDR) system to RT help overcome radioresistance. But just few is known of the potential of this combination to modulate the immunogenicity of treated tumor cells. We hypothesized that the combination of RT and DDR inhibitors increase the immunogenic potential of HPV-positive and HPV-negative HNSCC cell lines.

Methods: UD-SCC-2 and UM-SCC-47 (HPV-positive) and Cal33 and HSC4 (HPV-negative) HNSCC cell lines were used as model system. Cells were treated with either 1 µM AZD0156 (ATM inhibitor) or 0.1 µM VE-822 (ATR inhibitor) alone or in combination with

RT (2x5Gy). Cell death (AnnexinV/PI), immune checkpoint molecule (ICM) expression, and cell surface expression of activation markers on Natural Killer (NK) cells (isolated from healthy donor PMBCs) were measured using flow cytometry. Further, inflammation-associated cytokines were analyzed using multiplex ELISA by MSD multiplexing and first whole transcriptome analysis were performed with Cal33.

Results: Higher cell death rates were induced by the combination of both inhibitors and RT in a cell line-specific manner in contrast to monotherapy with inhibitors alone. The ICMs PD-L1 and ICOS-L were significantly upregulated by ATR inhibitor + RT in the HPV-negative cell lines. Cytokine analysis showed that IL-6 is predominantly triggered by combination of ATR inhibitor + RT. Finally, co-culture experiments with combination therapy-treated tumor cells and NK cells led to upregulation of activation marker CD314, CD335, CD336 and CD337 on NK cells.

Conclusion: Both inhibitors induce significant toxicity in HNSCC tumor cells only in combination with RT. Noticeably, inhibition of ATR via VE-822 + RT treatment shows greater immune modulating potential regarding surface markers on tumor cells, release of IL-6 and slight upregulation of activation markers on NK cells after co-culture.

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Session2-016

Development of an endoscope-based plasma source and new applications of cold atmospheric plasma (CAP) in head and neck carcinoma treatment

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Background: Over the past decade, cold atmospheric plasma (CAP), a near room temperature ionized gas has shown its promising application in cancer therapy. In contrast to conventional anti-cancer approaches and drugs, CAP is a selective anti-cancer treatment modality. Previous experimental studies have shown that CAP treatment leads to a significant growth inhibition of tumor cells and is able to trigger apoptosis up to a resulting specific immune response. In contrast to existing plasma sources, a new endoscope-based plasma source first had to be developed for applications in head and neck surgery.

Methods: Cold atmospheric plasma (CAP) was generated via a new developed endoscope-based atmospheric pressure plasma jet (PLASMASCOP). Physiological and molecular effects of CAP treatment on various human head and neck squamous cell carcinoma (HNSCC) cell lines compared to human epithelial cell lines were analyzed by cell counting, FACS analysis, COMET-Assays, Proteome and Western blot analyses.

Results: CAP treatment effectively attenuates malignant cell growth. The results demonstrated that even a single application of a short-term CAP treatment led to an attenuation of HNSCC cell growth and motility. We studied the detailed cellular adaptation reactions for a specified plasma intensity by time-resolved comparative proteome analyses of CAP treated vs. nontreated cells to elucidate the molecular mechanisms and to define potential biomarkers and networks for the evaluation of plasma effects on human tumor cells. Results were consistent in various HNSCC cell lines.

Conclusion: In summary, the CAP application in head and neck surgery leads to strong anti-proliferative effects and opens up novel opportunities for the treatment of HNSCCs. As an adjuvant intraoperative application, CAP may represent a promising option particularly for the treatment of tissue regions that are close to critical structures (e. g. nerves, adjacent organs).

Session2-017

Platelet-derived extra-cellular vesicles reprograms the platelet mitochondrial metabolism in a PD-L1 dependent manner

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Introduction:

Platelet-derived extracellular vesicles (pEVs) have been recently studied as potential modulators of metabolic reprogramming, contributing to the development of malignancies and evasion from immune surveillance. While mitochondrial function is crucial for cellular homeostasis, it serves as a key target for the exosome-induced metabolic reprogramming process, mediated by programmed death-ligand 1 (PD-L1).

Objectives:

This study aims to determine the impact of PD-L1-positive pEVs, isolated from HNSCC patients, on the mitochondrial respiration of platelets from healthy donors. The goal is to unravel potential pEV-mediated metabolic reprogramming controlled by PD-L1.

Materials & methods:

We isolated pEVs from freshly drawn blood of HNSCC patients and subjected them to flow cytometry analysis to determine PD-L1 expression status. The effect of the EVs, categorized into PD-L1-positive and negative groups, was assessed on the mitochondrial respiration of platelets from healthy donors using high-resolution respirometry with a substrate-uncoupler-inhibitor-titration protocol. To explore the restorative effects of PD-L1, an anti-PD-L1 antibody will be added during the measurements.

Results:

Mitochondrial respiration in platelets from healthy donors was reduced upon treatment with PD-L1-positive pEVs. In contrast, the addition of PD-L1-negative pEVs did not alter mitochondrial respiration. Interestingly, preliminary results suggest that the application of an anti-PD-L1 antibody could partially restore the decreased platelet mitochondrial respiration.

Conclusion and Outlook:

Our findings reveal the down-regulatory impact of tumor-influenced platelet-derived extracellular vesicles on the mitochondrial function and metabolic health of platelet mitochondria. This discovery contributes to a better understanding of the crosstalk between metabolic reprogramming and checkpoint proteins, offering potential therapeutic targets for modern cancer therapy.

Session2-018

Characterization and Functional Analysis of Exosomes Derived from the UD5 Cell Line in Head and Neck Cancer: Prospects for Immune Regulation and Biomarker Potential

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Introduction

Head and neck squamous cell carcinoma (HNSCC) is the seventh most prevalent cancer worldwide. Exosomes, which are small extracellular vesicles, play a major role in the progression of HNSCC. This study aimed to investigate the role of exosomes derived from the HNSCC cell line (UD5), with a focus on characterizing their surface proteins for potential biomarker identification and exploring their interactions with natural killer (NK) cells.

Material/Methods

Exosomes from the UD5 cell line were isolated via size exclusion chromatography from culture media. Their isolation and characteristics were verified using dynamic light scattering for size measurement and transmission electron microscopy for morphological assessment. To evaluate their binding affinity to the heat shock protein 70 (Hsp70), microscale thermophoresis was employed. Surface protein profiling of both UD5 cells and their exosomes was achieved through flow cytometry. Additionally, the relationship between these exosomes and NK cells was assessed using cytotoxicity assays.

Results

The analysis revealed that UD5 cells displayed various inhibitory receptors, such as PD-L1 and HLA-E. Correspondingly, the UD5-derived exosomes were characterized by the presence of tetraspanins (CD9, CD63, and CD81) and membrane-bound Hsp70 (mHsp70), typical of exosomal content. These exosomes demonstrated a consistent size around 100 nm and a high binding affinity to cmHsp70.1 monoclonal antibody. Additionally, interaction studies showed that co-incubation with NK cells resulted in adverse effects on the expression and functionality of the surface receptors of these immune cells.

Discussion

This research offers a thorough examination of the surface proteome of UD5 cells and their exosomes, shedding light on their interaction with immune cells in the context of HNSCC. The study underscores the significant role of exosomes in modulating immune responses within the cancer milieu.

Session2-019

RNA-binding proteins affect growth of HNSCC cell lines

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Introduction

RNA-binding proteins (RBPs) can affect tumor growth and metastasis through regulation of transcription of tumor-associated proteins. In that context, the polypyrimidine tract binding protein 1 (PTBP1), human antigen R (HuR, aka ELAVL1), cytoplasmic polyadenylation element binding protein 1 (CPEB1) and the RNA binding motif protein 38 (RBM38) have been associated with hypoxia-inducible factor 1 (HIF1) mRNA regulation.

Methods

We investigated the expression of these proteins in different head and neck squamous cell carcinoma (HNSCC) cell lines using qPCR and Western blot. We also assessed the effect of a knockdown of these genes on cellular proliferation, migration and expression of cancer-associated proteins, e.g. HIF1 or vascular endothelial growth factor (VEGF).

Results

We found that PTBP1, ELAVL1, CPEB1 and RBM38 are differentially expressed between the UD5, SAS and Cal27 cell lines. A knockdown of PTBP1 led to a strong reduction in UD5 and SAS proliferation. Furthermore, knockdown of the RBPs affected the expression of cancer-associated proteins.

Discussion

In HNSCC patients, increased levels of PTBP1 and ELAVL1 have been observed. Furthermore, the investigated RBPs are known to regulate the expression of cancer-associated proteins. Therefore, a downregulation or inhibition of these proteins might be beneficial in the treatment of therapy-resistant HNSCC patients.

ABSTRACTS ACCEPTED

Abstract-001

Peritumor mucosa in advanced laryngeal carcinoma exhibits an aberrant proangiogenic signature distinctive from the expression pattern in adjacent tumor tissue

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Field cancerization theory is an important paradigm in head and neck carcinoma as its oncological repercussions affect treatment outcomes in diverse ways. The aim of this study is to assess the possible interconnection between peritumor mucosa and the process of tumor neoangiogenesis. Sixty patients with advanced laryngeal carcinoma were enrolled in the study. The majority of patients express a canonical HIF-upregulated proangiogenic signature with almost complete predominancy of HIF-1α overexpression and normal expression levels of the HIF-2α isoform. Remarkably, more than 60% of the whole cohort exhibit a HIF-upregulated proangiogenic signature also in peritumoral benign mucosa. Additionally, the latter subgroup has a distinctly shifted phenotype towards HIF-2α upregulation compared to the one in tumor tissue, i.e., a tendency towards a HIF-switch is observed in contrast to the dominated by HIF-1α tumor phenotype. ETS-1 displays stable and identical significant overexpression in both proangiogenic phenotypes present in tumor and peritumoral mucosa. In the current study, we report for the first time the existence of an abnormal proangiogenic expression profile present in the peritumoral mucosa in advanced laryngeal carcinoma when compared to paired distant laryngeal mucosa. Moreover, we describe a specific phenotype of this proangiogenic signature that is significantly different from the one present in tumor tissue as we delineate both phenotypes, quantitively and qualitatively. This finding is per se cancer heterogeneity that extends beyond the “classical” borders of the malignancy and is proof of a strong interconnection between field cancerization and one of the classical hallmarks of cancer – the process of tumor neoangiogenesis.

Abstract-002

Paired evaluation of p16 and HPV DNA in Oral cavity squamous cell carcinoma

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Background/Objectives: The incidence of oral cavity squamous cell carcinoma(OSCC) is very high in Indian subcontinent. The main cause is the high incidence of tobacco chewing habit with or without smoking. However, Human Papilloma Virus(HPV) may play a confounding role in a minor subset of patients. OSCC is the leading type of cancer, even a minor component (10 -20%) will merit public health importance. Methods: A total of 1000 cases were included in the study. Among them 800 cases were of Oral squamous cell carcinoma and200 control cases consisted leukoplakia and lesion without the history of cancer.The patients were given a questionnaire comprising of questions about demographic details and habits.The biopsy samples of all the participated patients were collected. The immunohistochemistry for p16 (E6H4 clone, CINtec histology, Roche diagnostics) of all the biopsy samples was done. The cases with 2+/3+ positive nuclear staining with more than 75% cells immuno-positive were taken as p16 immuno-positive as per the AJCC criteria. The cases with positive immunohistochemistry for p16 were subjected to HPV DNA PCR. For real-time PCR, DNA was extracted from the formalin fixed paraffin embedded tissue. Results: The OSCC cases were compared with control cases in terms of staining and histomorphology. However, the grading of immunopositivity was seen among OSCC cases. Out of 800 OSCC cases 139 (17.37%) showed p16 immunopositivity as per AJCC criteria. The 104 (104/139, 74.8%) cases were positive by HPV DNA PCR for HPV-16/18. The patient's characteristics associated with a higher proportion of p16 and HPV DNA positivity were urban residence, vegetarian diet, illiteracy, graduate or higher education. There was no correlation noted with gender, tobacco smoking or chewing habits, religion, occupation and site of tumor. The p16 immunopositivity was higher in the younger age group with no tobacco habits. Conclusions: A significant proportion of OSCC cases in India are being associated with HPV infection. Out of total OSCC cases, younger patients with no tobacco habits showed higher percentage of p16 immunopositivity that indicated towards a distinct subset of patients in whom HPV may be contributing for carcinogenesis.