interactions within the tumor and its microenvironment. Interrogation of complex cellular interactions within the tumor microenvironment (TME) requires a multi-omics approach where multiple RNA and protein targets can be visualized within the same tumor sample and be feasible in FFPE sample types. Simultaneous detection of RNA and protein can reveal cellular sources of secreted proteins, identify specific cell types, and visualize the spatial organization of cells within the tissue. Examination of RNA by in situ hybridization (ISH) and protein by immunohistochemistry (IHC) or immunofluorescence (IF) are widely used and accepted techniques for the detection of biomarkers in tumor samples. Given the similarities in workflow, co-detection of RNA and protein by combining ISH and IHC/IF in a single assay can be a powerful multi-omics solution for interrogating the complex tumor and its microenvironment.

Methods In this report we combined the single cell, single molecule RNA ISH technology known as RNAscope with IHC/IF to simultaneously detect RNA and protein in the same FFPE tumor section using both chromogenic and fluorescence detection methods.

Results We demonstrate co-localization of target mRNA and the corresponding protein in human cancer samples, visualize infiltration of immune cells into the TME, characterize the activation state of immune cells in the TME, identify single cell gene expression within cellular boundaries demarcated by IHC/IF, examine cell type-specific expression of multiple immune checkpoint markers, and distinguish endogenous T cells from activated CAR+ T cells. Overall, we show that co-detection of RNA by the RNAscope ISH assay and protein by the IHC/IF assay in the same FFPE section is a feasible methodology. The combined RNAscope ISH-IHC/IF workflow is a powerful technique that can be used to study gene expression signatures at the RNA and protein level with spatial and single cell resolution.

Conclusions By leveraging the strength of the similar workflows of RNAscope ISH and IHC/IF assays, this methodology combines transcriptomics and proteomics in the same tissue section, providing a multi-omics approach for characterizing complex tissues and revealing cell type specific gene expression with spatial and single cell resolution.

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87 TRANSCRIPTION FACTOR RUNX1 ACTIVATES OPN TO PROMOTE TUMOR PROGRESSION VIA MAPK SIGNALING IN HEAD AND NECK CANCER

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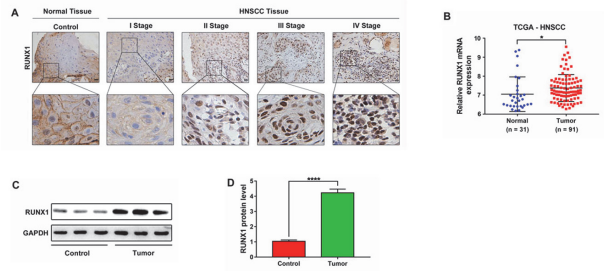
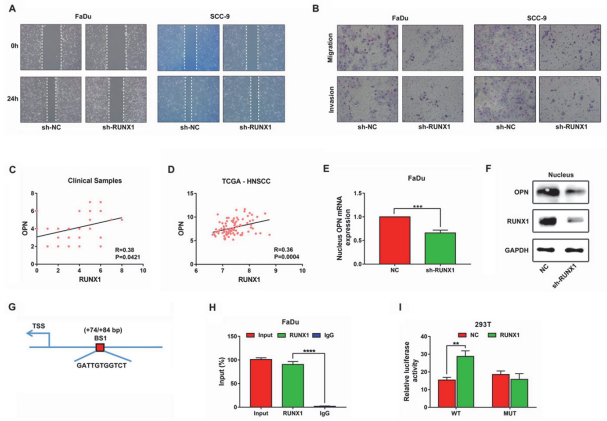
Background Tumor progression and metastasis are still major burdens for head and neck squamous cell carcinoma (HNSCC) and are associated with eventual resistance to prevailing therapies. Complex molecular transcription and downstream signaling pathways have been implicated in the development, progression, invasion, metastasis, and treatment resistance of HNSCC. Runt-related transcription factor 1 (RUNX1) are involved in aggressive phenotypes in several cancers, while the molecular role of RUNX1 underlying cancer progression and metastasis of HNSCC remains largely unknown.

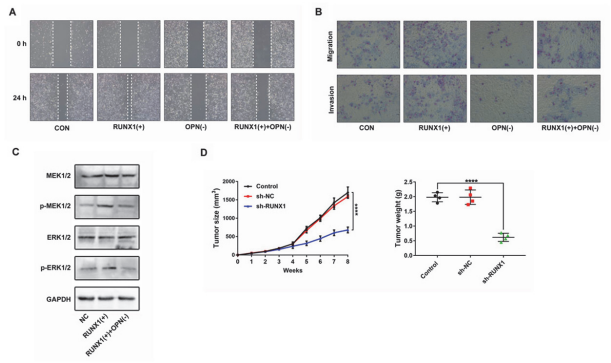
Methods RUNX1 expression levels in HNSCC cells and tissues were detected by quantitative real-time PCR (qPCR), Western blotting and immunohistochemistry (IHC). In vitro and in vivo assays were performed to investigate the function of RUNX1 in the metastatic phenotype and the tumorigenic capability of HNSCC cells. Luciferase reporter and chromatin immunoprecipitation (ChIP)-qPCR assays were performed to determine the underlying mechanism of RUNX1-mediated HNSCC aggressiveness.



Abstract 87 Figure 1 RUNX1 expression in cancer progression of HNSCC. (A) Representative images of RUNX1 immunohistochemical staining between normal tissues and HNSCC tissues (scale bar 20μm). Insets (bottom) are lower magnification (15×) images of respective cores to show a more global view of individual samples. (B) The RUNX1 mRNA expression in tumor versus normal tissues from the TCGA database, which contains 31 normal samples and 91 HNSCC samples. (C) Immunoblotting analysis of RUNX1 expression in 3 pairs of HNSCC and non-tumoral laryngeal tissues. (D) Quantitative and statistical analysis of the immunoblotting analysis. *P<0.05, **P<0.01



Abstract 87 Figure 2 Effect of RUNX1 on progression and the interrelationship between RUNX1 and OPN in HNSCC. (A) The migration ability of FaDu and SCC-9 cells transfected as above were assessed by wound-healing assay. Representative images were obtained at 0h and 24h (upper, magnification 40×) and quantified (bottom). (B) The migration and invasion ability detected by transwell assays. Representative images of FaDu and SCC-9 cells from migration and invasion assays experiment were obtained at 24h (upper, magnification 12×) and quantified (bottom). (C) Correlation analysis was performed between RUNX1 expression and OPN expression in HNSCC tissues (n = 29) and (D) in TCGA HNSCC database (n = 91). All P values are shown in the graphs. (E) Levels of nucleus OPN mRNA and (F) protein in the FaDu cells transfected with lentiviral vector encoding shRUNX1 or scrambled control were determined by real-time RT-PCR and immunoblotting analysis. (G) The predicted OPN promoter sequence bound by RUNX1 and their ChIP-PCR primers. (H) The binding of RUNX1 to predicted OPN promoter binding region was confirmed in FaDu using ChIP-qPCR and ChIP-PCR. IgG was used as the control. (I) Relative OPN activity was detected by luciferase assay in 293T cells co-transfected with RUNX1 and luciferase reporter. **P<0.01, ****P<0.0001



Abstract 87 Figure 3 RUNX1-mediated HNSCC cell metastasis in MAPK pathway via stimulating OPN. (A) The migration ability of FaDu cells transfected as above were assessed by wound-healing assay. Representative images were obtained at 0h and 24h (magnification 40×). (B) The migration and invasion ability detected by transwell assays. Representative images of FaDu cells from migration and invasion assays experiment were obtained at 24h (magnification 12×). (C) Immunoblotting analysis for protein markers expression levels of the MAPK pathway in FaDu cells transfected as above. (D) The graph of tumor growth/volumes curve at the indicated time intervals (left). Tumor weights were quantified at the end of the experiment (right). *P<0.05, **P<0.01, ***P<0.001, ****P<0.0001

Results In our study, RUNX1 expression was increased with disease progression in patients with HNSCC (figure 1). The silencing of RUNX1 significantly decelerated the malignant progression of HNSCC cells, reduced Osteopontin (OPN) expression in vitro, and weakened the tumorigenicity of HNSCC cells in vivo (figure 2). Moreover, we demonstrated that RUNX1 activated the MAPK signaling by directly binding to the promoter of OPN in tumor progression and metastasis of HNSCC (figure 3).

Conclusions Our results may provide new insight into the mechanisms underlying the role of RUNX1 in tumor progression and metastasis and reveal the potential therapeutic target in HNSCC.

Ethics Approval The study was approved by the Ethics Board of BenQ Medical Center, the Affiliated BenQ Hospital of Nanjing Medical University.

Consent Written informed consent was obtained from the patient for publication of this abstract and any accompanying images. A copy of the written consent is available for review by the Editor of this journal.

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88 DEVELOPMENT OF A 3D ORGANOID AUTOLOGOUS TIL CO-CULTURE PLATFORM FOR HIGH THROUGHPUT IMMUNO-ONCOLOGY STUDIES

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Background The preclinical screening of immune-modulatory therapies suffers from the absence of models that recapitulate in vivo heterogeneous tumor microenvironment (TME). 3D tumor organoid cultures provide a model that closely mimics in situ tumor architecture and is being aggressively used to evaluate therapeutic efficacy ex vivo. A vastly heterogenous TME impacts patient treatment response, and there is a dearth of human tumor models (2D or 3D), that mimic in vivo diversity of TME, including infiltrating immune populations. 3D organoid cultures typically contain neoplastic epithelium; however, they fall short in representing tumor to tumor-infiltrating lymphocytes (TILs) interactions, limiting their ability to generate a clinically relevant response to immunotherapeutics. Addition of immune cells from unrelated donors to organoids can simulate that microenvironment but is complicated by T cell alloreactivity. Here we describe 3D patient-derived xenograft organoid (PDXO) co-cultures with matching autologous human TILs to recapitulate the tumor-specific immune response, leveraging confocal high content analysis and luminex multiplex assays. This platform allows the evaluation and high throughput screening of novel immune targeting agents to determine impacts on patient-derived T cell function, T cell infiltration, and tumor cytotoxicity.

Methods Surgical resections from patients were used to generate patient-derived xenografts and tumor-infiltrating lymphocytes in parallel. PDX were resected and digested to establish PDXO. TILs and organoids from the same patient were fluorescent labeled and cultured together for four days to evaluate tumor infiltration and drug cytotoxicity in 3D cultures. CellInsight CX7 high content imaging platform was used to trace TILs and cancer cells and evaluate T cell infiltration and tumor cell killing in the presence and absence of immuno-modulatory therapies.

Results PDXO were established to mimic in vivo tumor biology. Tumor-specific TILs were successfully expanded and characterized by flow cytometry. Co-culture resulted in TIL infiltration in organoids from day one in culture and increased over four days. Cytotoxicity and TIL infiltration were quantified using fluorescent dyes via high throughput imaging platform. Significantly enhanced TIL infiltration was observed in autologous co-cultures compared to non-autologous co-cultures. The established unique autologous PDXO immune organoid co-cultures could be used as an improved simulation of the modulatory activity of therapeutic agents in patient-specific T cells against their own tumors.

Conclusions Patient autologous TILs – PDXO co-culture platform is an advanced model for evaluating IO therapeutics with the tumor-specific immune microenvironment. The platform provides an opportunity for precision medicine and high throughput drug screening of immuno-modulatory therapies.

Ethics Approval The study was approved by Champions Oncology's Institutional Animal Care and Use Committee (IACUC).

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Cellular therapies

89 FIRST-IN-HUMAN CAR T TARGETS CLEAVED MUC1, THE GROWTH FACTOR RECEPTOR FORM

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Background Minerva Biotechnologies has opened a Phase I 1st-in-human CAR T clinical trial, NCT-04020575, for metastatic breast cancers at the Fred Hutchinson Cancer Research Center. huMNC2-CAR44 targets the truncated extra cellular domain of MUC1* (muk 1 star), which is the transmembrane cleavage product that remains after MUC1 is cleaved and the