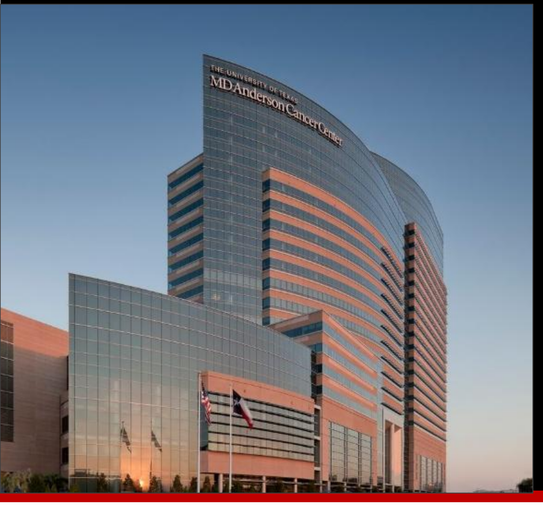

P300: Assessing the tumor immune microenvironment of sinonasal NUT carcinoma

Yoko Takahashi, PhD[1], Diana Bell, MD[2], Jennifer L. Anderson, MD, PhD[1], Jared Burks, PhD[3], Matheus Sewastjanow-Silva, MD[1], Shirley Y Su, MD[1], Ehab Y Hanna, MD[1]

[1]Head and Neck Surgery and [3]Hematopoietic Biology & Malignancy, The University of Texas MD Anderson Cancer Center, Houston, Texas
[2]Pathology, University of Pittsburgh Medical Center, Pittsburgh, Pennsylvania

THE UNIVERSITY OF TEXAS MD Anderson Cancer Center

Background

NUT carcinoma is a rare, highly aggressive, poorly differentiated carcinoma defined by rearrangements of the *NUT midline carcinoma family member 1 (NUTM1)* gene. In approximately 80% of cases, the disease is characterized by a chromosomal translocation resulting in the fusion of *NUTM1* with *bromodomain-containing protein 4* (*BRD4*), t(15;19)(q14;p13.1), producing an in-frame *BRD4::NUTM1* fusion oncogene driven by the *BRD4* promoter. Despite aggressive treatment, patients have a median survival of only seven months. Currently, there is no standard treatment for NUT carcinoma, highlighting the need for new experimental models and improved treatment strategies. In previous research, we established and characterized two novel sinonasal NUT carcinoma cell lines, MDA-NUT87 and MDA-NUT88, from a surgical specimen harboring *BRD4::NUTM1* (exon 11: exon 2) fusion. To gain a deeper understanding of the disease, we conducted a multiplex immunofluorescence study on the surgical specimen from which these cell lines were derived.

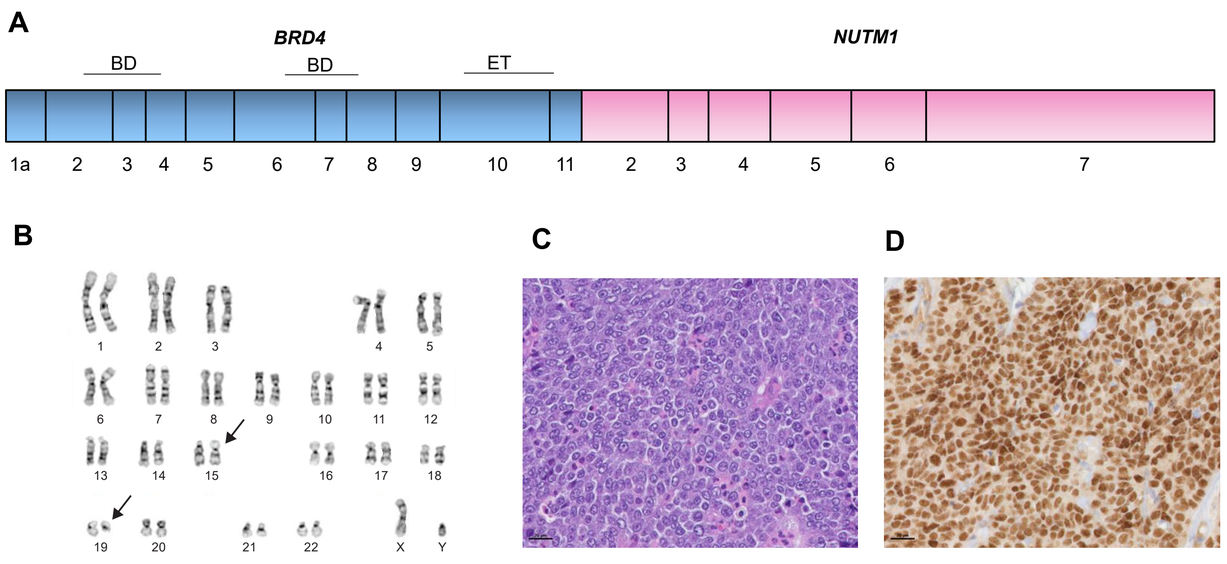

Figure 1: Molecular characteristics of the sinonasal NUT carcinoma specimen used in this study. A. Schematic representation of the *BRD4::NUTM1* fusion (exon 11: exon 2), which was confirmed by next-generation sequencing. BD: Bromodomain, ET: Extra-terminal domain. (Modified from K. Thompson-Wicking et al., 2013.) **B.** Cytogenetic analysis of a cell line derived from the patient's specimen revealed a t(15;19) chromosomal translocation. **C.** H&E staining of the resected tumor revealed a high-grade malignancy with heterogeneous morphology—predominantly basaloid and round cells arranged in lobules and sheets, intermixed with areas of clear cells, as well as epithelioid regions —features consistent with NUT carcinoma morphology. **D.** Immunohistochemistry using an anti-NUT monoclonal antibody (clone C52B1/3625) demonstrated strong nuclear staining. Scale bars: 20 μm

Materials & Methods

A formalin-fixed, paraffin-embedded surgical specimen from a 28-year-old man who was diagnosed with T4bN0M0 sinonasal NUT carcinoma was used in this study. The patient underwent two cycles of induction chemotherapy with docetaxel, cisplatin, and 5-fluorouracil at another institution, followed by surgical resection at the University of Texas MD Anderson Cancer Center. Opal 7-color multiplex immunofluorescence was performed on the surgical specimen, using antibodies for pan-cytokeratin (CK, clone 80), CD3, CD8, PD-L1, CD68, CD56/NCAM (an NK cell marker), and DAPI. Tissue identification, marker detection, and data extraction were conducted using QuPath (version 0.5.1). Tumor cells with low CK expression were categorized as 'CK^{low}' cells, and their proportion within the tumor was calculated as CK^{low} / (CK^{low} + CK^{+}) x 100.

Results

54% of the tumor cells were classified as CK^{low}, indicating heterogeneity within this tumor sample. $CD8^{+}$ tumor-infiltrating lymphocytes were present at only 0.14%, suggesting that this specimen exhibits a less immunogenic 'cold' tumor profile. The percentage of PD-L1-positive tumor cells, which is associated with a better response to immunotherapy in various tumor types, was less than 0.2%. Furthermore, the percentage of $CD68^{+}PD\text{-}L1^{+}$ macrophages, another indicator for response to certain types of immunotherapies, was found to be less than 5%.

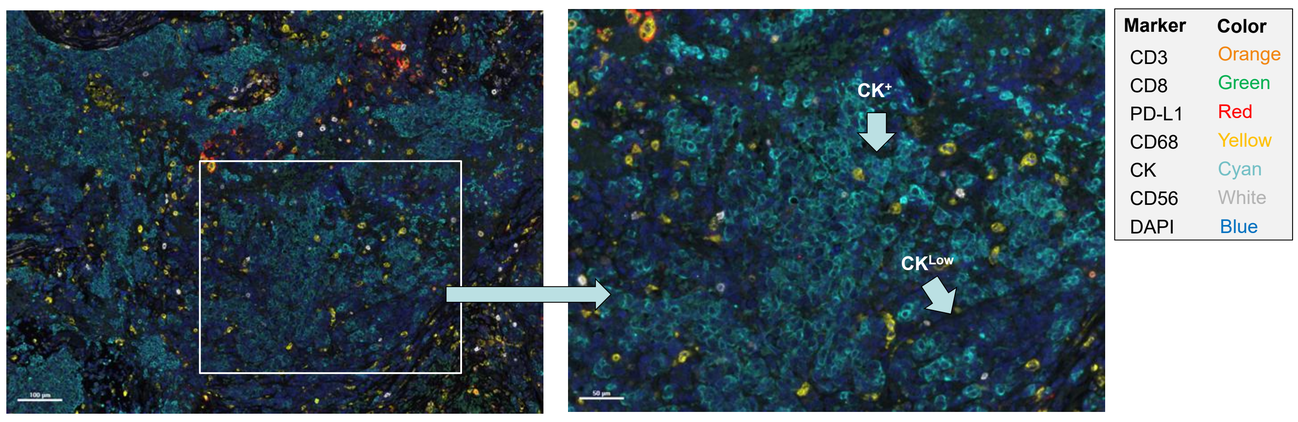

Figure 2: Multiplex immunofluorescent staining of the sinonasal NUT carcinoma patient specimen. A. A lower power image showing both the tumor and stromal areas. Scale bar: 100 μm. **B.** Examples of CK-positive (CK^{+}) and CK^{low} cells found in the tumor area. Scale bar: 50 μm.

Conclusions

The sample of sinonasal NUT carcinoma that we analyzed exhibited heterogeneous cytokeratin expression and low immunogenic characteristics. Given that NUT carcinoma is primarily driven by a single fusion oncoprotein, it has a low tumor mutational burden, which may align with these findings. Since conventional treatments for NUT carcinoma, such as chemotherapy and radiation therapy, have shown very limited success, it is essential to develop strategies to address the tumor immune microenvironment and to incorporate immunotherapy and potential new targetable therapies effectively. We plan to create an advanced panel with additional markers and include more tumor samples to further investigate the biology of sinonasal NUT carcinoma in our future studies.

References

1) Bauer DE, Mitchell CM, Strait KM, Lathan CS, Stelow EB, Lüer SC, et al. Clinicopathologic Features and Long-term Outcomes of NUT Midline Carcinoma. Clinical Cancer Research. 2012;18:5773-9.
2) Chau NG, Ma C, Danga K, Al-Sayegh H, Nardi V, Barrette R, et al. An Anatomical Site and Genetic-Based Prognostic Model for Patients With Nuclear Protein in Testis (NUT) Midline Carcinoma: Analysis of 124 Patients. JNCI Cancer Spectr. 2020;4:pkz094.
3) French CA, Stelow EB, Minato H. Nasal, paranasal, and skull base tumors-NUT carcinoma. In: Justin A. Bishop LDRT, Elizabeth L. Loney, editor. WHO Classification of Tumours Editorial Board Head and neck tumours [Internet; beta version ahead of print]. 5 ed. Lyon (France): International Agency for Research on Cancer; 2022.
4) French CA, Miyoshi I, Kubonishi I, Grier HE, Perez-Atayde AR, Fletcher JA. BRD4-NUT fusion oncogene: a novel mechanism in aggressive carcinoma. Cancer Res. 2003;63:304-7.
5) Takahashi Y, Bell D, Corona A, Su SY, Ferrarotto R, Gunn B, DeMonte F, Raza S, Hanna EY. Establishment of novel stable human sinonasal NUT carcinoma cell lines. Oral Oncol. 2026.

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