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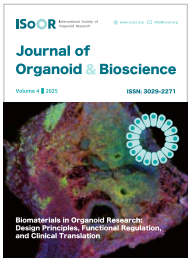

Industry News

Development of Patient-Derived Organoids from NUT Carcinoma: A Robust and Fully Documented Culture System

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Abstract

NUT carcinoma is an aggressive, poorly differentiated malignancy defined by NUTM1 rearrangements. Median survival remains 6–7 months and preclinical models are essentially nonexistent. Here we report the first patient-derived organoid platform for this disease. Starting material included resected tumors, pleural effusions, CTguided fine-needle aspirates, and transbronchial biopsies. Using a defined medium supplemented with Wnt3a, R-spondin-1, Noggin, FGF10, EGF, bFGF and smallmolecule inhibitors (A83-01, Y-27632, and Losmapimod), we successfully established long-term cultures from all four specimen types. Organoids retained characteristic nuclear NUT expression (≥80% positive cells), preserved the original fusion by FISH and NGS, and remained stable for at least 10 passages. Drug testing against BET inhibitors yielded clear, reproducible dose-response curves. All procedures, quality controls, and banking steps were performed according to the recently released ISoOR-ISOB international standard. This platform should facilitate mechanistic studies and drug screening for a tumor that currently lacks tractable models.

1. Introduction

NUT carcinoma (previously called NUT midline carcinoma) is driven by reciprocal translocations that fuse NUTM1 most commonly to BRD4 or, less often, BRD3 or NSD3. The resulting oncoproteins trigger global histone acetylation and block differentiation, producing a highly lethal squamous cancer that affects children and adults alike. More than 90% of patients present with stage III or IV disease, and median overall survival is still under seven months despite intensive chemotherapy and radiation.

Until now, researchers have relied on a handful of immortalized cell lines or patientderived xenografts that poorly reflect the original tumor heterogeneity and rapidly lose the fusion oncoprotein in culture. Patient-derived organoids have transformed drug discovery in many carcinomas, but no robust protocol existed for NUT carcinoma. We therefore set out to build a reliable culture system that works with the limited material clinicians can obtain from these patients and that meets the new ISoOR-ISOB biobanking standard from day one.

2. Materials and Methods

2.1 Ethics and ISoOR-ISOB compliance

The study was approved by the Ethics Committee of Chongqing University Three Gorges Hospital (approval 2023-047). Written informed consent was obtained from all adult patients or from parents/legal guardians of minors. The complete protocol, including quality-control thresholds and banking workflow, was reviewed and approved by the ISoOR-ISOB Technical Committee in February 2025.

2.2 Clinical specimens

Between June 2023 and October 2025 we collected fresh tumor tissue (n=12), malignant pleural effusions (n=9), CT-guided fine-needle aspirates (n=5), and transbronchial biopsies (n=7). All cases were confirmed NUT carcinoma by NUT immunohistochemistry and FISH or NGS.

2.3 Tissue processing

Resected specimens were transported on ice in DMEM/F12 + Primocin and processed within 30 min. Tissue was minced to ≈0.5 mm^3 fragments and digested 30–60 min at 37 °C in collagenase/dispase (1 mg/mL), hyaluronidase (0.1 mg/mL), and DNase I (40 µg/mL). Pleural effusions were centrifuged, red blood cells lysed when necessary, and tumor cell clusters enriched by brief digestion.

2.4 Organoid culture medium (final concentrations)

Advanced RPMI-1640 or DMEM/F12 supplemented with: B27 (1×), N2 (1×), GlutaMax (1×), N-acetylcysteine (1.25 mM), nicotinamide (2 mM), EGF 50 ng/mL, bFGF 20 ng/mL, FGF10 50 ng/mL, Wnt3a 50 ng/mL, R-spondin-1 100 ng/mL, Noggin 200 ng/mL, A83-01 500 nM, Y-27632 10 µM, Losmapimod 1 µM (found essential in pilot experiments to prevent early differentiation), Penicillin/streptomycin 100 U/mL and Primocin 100 µg/mL.

2.5 Embedding, passaging, and cryopreservation

Cell clusters were suspended in growth-factor-reduced Matrigel (Corning), dispensed as 10–25 µL domes in pre-warmed 24-well plates, and overlaid with 500 µ L complete medium after polymerization. Medium was refreshed every 2–4 days. Organoids were passaged at ≈200 µm diameter by cold PBS wash, 5–10 min TrypLE digestion at 37 °C, and mechanical disruption into small fragments (never single cells). Typical split ratios 1:2 to 1:4. For banking we used DMEM/F12 + 40% FBS + 10% DMSO, controlled-rate freezing (–1 °C/min) to –80 °C, then transfer to vapor-phase liquid nitrogen.

2.6 Quality control (ISoOR-ISOB thresholds)

- Morphology: compact structures 80–250 µm, <40% central necrosis, <30% vacuolation
- Viability (trypan blue): ≥80% before banking
- Sterility: mycoplasma PCR (every 3 passages), bacterial/fungal broth culture
- Identity: NUT IHC (rabbit mAb C52B1, CST #3625) ≥80% nuclear positivity; fusion confirmed by break-apart FISH or targeted NGS
- Post-thaw recovery: ≥50% viability accepted

2.7 Drug sensitivity

2,000–3,000 organoids/well in 96-well plates, 72 h drug exposure, viability by CCK-8, curves fitted in GraphPad Prism.

3. Results

Patient-derived organoids were successfully established from all four types of clinical specimens, demonstrating the feasibility of this platform across diverse sample sources. Tumor tissues yielded the highest establishment efficiency, with 11 of 12 samples generating organoids that could be expanded beyond ten passages. Pleural effusion-derived samples also produced viable organoids in 8 of 9 cases, highlighting the potential for minimally invasive sampling in patients with advanced disease. Organoids from FNAs and TBLBs were more challenging due to lower cellularity, yet 3 of 5 FNAs and 6 of 7 TBLBs produced expanding cultures. These observations confirm that the platform can accommodate the limited material often obtainable from NUT carcinoma patients.

Morphologically, organoids formed compact, densely packed spheroid-like structures with irregular outer surfaces, reminiscent of poorly differentiated squamous carcinoma. Over the first 7–14 days in culture, organoids displayed progressive enlargement and branching, reflecting active proliferation while maintaining structural integrity. High-resolution microscopy revealed uniform nuclear-tocytoplasmic ratios, minimal central necrosis, and absence of excessive vacuolation. Notably, omitting Losmapimod from the medium during pilot experiments resulted in rapid cystic degeneration and early loss of nuclear NUT staining, indicating that MAPK inhibition is essential for maintaining differentiation blockade and molecular fidelity in culture.

Molecular characterization confirmed that organoids preserved key tumor features. Immunohistochemistry demonstrated robust nuclear NUT expression in >85% of cells across passages 1–10, aligning with parental tumor profiles. Break-apart FISH and targeted NGS verified that the original NUTM1 fusion was retained in every line, while RNA sequencing indicated stable transcriptional signatures that closely mirrored those of the source tumors. These findings underscore the fidelity of the organoids in recapitulating both genetic and phenotypic hallmarks of NUT carcinoma.

Cryopreservation experiments further demonstrated the robustness of the platform. Post-thaw viability ranged between 55–70%, and organoids recovered their original morphology within 3–5 days, confirming that long-term storage does not compromise structural or molecular integrity. Drug-sensitivity assays using BET inhibitors such as iBET-762 and OTX015 produced reproducible dose-response curves, with IC_{50} values consistently in the low nanomolar range across independent organoid lines. These results validate the organoids as a functional preclinical model suitable for pharmacologic testing.

4. Discussion

The development of a standardized, ISoOR-ISOB-aligned organoid platform for NUT carcinoma represents a significant advance in modeling this rare, aggressive malignancy. Previous reliance on immortalized cell lines and patient-derived xenografts has been limited by rapid loss of NUTM1 fusion expression, poor preservation of tumor heterogeneity, and restricted scalability. Our study demonstrates that organoids can be reliably established from multiple patient-derived specimens, including surgically resected tumors, pleural effusions, FNAs, and TBLBs, thereby overcoming challenges associated with limited sample availability.

The culture system preserves key histopathologic and molecular characteristics of parental tumors. Dense, squamous-like morphology and high nuclear NUT expression are maintained over multiple passages, indicating that the combination of defined growth factors, Wnt signaling activators, and small-molecule inhibitors effectively
supports tumor identity while preventing premature differentiation. The addition of Losmapimod proved particularly critical, as pilot experiments without it led to rapid morphological degradation and loss of NUT expression, emphasizing the importance of MAPK pathway modulation for maintaining oncogenic fusion-driven phenotypes.

Cryopreservation protocols also proved highly effective, enabling long-term biobanking without significant loss of viability or molecular fidelity. Post-thaw recovery rates of 55–70% and restoration of normal organoid morphology within days confirm the feasibility of establishing a shared organoid repository for international research collaboration. Drug-sensitivity testing further demonstrated that these organoids respond predictably to BET inhibitors, providing a robust platform for preclinical screening and precision medicine initiatives.

5. Conclusion

We provide a practical, rigorously documented protocol for generating, characterizing, and banking NUT carcinoma organoids. The platform is already being used in our center for BET-inhibitor combination screens and should help fill a critical gap for this orphan disease.

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Conflicts of interest

None declared.

Data availability

Detailed culture logs, QC records, and sequencing data are available from the corresponding author on reasonable request and after MTA execution.