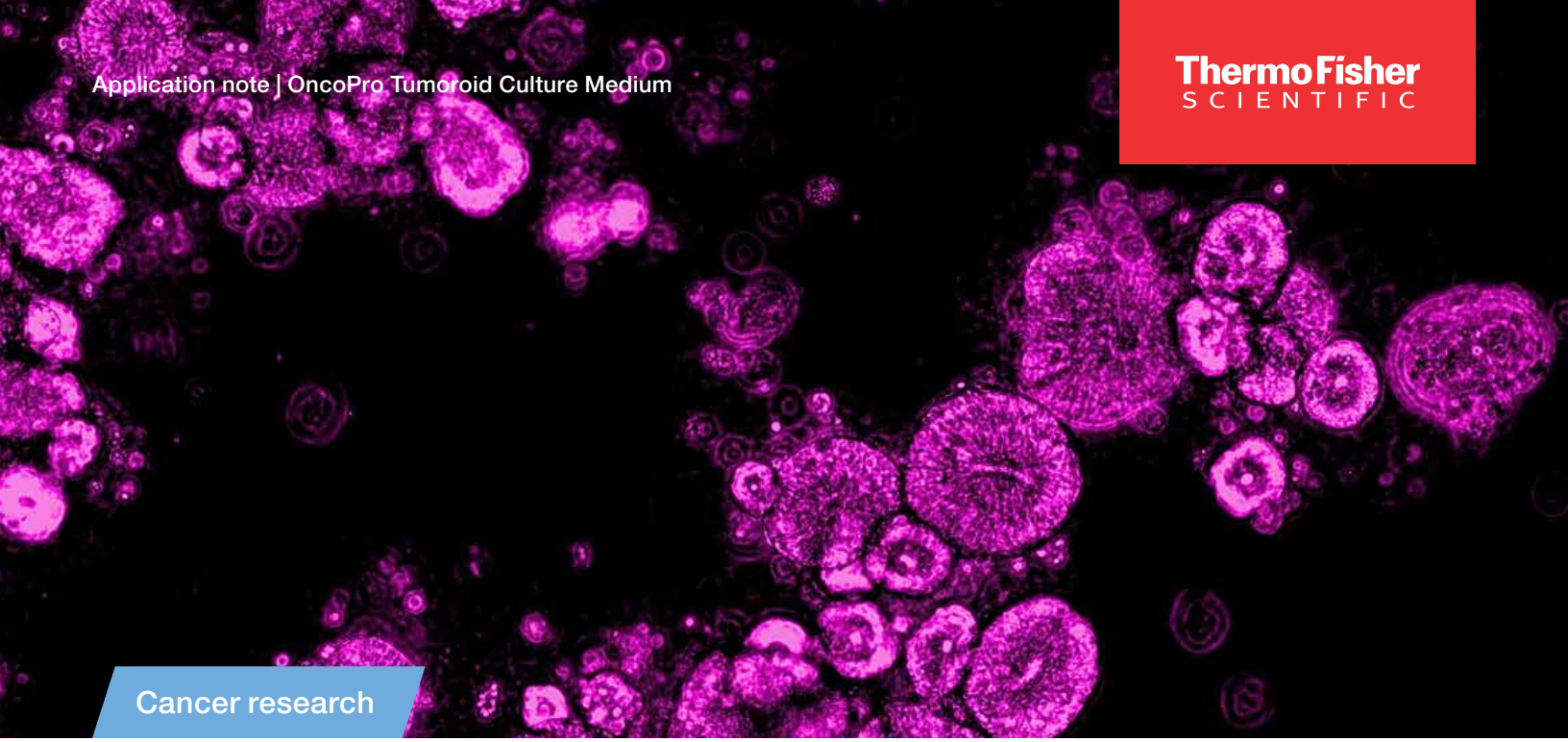

Cancer research

Culture of triple-negative breast cancer tumoroid lines in OncoPro Tumoroid Culture Medium

Highlights

- OncoPro Tumoroid Culture Medium can be supplemented with heat-stable FGF-10 and beta-estradiol to support expansion of triple-negative breast cancer (TNBC) tumoroids
- OncoPro medium maintains patient-specific characteristics of TNBC tumoroid lines, such as bulk morphologies, growth rates, and mutational and transcriptional profiles

Keywords

Tumoroids, cancer organoids, patient-derived cancer models, triple-negative breast cancer, targeted mutation analysis, bulk RNA sequencing

Introduction

The Gibco™ OncoPro™ Tumoroid Culture Medium is an easy-to-use medium designed for the expansion of tumoroid lines. To date, OncoPro medium has been validated for colorectal, lung, pancreatic, and head and neck tumoroid lines. However, the medium has utility beyond these four cancer indications. In this study, we investigated the potential of using OncoPro medium for expanding established triple-negative breast cancer (TNBC) tumoroid lines. The tumoroid lines listed in Table 1 were procured from the National Cancer Institute (NCI) Patient-Derived Models Repository (PDMR). Here, we present results demonstrating the retention of line-specific morphologies, growth rates, mutation profiles, and transcriptional characteristics during culture of these lines in OncoPro medium.

Table 1. Tumoroid lines developed by the NCI PDMR and tested for compatibility with OncoPro medium.

NCI PDMR name	Passage received	Abbreviated name	Diagnosis	Site of tissue collection	NCI-recommended medium
128162-247-R-V1-organoid	P12	NCI128162	Invasive breast carcinoma, TNBC	Metastatic site (lymph node)	Breast #2
868763-120-R-V1-organoid	P8	NCI868763	Invasive breast carcinoma, TNBC	Primary	Breast #1

Study design

Breast cancer tumoroid lines were initially expanded in the complete medium and with the culture method (encapsulated in basement membrane extract (BME) domes, known as embedded culture) recommended by the NCI PDMR* until a working cryobank was established. From the bank, cells were thawed into three conditions in parallel to test the compatibility of OncoPro Tumoroid Culture Medium supplemented for breast cancer tumoroid culture (Table 2):

- Complete OncoPro medium in embedded culture
- Complete OncoPro medium in suspension culture (cells free-floating in a cell culture vessel (non-tissue-culture treated) with 2% BME added to the medium)
- NCI-recommended complete medium in embedded culture (control condition)

For each of the cultures, we analyzed five readouts over the course of 5–6 passages:

- Bulk tumoroid morphology
- Growth rate
- Maintenance of patient-specific mutations (via the Ion Torrent™ Oncomine™ Comprehensive Assay v3C) for targeted mutation analysis
- Maintenance of patient-specific gene expression (via the Ion AmpliSeq™ Transcriptome Human Gene Expression Panel, Chef-Ready Kit)
- Maintenance of the molecular subtype of breast cancer

Table 2. Complete OncoPro medium recommended for breast and endometrial tumoroids.

Component	Cat. No.	Final concentration
OncoPro Tumoroid Culture Medium Kit	A5701201	1X
Penicillin-Streptomycin (10,000 U/mL)	15140122	2%
Primocin antimicrobial agent for primary cells (InvivoGen)	NC9392943 (Fisher Scientific)	100 µg/mL
Heat Stable FGF-10 Recombinant Protein	PHG0372	10 ng/mL
Beta-estradiol	501848155 (Fisher Scientific)	10 nM
Y-27632 2HCl (added day of use)	50-863-6 (Fisher Scientific)	10 µM

* With use of recombinant Wnt surrogate-Fc fusion protein, RSPO1, and noggin instead of conditioned medium.

Results

The patient-specific bulk morphology of each tumoroid was consistent between all three experimental conditions over the course of the culture (Figure 1A). Both tumoroid lines tended to form fairly solid, spherical tumoroids, with model NCI128162 generally organizing into smaller tumoroids than model NCI868763 across culture formats. As observed in other cancer indications and cell models, tumoroid aggregation typically increased in suspension culture. Additionally, both OncoPro medium conditions maintained comparable growth rates to the NCI-recommended conditions (Figure 1B).

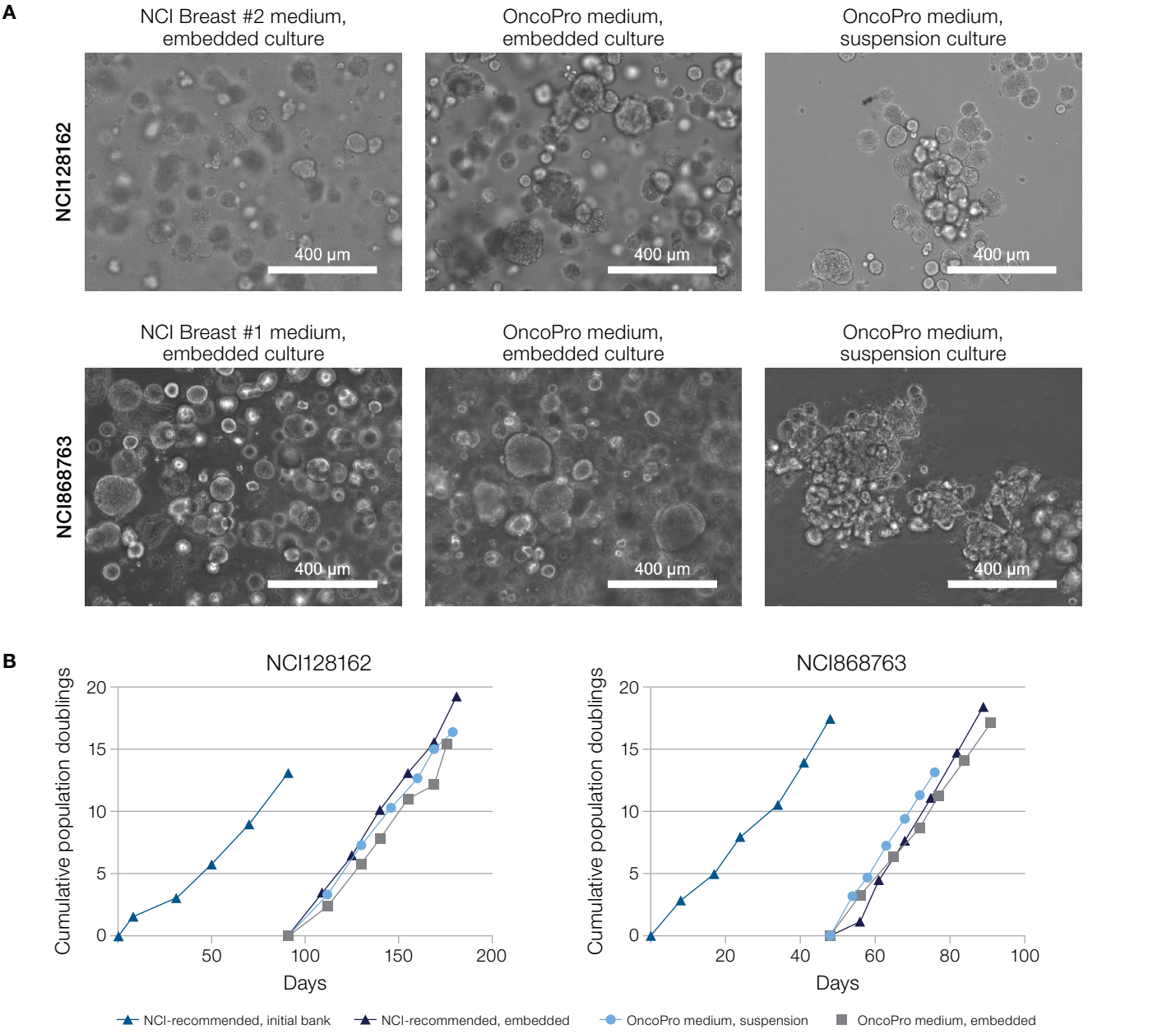

Figure 1. OncoPro medium maintained the patient-specific bulk tumoroid morphology and growth rate of each TNBC line tested. **(A)** Photomicrographs of the tumoroid lines in the NCI-recommended and two OncoPro medium conditions imaged immediately before passaging. All conditions have consistent morphologies. **(B)** Cumulative population doublings over time in culture for initial expansion to establish a bank in the NCI-recommended culture conditions, and then after thawing into the three experimental conditions. The OncoPro medium embedded and OncoPro medium suspension culture conditions maintained a comparable growth rate to that of the NCI-recommended conditions.

Beyond the morphological and growth characteristics of the TNBC tumoroid lines, the allelic frequency of somatic and germline single-nucleotide variants (SNVs) and ploidy values (copy number variants, CNVs) were examined for consistency between the original banks of cells and those expanded in the three experimental conditions (Figure 2). Multivariate analysis of the SNVs and CNVs revealed a high degree of correlation with the original banked cells received from the PDMR (Pearson $r \geq 0.95$ and Pearson $r \geq 0.85$, respectively) for both tumoroid lines following expansion in OncoPro medium (Figure 2).

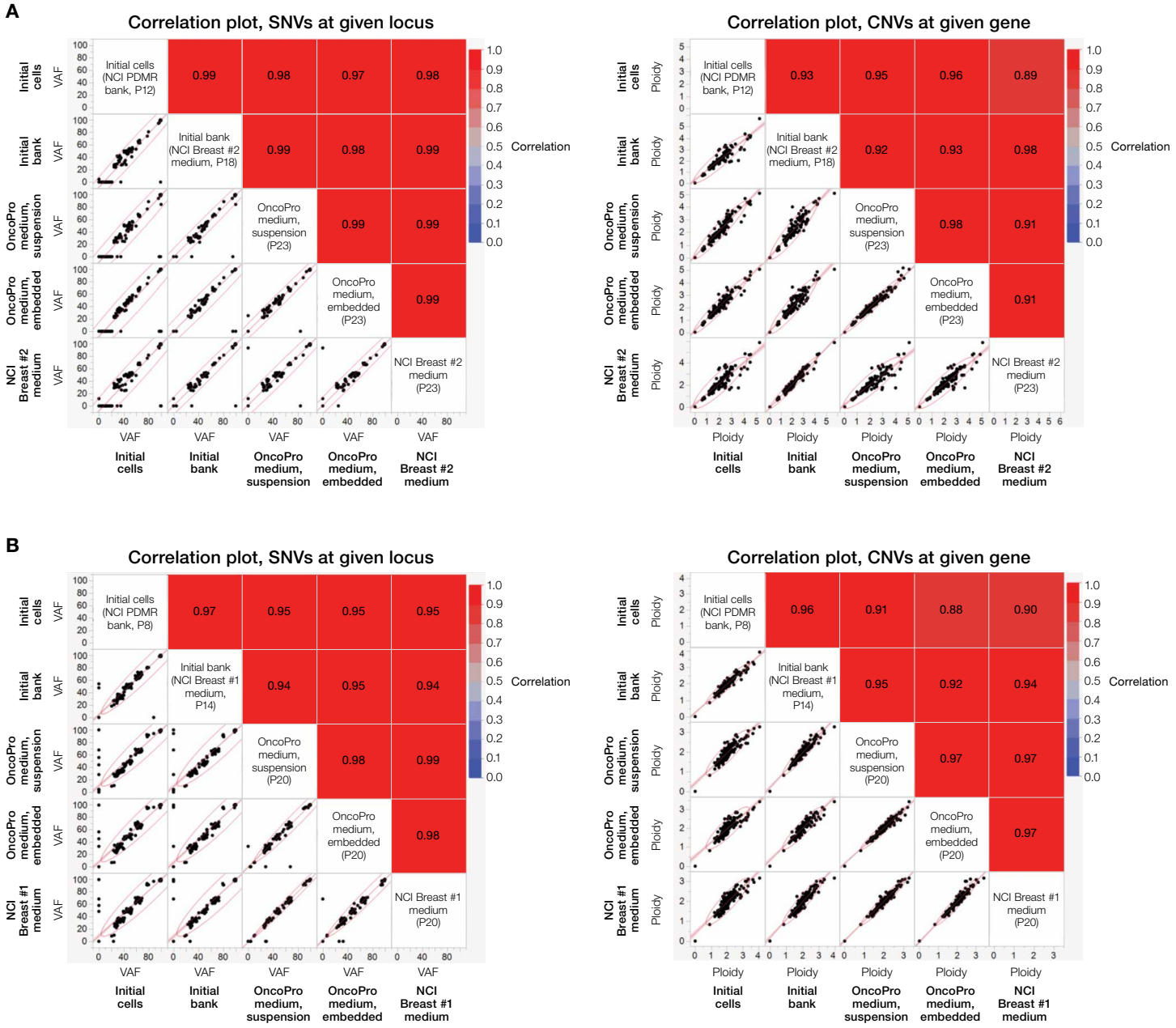

Figure 2. OncoPro medium maintained patient-specific mutational profiles of cancer-related genes. Correlation matrices of the variant allele frequencies (VAFs) of SNVs and ploidy values of CNVs of the **(A)** NCI128162 tumoroid line and **(B)** NCI868763 tumoroid line are shown for cells collected at time of thaw of the original material provided by the NCI PDMR, when an initial bank was established using the NCI-recommended condition, and after expansion in our three experimental conditions. Genomic DNA analysis was completed with the Oncomine Comprehensive Assay v3C.

To further examine the response of TNBC tumoroid lines when cultured in OncoPro medium, gene expression profiles were generated for cells sampled before and after expansion in the experimental conditions. Gene expression levels across either more than 20,000 RefSeq genes or a curated list of cancer-associated genes were highly correlated for tumoroid lines expanded in NCI PDMR conditions and tumoroids grown using OncoPro medium suspension or embedded culture (Pearson r >0.85; Figure 3).

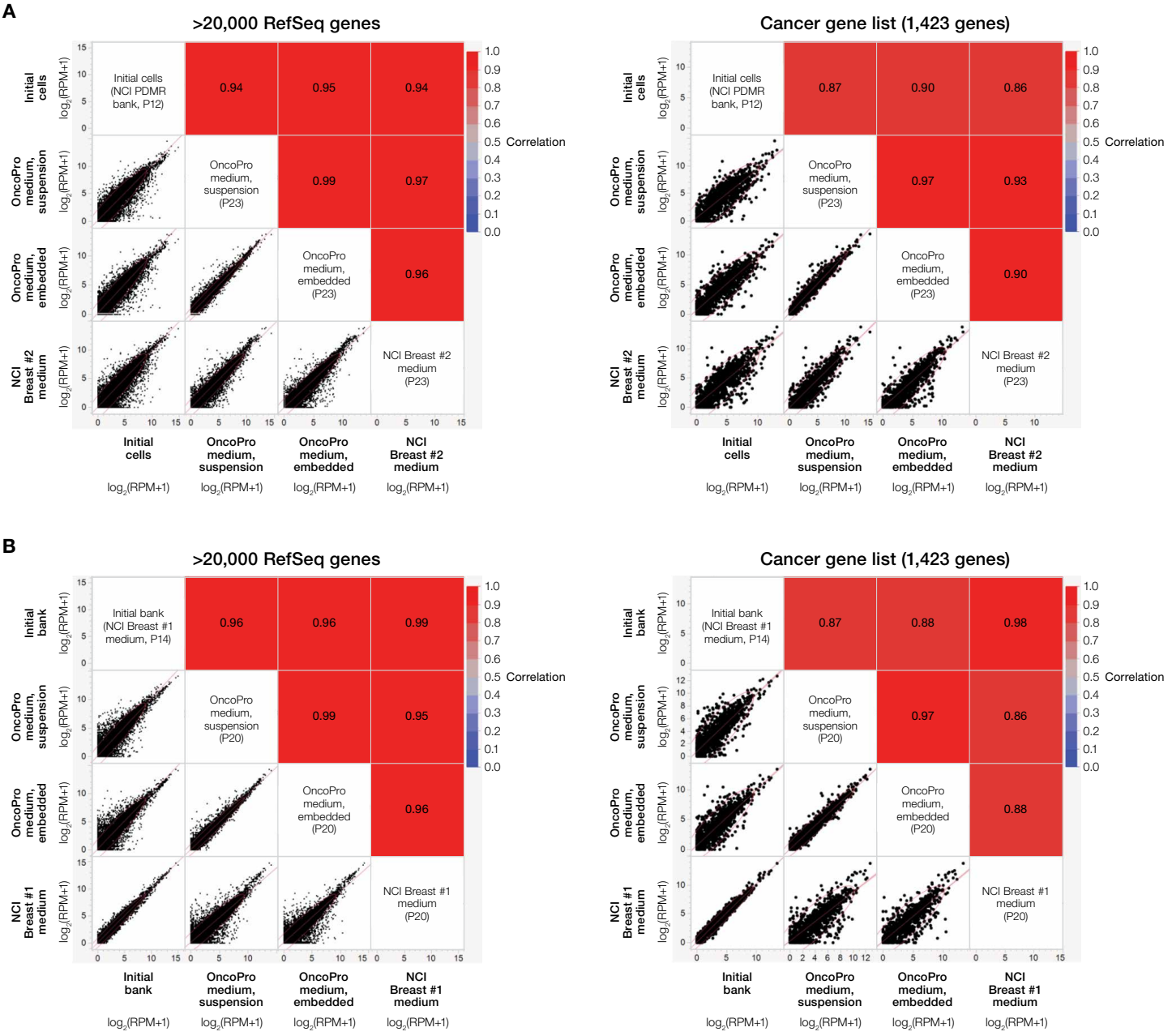

Figure 3. OncoPro medium maintained patient-specific transcriptional profiles. Correlation matrices of log-transformed normalized reads per million (RPM) for over 20,000 human RefSeq genes (left) and for 1,423 cancer-related genes (right) of the **(A)** NCI128162 tumoroid line and **(B)** NCI868763 tumoroid line are presented. Samples were collected at the indicated passages. Bulk RNA sequencing was performed using the Ion AmpliSeq Transcriptome Human Gene Expression Panel, Chef-Ready Kit.

Furthermore, differential gene expression analysis was performed between cells expanded in OncoPro medium suspension culture and the NCI-recommended (embedded) culture conditions for the two TNBC tumoroid lines (Figure 4). Over 95% of the >14,500 genes analyzed, after filtering low-quality genes, were not expressed at significantly different levels between these two conditions across the TNBC tumoroid lines tested. Interestingly, the Wnt signaling pathway was overrepresented in the list of differentially expressed genes (DEGs) upregulated for cells grown in the NCI-recommended media formulations, which contain Wnt pathway agonists in the form of Wnt surrogate-Fc fusion protein and RSPO1. Similar results were obtained when comparing gene expression of TNBC tumoroids expanded in OncoPro medium embedded culture to tumoroids in NCI-recommended formulations (data not shown).

Prior experiments comparing gene expression patterns for lines from various indications and donors cultured in OncoPro medium versus NCI-recommended (embedded) culture conditions have not shown differential regulation of Wnt signaling by KEGG analysis (data not shown). Of note, both TNBC tumoroid lines examined here retained expression of endogenous Wnt genes when cultured in OncoPro medium. Additionally, we have not observed that other signaling pathways are consistently differentially regulated when comparing tumoroids from other indications in OncoPro medium vs. NCI-recommended embedded culture. Furthermore, they maintained similar growth rates, as well as overall genomic and transcriptomic stability.

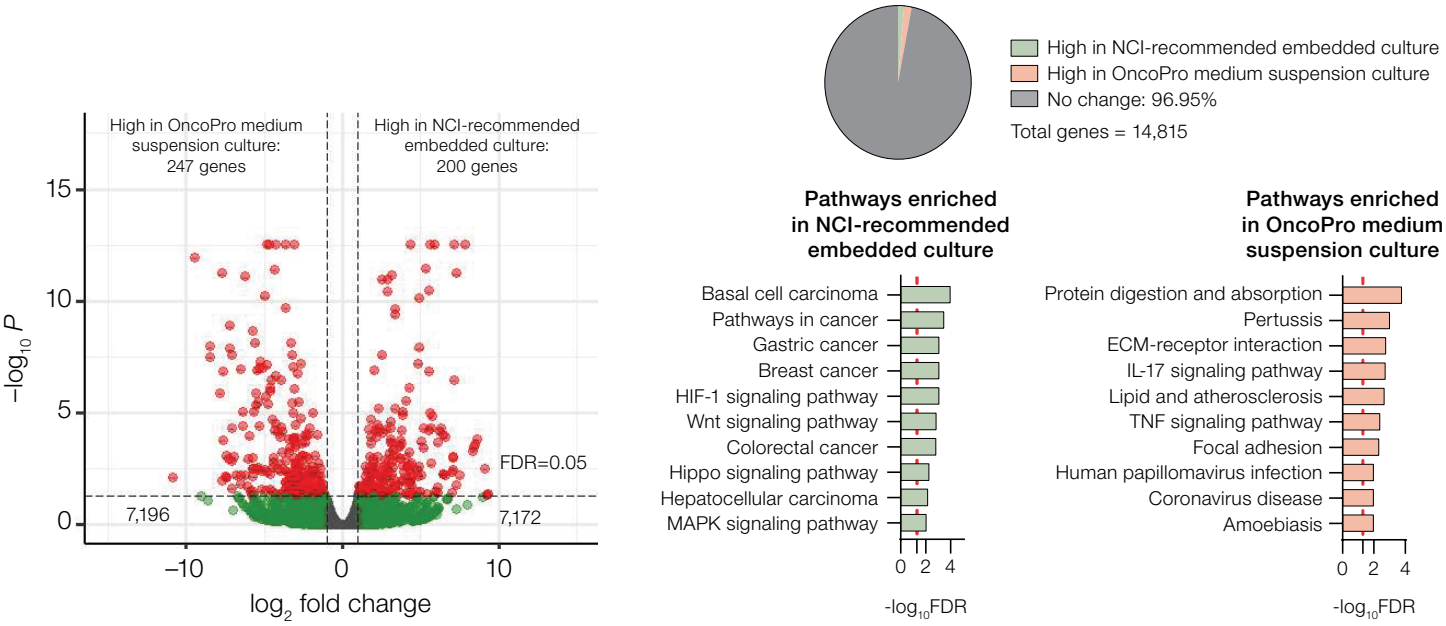

Figure 4. Transcriptomic comparison of NCI PDMR breast cancer lines (NCI128162 and NCI868763) cultured in the NCI-recommended condition (embedded culture) and the OncoPro medium suspension culture condition showed minimal differences in gene expression. Differential gene expression analysis revealed only 3.05% of the total genes analyzed were significantly differentially expressed (>2-fold change and false discovery rate (FDR) of <0.05). Gene ontology using Kyoto Encyclopedia of Genes and Genomes (KEGG) pathways showed some significant differences (FDR <0.05) in signaling pathways. Bulk RNA sequencing was performed using the Ion AmpliSeq Transcriptome Human Gene Expression Panel, Chef-Ready Kit.

Breast cancers can also be classified into normal-like (Normal), luminal A (LumA), luminal B (LumB), Her2-enriched (Her2), and basal-like (Basal) subtypes according to expression levels of PAM50 genes [1]. Importantly, these tumoroids also retained gene signatures of a basal breast cancer subtype when expanded in OncoPro medium, with similar expression levels of genes associated with the basal PAM50 breast cancer subtype observed across culture formats (Figure 5).

Although supplementation with beta-estradiol is not expected to be required for TNBC tumoroid lines, we have observed that it is required for expansion of some endometrial cancer tumoroid models. Additionally, inclusion of beta-estradiol did not drive estrogen receptor expression in endometrial tumoroid lines or in the TNBC lines examined here (ESR1, Figure 5). Therefore, we recommend inclusion of beta-estradiol during culture of endometrial and breast cancer tumoroids, especially when derivation of models begins before pathology reports containing analysis of hormone receptor status are obtained.

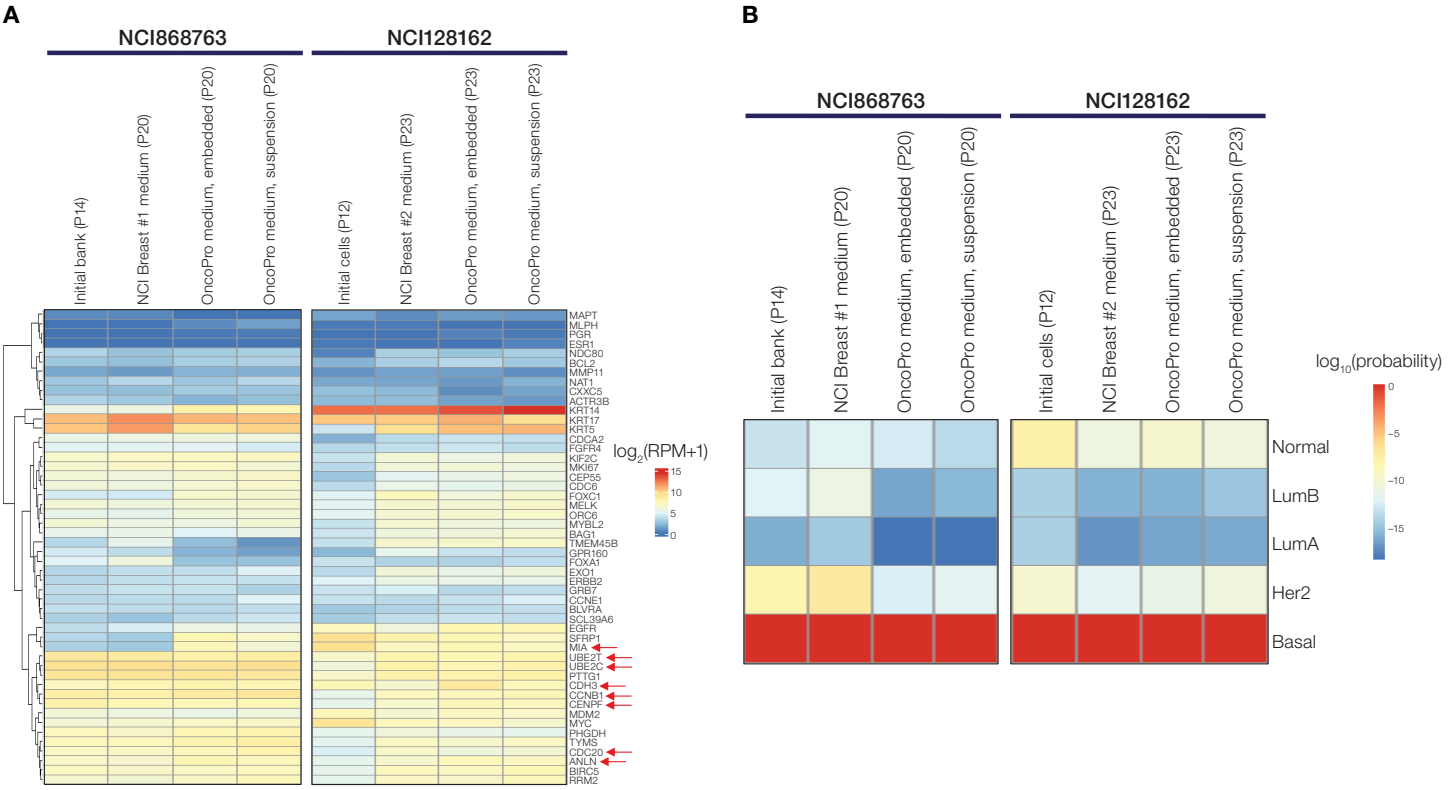

Figure 5. OncoPro medium maintained basal-like breast cancer molecular subtype. (A) Unsupervised hierarchical clustering of the expression levels of PAM50 genes, which are 50 genes used to classify breast cancer into five molecular subtypes. The gene expression profiles of the original NCI PDMR material provided are conserved in the NCI-recommended and OncoPro medium culture conditions. Genes typically expressed at a high level in basal-like breast cancer are marked with arrows. **(B)** Gene expression–based PAM50 breast cancer subtype classifications of breast cancer tumoroids show maintenance of basal subtype in all culture conditions [1]. Bulk RNA sequencing was performed using the Ion AmpliSeq Transcriptome Human Gene Expression Panel, Chef-Ready Kit.

Conclusion

The OncoPro Tumoroid Culture Medium Kit is an adaptable and easy-to-use media system for the culture of patient-derived tumoroid cells across culture formats. Here, we demonstrate that growth rates, mutational profiles, and gene expression levels are conserved when culturing previously established TNBC tumoroid lines in OncoPro medium. Additionally, we have derived a breast tumoroid line directly from patient tumor material using OncoPro medium, which is available for purchase through our Cell Biology Services team. Donor demographics, growth rate data, and genetic characterization for this line are **available online**. The adaptability of the OncoPro media system will enable breast cancer researchers to culture physiologically relevant cell models.

Acknowledgements

Established tumoroid models were provided by NCI PDMR. We thank the PDMR and their contributing institutions for their contributions to this work.

Reference

1. Parker JS, et al. (2009) Supervised risk predictor of breast cancer based on intrinsic subtypes. *Journal of Clinical Oncology* 27(8):1160-1167.

Ordering information

Product	Cat. No.
OncoPro Tumoroid Culture Medium Kit	A5701201
OncoPro Tumoroid Cell Lines	Submit an inquiry
Heat Stable FGF-10 Recombinant Protein	PHG0371
Geltrex LDEV-Free Reduced Growth Factor Basement Membrane Matrix	A1413201
StemPro Accutase Cell Dissociation Reagent	A1110501
TRIzol Reagent	15596026
Hibernate-A Medium	A1247501
Hanks' Balanced Salt Solution (no calcium, no magnesium)	14175095
Collagenase Type IV	17104019
TrypLE Express Enzyme	12604013
Nunc Non-treated Flasks	156800
Nunc Non-Treated Multidishes	150239
Nunc 15 mL and 50 mL Conical Sterile Polypropylene Centrifuge Tubes	339653
Oncomine Comprehensive Assay v3C	A35806
Ion AmpliSeq Transcriptome Human Gene Expression Panel, Chef-Ready Kit	A31446
Penicillin-Streptomycin (10,000 U/mL)	15140122

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