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Long-term acclimation of target cell lines to tumor microenvironment culture condition provides mechanistic insights into cell therapy effectiveness

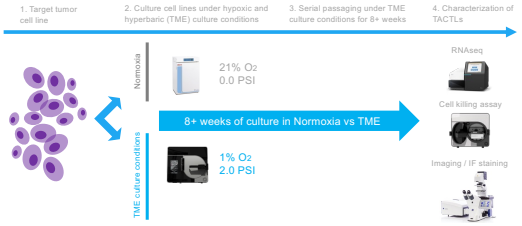
James Lim, Yunmin Li, Albert Wong, Yewei Xing*, Ningchun Liu*, Natalie Czeryba*, Evan Massi, Candy Garcia, Scott Wise*

*Labcorp Early Development Laboratories, Ann Arbor, Michigan; Xcellbio, San Francisco, California

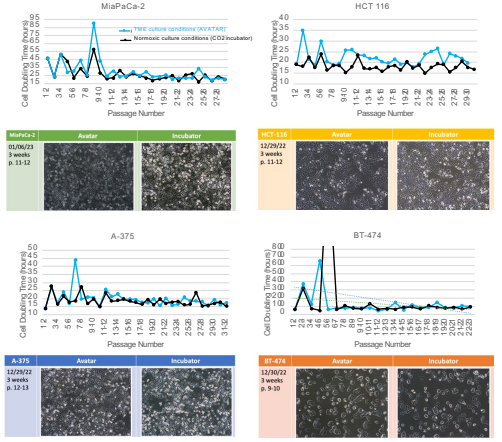
Abstract: Predict CAR T potency in solid tumor microenvironment

The tumor microenvironment (TME) has several characteristics that distinguish it from normal tissue, including elevated interstitial fluid pressures and hypoxia. To study the effects of TME culture conditions, commonly used target cell lines were serially passaged under hyperbaric and hypoxic conditions for a period of eight weeks. Conventionally expanded parental cell lines (normoxia) were compared against TME-acclimated tumor cell lines (TACTLs; hyperbaric and hypoxic), using RNAseq and flow cytometry analysis. Commonly used target cell lines for cell therapy development were selected for TME acclimation (SKOV3, A549, JEKO1, and NALM6). TACTLs were generated using an AVATAR system, with culture conditions set at 1% O2 and 2.0 PSI, and serially passaged twice a week for a minimum of 8 weeks. Cell doubling times were measured weekly and compared against parental lines maintained under normoxic culture conditions in a conventional CO2 incubator. In-depth characterization was performed on TME-Acclimated Tumor Cell Lines (TACTLs), utilizing a multi-omic approach. Differential gene expression analysis was performed using RNAseq datasets, and surface biomarkers/targets used for cell therapy development were assessed via nanostring and flow cytometry. Growth kinetics and cell doubling times of TACTLs were initially inhibited during the first two weeks of culture under TME conditions, but eventually reached parity with their normoxia maintained parental cell lines at 6 to 8 weeks, signaling a successful adaptation of the tumor lines to low oxygen and hyperbaric conditions. RNAseq analysis revealed upregulation in glycolytic pathways and epithelial-to-mesenchymal signaling, accompanied by altered metabolic profiles. Surface target expression showed increased expression of checkpoint ligands, such as PD-L1. Cell therapy targets, such as ROR1 were also upregulated in A549 and JEKO1 cell lines passaged under TME. Preliminary drug screening experiments were conducted using CAR-T candidates on TACTLs, revealing significant changes in killing efficiencies when compared to parental tumor lines maintained under normoxia. In summary, acclimation of tumor cell lines to TME culture conditions can provide unique mechanistic insights that can facilitate drug development efforts. Future studies will incorporate TACTLs for CDX tumor models with the goal of identifying cell therapies that work effectively in the tumor microenvironment.

TME-acclimated tumor cell lines (TACTLs) were generated through serial passaging under hypoxic and hyperbaric culture conditions

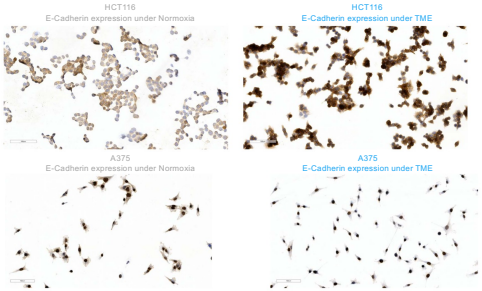


Target get cell lines cultured under TME conditions initially exhibit decreased proliferation rates, but recovers after 10 passages

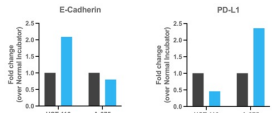


Cell doubling time was calculated based on cell count and the length of time between each passage. Cells grown in hypoxia condition had a longer doubling time in the beginning and gradually shortened to reach near parity with cells cultured under normoxic culture conditions. Cell morphology pictures: Cells were cultured in standard CO2 incubator or AVATAR incubator (1% O2 + 2 PSI + 5% CO2 + 37 degrees Celsius (TME culture conditions) for 4 weeks before pictures were taken. A375 cell started to exhibit more elongated and spiky cell shapes after growing in AVATAR system for 4 weeks, while larger cell body and more angular/ various shape multinucleated cells were seen in HCT-116 population under the same condition.

Target get cell lines cultured under TME conditions exhibit differences in EMT and immune checkpoint expression

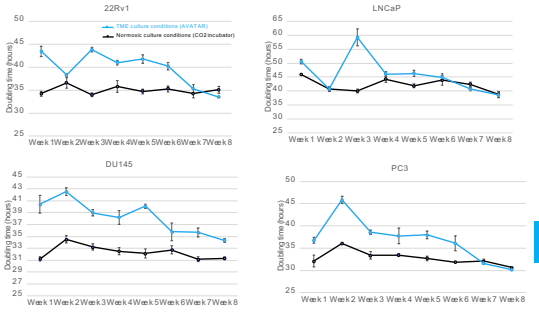


Cells were seeded on tissue culture treated chamber slides at proper seeding density and allowed to attach and grow until 70% confluent (between 24-48 hours). At 70% confluency, cells were fixed with 2 mL ice-cold methanol and placed in a -20°C freezer for 10 minutes. After fixing, cells were permeabilized and stained with primary antibodies E-cadherin (Cell Signaling Tech, #3195) or Beta Actin (Cell Signaling Tech, #4970). The staining was visualized by DAB chromogen method and imaged using the Leica Versa microscope with brightfield settings and 40x magnification. Cell area and intensity was analyzed using ImageJ software. E-cadherin protein expression was lower in A375 cells after 6-8 weeks in AVATAR incubator compared to normoxic culture condition, however the opposite effects were observed for HCT-116 cells.

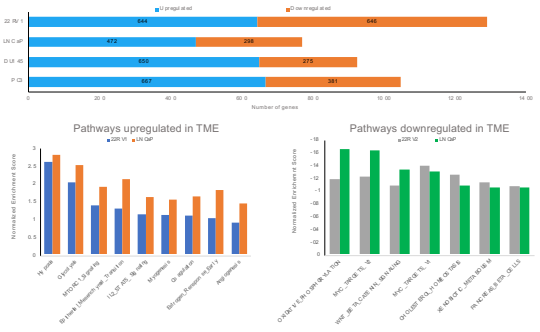


Gene expression results of qPCR. XX were cultured in a CO2 incubator or AVATAR incubator (1% O2 + 2 PSI + 5% CO2 + 37 degrees Celsius (TME culture conditions) for 6-8 weeks and RNA was harvested for RT-qPCR. A 2.4-fold increase in PD-L1 gene expression was found in A375 cells after long term acclimation in AVATAR incubator compared to CO2 incubator. HCT-116 cells on the other hand, had decreased expression of PD-L1, but doubled the level of E-cadherin mRNA.

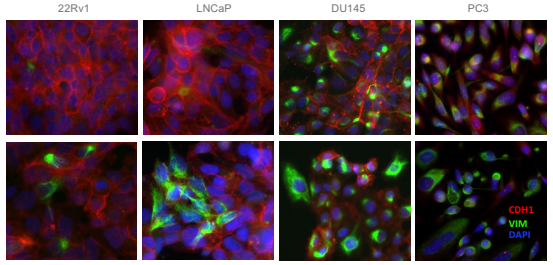
Prostate cancer cell lines cultured under TME conditions initially exhibit decreased proliferation rates, but recovers after 8+ weeks in culture

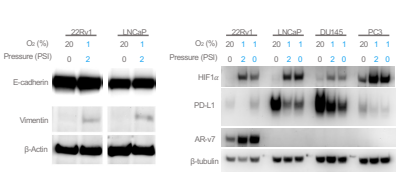


RNAseq reveals altered signaling pathways in prostate cancer cell lines acclimated to TME culture conditions



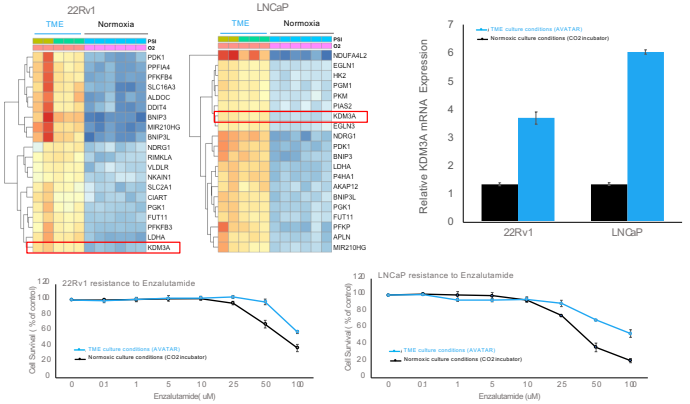
Prostate cancer cell lines acclimated to TME culture conditions exhibit increased vimentin, HIF1a, and decreased PD-L1 expression

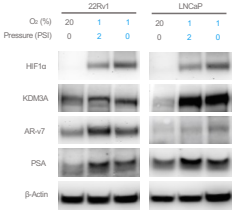




Prostate cancer cell lines were cultured under 20% O2 + 0 PSI and 1%O2 + 2PSI for 3 days. Vimentin expression was upregulated in 22Rv1 and LNCaP cells maintained in TME culture conditions. Androgen receptor variant 7 (AR-v7), a treatment resistant marker for metastatic castration resistant prostate cancer, was also upregulated under TME culture conditions for the AR-positive cell line, 22Rv1. Programmed death-ligand 1 (PD-L1), which binds to the inhibitory checkpoint molecule PD-1, was shown to be decreased in the four prostate cancer cell lines acclimated to TME culture conditions.

Prostate cancer cell lines acclimated to TME culture conditions exhibit increased KDM3A expression leading to increased expression of the treatment resistance marker Androgen Receptor Variant 7 (AR-v7)

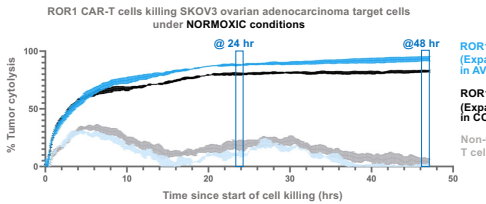


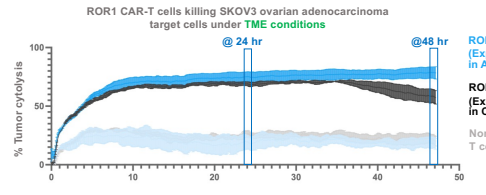


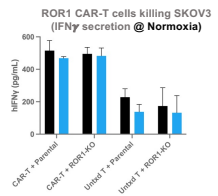
Prostate cancer line:	Tumor site of origin	Androgen receptor
22Rv1	derived from xenograft, CWR22	+
LNCaP	metastatic site: lymph node	+
DU145	metastatic site: brain	-
PC3	metastatic site: bone	-

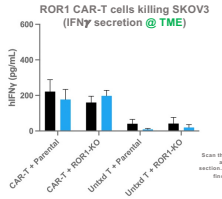
The histone demethylase KDM3A is involved in regulating alternative splicing of the androgen receptor, including androgen receptor variant 7 (AR-v7), in response to androgen deprivation therapy. Here, we show that KDM3A expression is increased under TME culture conditions in 22Rv1 and LNCaP, which is also accompanied by increased expression of AR-v7. These results support the hypothesis that hypoxic prostate cancer cells are preprogrammed to resist androgen deprivation and anti-androgen therapies (Enzalutamide).

CAR-T potency screening against TME-acclimated cell lines can provide insights into immunosuppression and cell therapy resistance









Tumor cytolysis assay:
Target cells, SKOV3 (ATCC) were seeded onto an AVATAR AI E-plate at 5K cells per well (96 well format). Target cells were allowed to adhere for 24 hours before effector cells (ROR1 CAR-T cells) were introduced at 10:1 effector to target ratio (50K). Cell killing assays were performed under **Normoxia** (21% O2 & 0 PSI) or **TME** (1% O2 & 2 PSI). ROR1 CAR-T cells were transduced and expanded for 9 days, either in a **CO2 incubator** or an **AVATAR System** (Ambient O2 + 4 PSI). IFNγ secretion assays was performed at 48 hours after co-culture (BD cat# 555142). **Flow cytometry** was performed using a BD Fortessa X-20. Naïve cells (CCR7+CD45RA+), Central Memory (CCR7+CD45RA-), Effector Memory (CCR7-CD45RA-), Terminally Differentiated Effector Memory (CCR7-CD45RA-). Details on flow cytometry antibodies used are found by accessing the QR code below.

Scan the QR Code for a copy of this poster, and access to materials and methods section. Visit us at Xcellbio.com/avatar-ai to find out how you can enhance your cell therapy potency in the solid tumor microenvironment.