

Cancer stem cell proliferation in human prostate and breast cancer cell lines utilizing a new defined serum-free 3D spheroid media

MERCK

Kan Saito, Nick Asbrock, Vi Chu

Cellular Assays, Biological Reagents & Kits, Merck KGaA, Temecula, CA

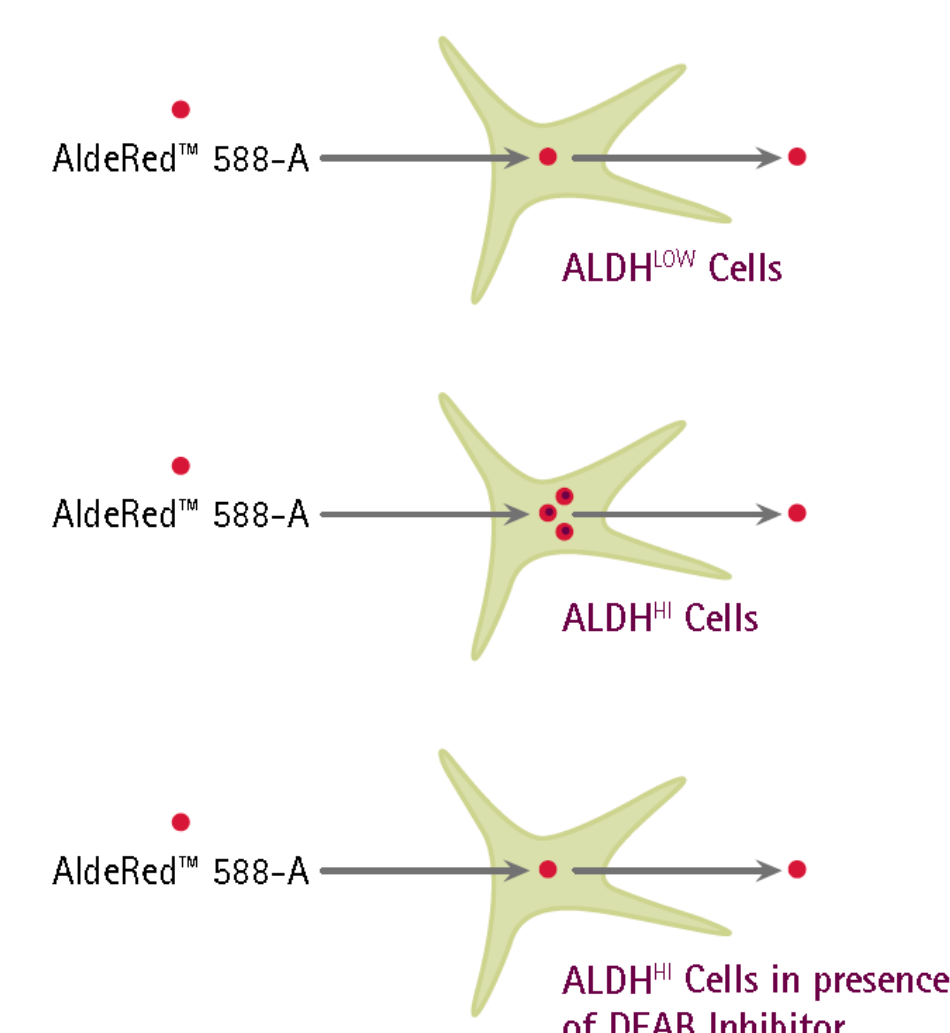
Introduction

Solid tumors grow in a three-dimensional (3D) spatial conformation, resulting in a heterogeneous exposure to oxygen and nutrients as well as to other physical and chemical stresses. To mimic the 3D spatial conformation, 3D in vitro culture models have been used in cancer research since the diffusion-limited distribution of oxygen (hypoxia), nutrients, metabolites, and signaling molecules is not mimicked in conventional two-dimensional (2D) monolayer cultures. One of the 3D in vitro culture models, the tumorsphere culture is an emerging model for studying and expanding the cancer stem cell (CSC) population. E006AA cell line is a spontaneously immortalized cell line derived from a Gleason 6 localized prostate cancer in a hormone-naïve prostate cancer patient of African American descent. This cell line expresses androgen receptor and was repeatedly reported to be non-tumorigenic in nude mice. However this cell line forms continuously growing tumor in NOG-SCID triple-deficient mice (i.e., NOG-SCID mice not having NK, B, and T-cells) and an establishment of highly tumorigenic subline E006AA-hT was reported suggesting this cell line has a potential for CSC proliferation by 3D sphere cultures. Here we report tumorsphere cultures of E006AA and MCF-7 cells by a new defined serum-free 3D sphere culture media system. The cells showed continuous proliferation supported during serial passage of 3D tumorsphere cultures. In addition to the stable proliferation, increases of Aldehyde Dehydrogenase (ALDH) expressing CSC population were observed with the increase of passages of 3D tumorsphere cultures.

AldeRed™ ALDH Detection Assay

Method principal:

Uncharged ALDH-substrate has the ability to diffuse passively into live cells. Intracellular ALDH enzymes convert the substrate into a negatively charged reaction product, which is trapped inside cells, causing ALDH^{hi} cells to become brightly fluorescent.



AldeRed 588-A staining and detection

Dry AldeRed 588-A reagent was resuspended in DMSO and activated by addition of 2N HCl. Each cell sample was suspended in the Assay Buffer and AldeRed 588-A reagent was added. Immediately, the sample was split between two tubes, one of which contained DEAB, an inhibitor of ALDH. Reactions were carried at 37°C for 30 min followed by a round of wash in Assay Buffer.

Detection of AldeRed 588-A was carried on cytometry instruments equipped with blue and/or green laser with PE and/or PE-TexasRed detectors (Guava, Muse, ACEA Novocyte, Beckman).

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Methods

40,000 cells (10,000 cells/mL) per well of **E006AA Human Prostate Cancer Cell Line (Merck Cat. # SCC102)** or **MCF7 Cell Line human (SIGMA Cat. # 86012803-1VL)** were plated in triplicate in the new chemically defined cell culture Media, **3dGRO™ CSC Spheroid Media (SIGMA Cat. # S3077-1KT)** using **Greiner CELLSTAR® 6-well suspension culture plates (SIGMA Cat. # M9062-100EA)**. Serial passage by enzymatic dissociation with Trypsin-EDTA was performed every 7 days for E006AA cells and every 9 days for MCF7 cells. Tumorsphere formation and proliferation were maintained during the culture, which was discontinued after passage 5 or 6.

E006AA cells and MCF7 cells were also maintained in EmbryoMax® DMEM - High Glucose, Low Bicarbonate w/o Sodium Pyruvate (Merck, Cat. # SLM-220-M) with 10% EmbryoMax® ES Cell Qualified Fetal Bovine Serum (Merck Cat. # ES-009-B) in adherent (2D) culture as controls.

After the passages of 1, 3, and 5 the proliferations were measured with **MTT Cell Growth Assay Kit (Merck Cat. # CT01)** and Aldehyde Dehydrogenase (ALDH) Expression was analyzed by flow cytometry with **AldeRed ALDH Detection Assay (Merck Cat. # SCR150)**

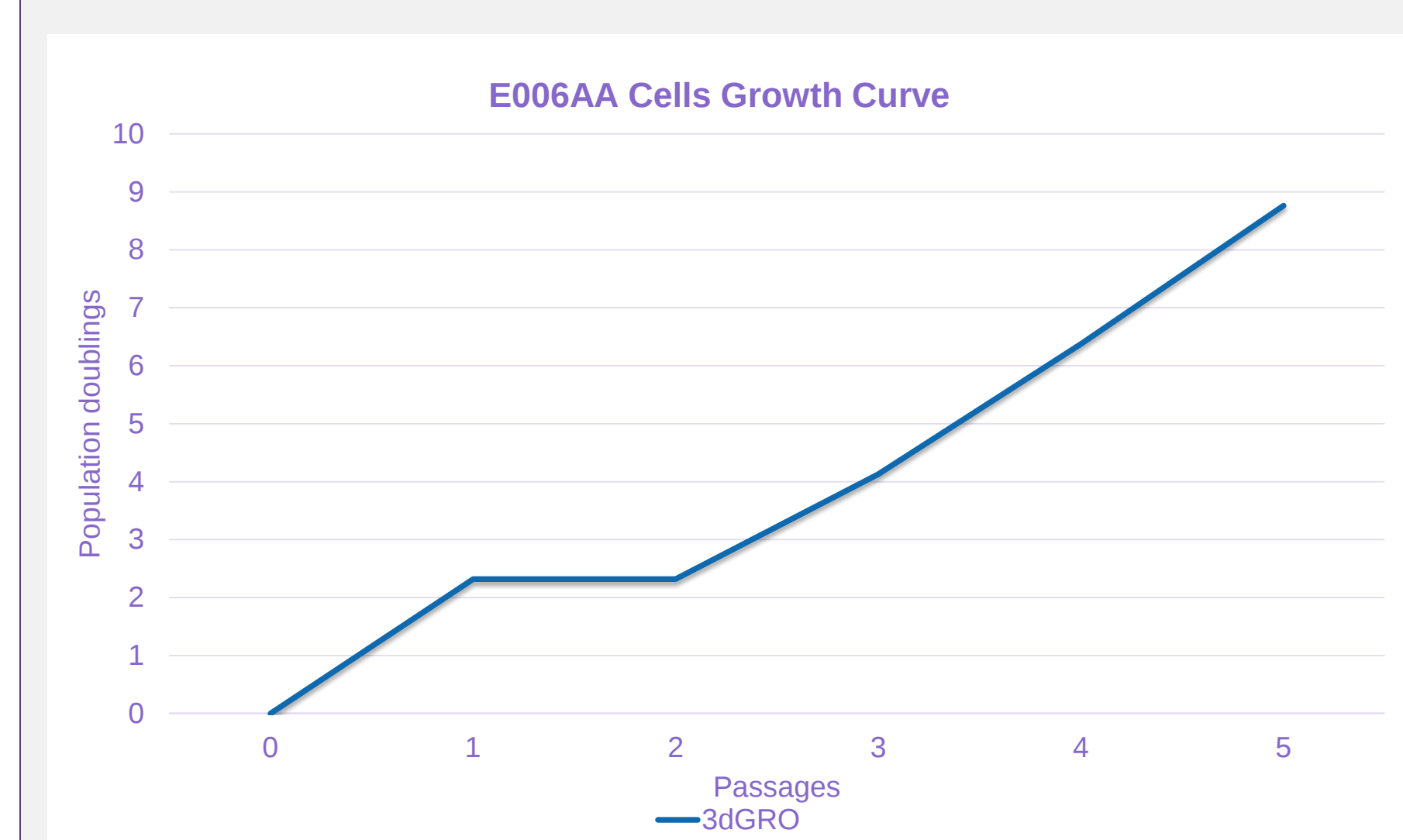
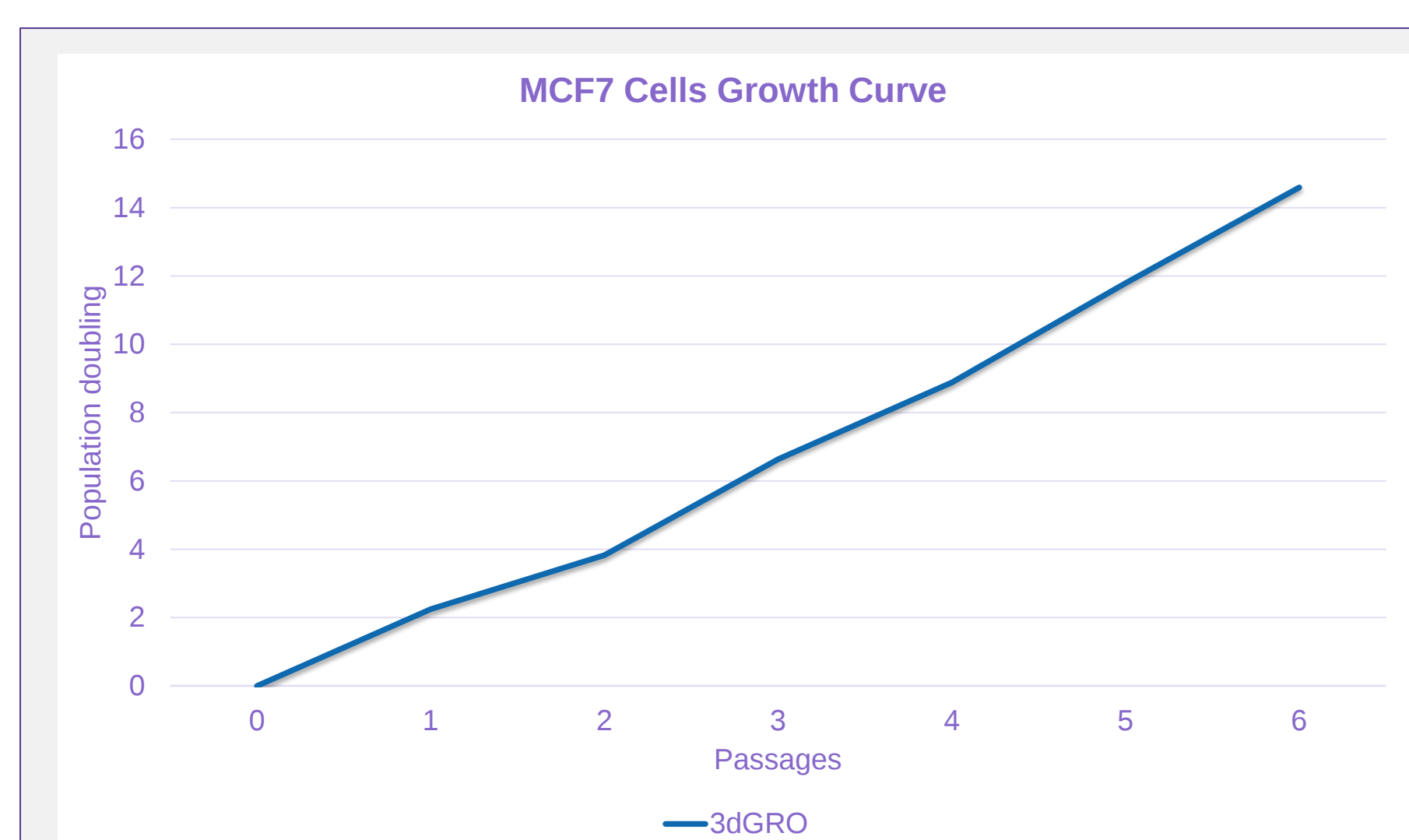


Figure 1. Stable Growth of Cancer Cells in 3dGRO™ CSC Spheroid Media (SIGMA Cat. # S3077-1KT)

Cumulative population doublings of MCF7 cells and E006AA cells during serial passage of 3D tumorsphere culture was plotted.

Results

- Tumorsphere formation and proliferation were maintained during the culture of both MCF7 and E006AA cells in 3dGRO™ CSC Spheroid Media. (Figure 1, 2, 3)
- E006AA cells attached to the plates at the first passage (Figure 3A) and the cells didn't proliferate well until the 2nd passage however at the 3rd passage the proliferation took off (Figure 1) and the cells started to form tumorsphere formation (Figure 3B) in 3dGRO™ CSC Spheroid Media.
- In 3dGRO™ CSC Spheroid Media the proliferation of MCF7 and E006AA were maintained around 50 to 90 % (MCF7) and 140 to 180 % (E006AA) of proliferation compare to the standard DMEM 2D culture at the all three passages. (Figure 4)
- At the passage 3 and 5 ALDH^{High} stem cell population of MCF7 cells was increased to around 8 % in 3dGRO™ CSC Spheroid Media compared to less than 4% of normal (adherent) cultured cells. (Figure 5A)
- At passage 1, 3, and 5 ALDH^{High} stem cell population of E006AA cells was increased to 7-13 % in 3dGRO™ CSC Spheroid Media compared to less than 4% of normal (adherent) cultured cells. The ALDH^{High} populations of E006AA cells were increased in 3dGRO™ CSC Spheroid Media at higher passages. (Figure 5B)



Figure 2. Tumorsphere formation of MCF7 Cells at Passage 1 (A) and Passage 5 (B)

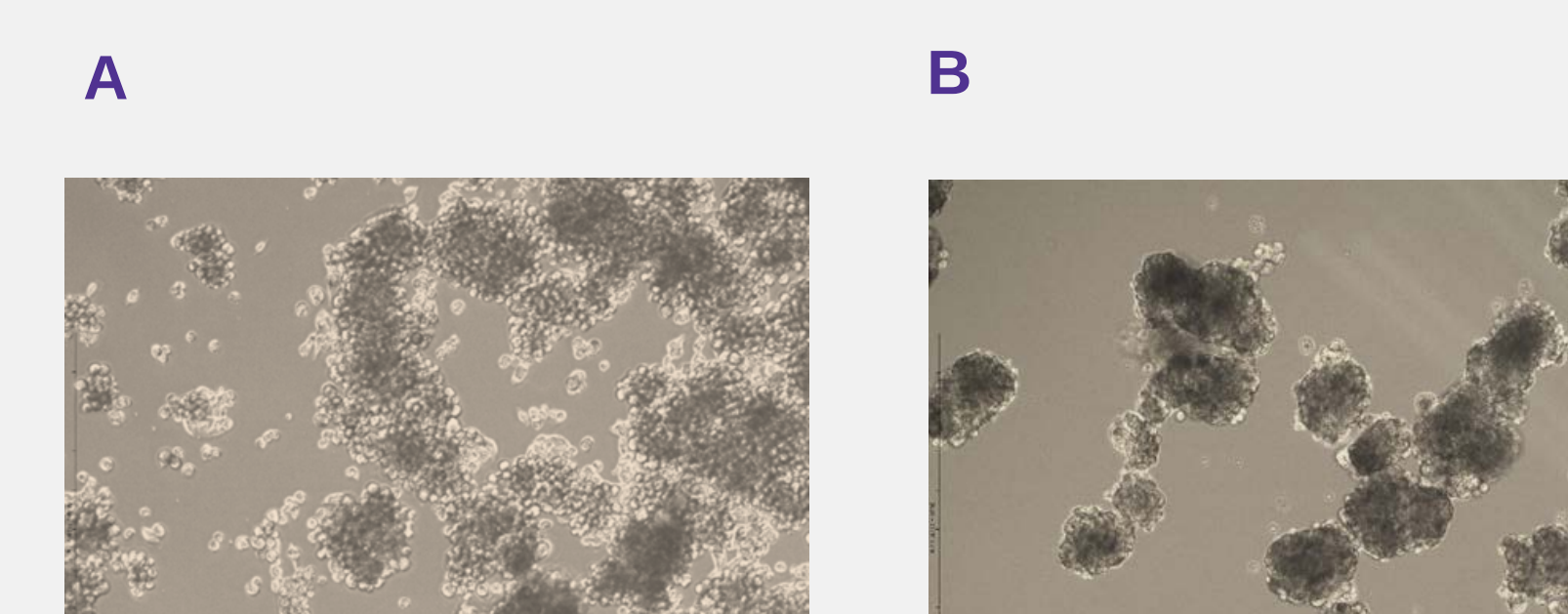


Figure 3. Tumorsphere formation of E006AA Cells at Passage 1 (A) and Passage 5 (B)

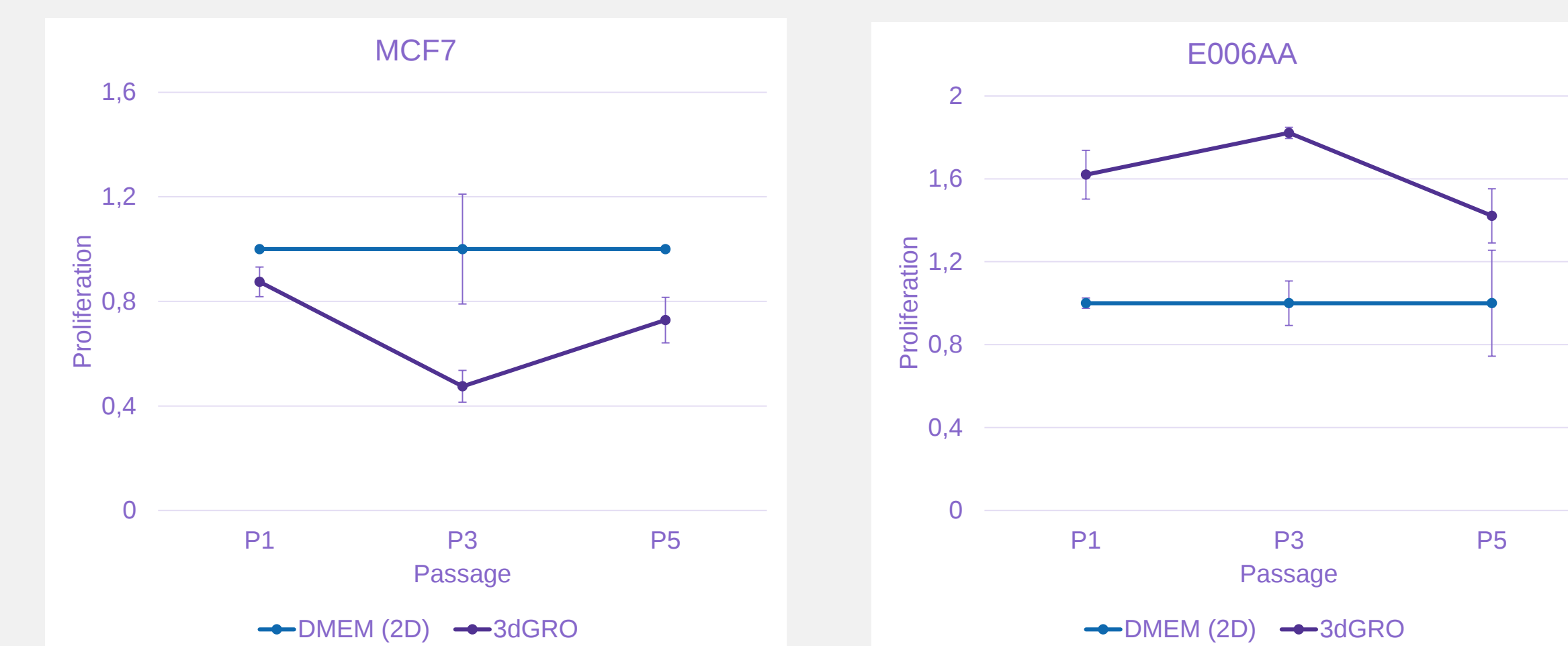


Figure 4. Constant Proliferation of the Cancer Cells in 3dGRO™ CSC Spheroid Media

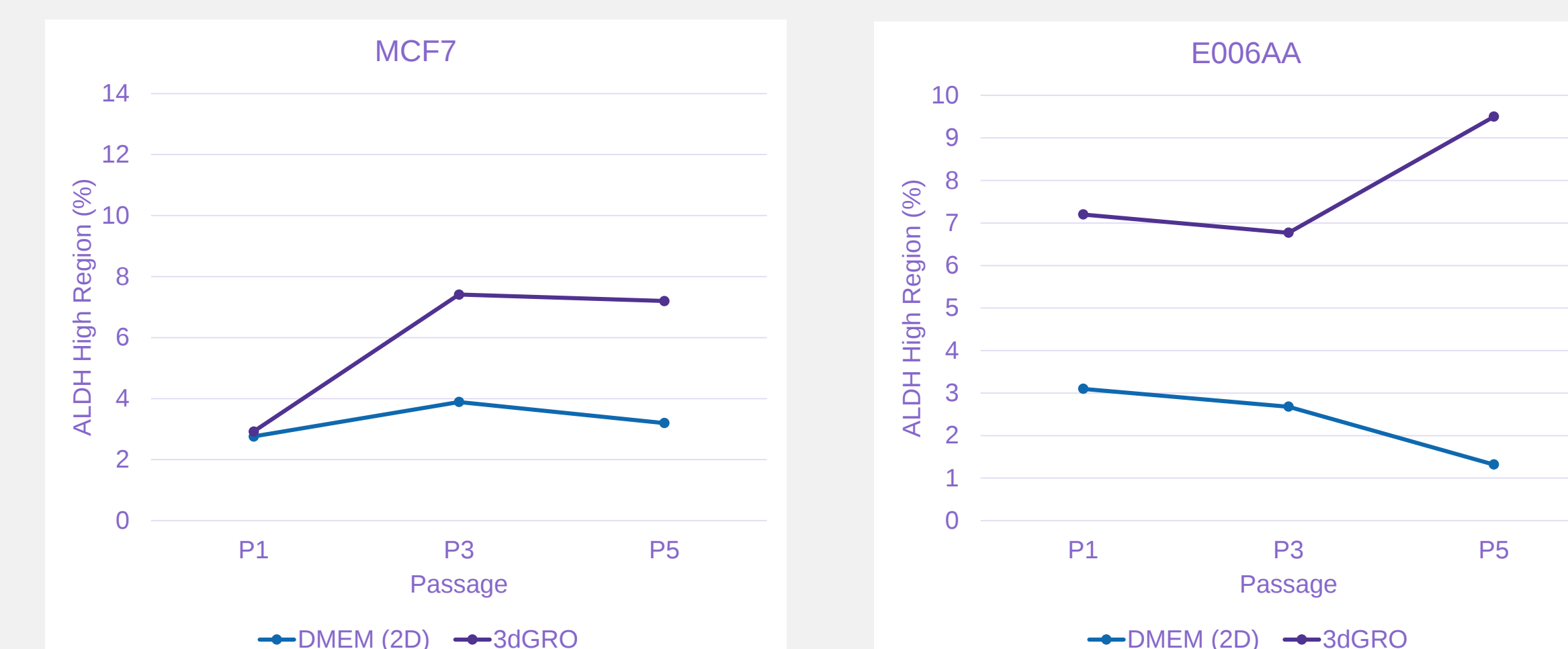


Figure 5. Increase of ALDH^{High} Stem Cell Population in 3dGRO™ CSC Spheroid Media

Summary

- Tumorsphere formation and proliferation were maintained during the culture of both MCF7 cells and E006AA cells in 3dGRO™ CSC Spheroid Media (SIGMA Cat. # S3077-1KT)
- By increasing the passage both MCF7 cells and E006AA cells form more sphere structures. The changes were more obvious with E006AA cells.
- Increase of ALDH^{High} stem cell population was observed with both MCF7 cells and E006AA cells maintained in 3dGRO™ CSC Spheroid Media. The increase was more obvious in E006AA cells.

Corresponding Authors: kan.saito@emdmillipore.com