



Short Communication

The Ability to Deal with Metabolic Stress Correlates with Cell Proliferation Rates under 2D or 3D Cell Culture System

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ABSTRACT

Different cancer cell lines display altered—either decreased or increased—proliferation rates when cultivated as 3D tumor spheroids. The presented work aimed to study the interplay between cell proliferation rates and metabolic stress in 2D and 3D cell culture systems. Two cell line models (HepG2; liver cancer cell line and HCT-116; colorectal cancer cell line) were selected that showed inverse proliferation trends under 2D and 3D cell culture systems: HCT-116 displays decreased, while HepG2 displays increased proliferation rates under 3D system. The presented data indicated that the proliferative capacity of the given cell line was associated with its ability to deal with metabolic stress under 2D or 3D system. HCT-116 cells coped better with metabolic stress in 2D system while HepG2 in 3D system.

Article Information

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Key words

3D cell culture system, Metabolic stress

One of the most obvious differences in cells under 2D or 3D cell culture systems is their altered proliferation capacity. In 3D cell culture systems different cancer cell lines displayed altered—either decreased or increased—proliferation rates. Number of cell lines showed reduced proliferation rates in 3D systems (Wong *et al.*, 2007; Gurski *et al.*, 2010; Maria *et al.*, 2011; Chitcholtan *et al.*, 2012; Fallica *et al.*, 2012; Luca *et al.*, 2013). The underlying mechanisms for the differences in proliferation rates of cancer cells in 2D and 3D cell culture systems are not widely explored. 3D spheroids are known to possess zones of differential proliferation. It has been shown that cells at the periphery of tumor spheroid—that have better access to nutrients and oxygen—divide more rapidly (Lin and Chang, 2008). Cancer cells within *in vivo* tumors are also exposed to oxygen- and nutrient-gradients depending on their distance from the nearest blood vessels. Hence, the cancer cells are often exposed to metabolically challenging environment where oxygen and nutrient supply is scarce. This metabolic stress induced by oxygen and nutrient-deprivation is shown to have differential effect on cell proliferation rates in different cancer cell lines. The presented work was designed to study the interplay between cell proliferation rates and metabolic stress in 2D and 3D cell culture systems. Two cell line models

(HepG2; liver cancer cell line and HCT-116; colorectal cancer cell line) were selected that show inverse proliferation trends under 2D and 3D cell culture system. In order to study the effects of metabolic stress—induced by oxygen and nutrient—both 2D and 3D cultures were exposed to different cell culture conditions.

Materials and methods

HCT-116 (Colon Adenocarcinoma Cell Line) and HepG-2 (Liver Hepatocellular Carcinoma) cell lines were purchased from the American Type Culture Collection (ATCC). Cell lines were cultivated in standard cell culture conditions according to the ATCC guidelines. For low-serum culture conditions RPMI 1640 medium was supplemented with 2%, 4% and 6% FBS. For inducing hypoxia cell culture plates were sealed with paraffin film and placed in anaerobic jar (Oxoid, HP0011) and incubated at 37 °C. Monolayers of cells (2D) were cultured in 24-wells culture plates. Multicellular tumor spheroids (MCTS) were generated by liquid overlay method (Casey *et al.*, 2001) in flat bottom 96-wells plates.

DNA was extracted using Trizol reagent according to manufacturer's guidelines. For DNA quantification absorbance was measured using Nanodrop spectrophotometer (Thermo Scientific NanoDrop Lite spectrophotometer). Protein was extracted using NP40

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Abbreviations

2D, Two dimensional; 3D, Three dimensional.

buffer and quantified by Bradford method (Merck Bradford Kit, 110306) according to manufacturer's guidelines.

For tracking of spheroid growth diameters of 3-6 spheroids were measured for each condition at alternate days (Day 3, 5, and 7). Spheroids were imaged via IRMECO IM-200 inverted light microscope and their diameters were assessed by Scope image 9.0(X5) software.

For determination of triglyceride content Lipids were extracted from cell pellets using a methanol/chloroform extraction method (De Schrijver *et al.*, 2003) and was spectrophotometrically determined using commercially available kit (Analyticon Biotechnologies AG, Catalogue # 5052) against a calibration-curve generated using known concentrations of triglyceride standard (SUPELCO, 17811-1AMP).

GraphPad Prism version 6 was used for statistical analysis.

Results and discussion

Previous studies have shown that colorectal cancer cells display decreased (Maria *et al.*, 2011), while HepG2 cells display increased proliferation rates under 3D system (Chang and Hughes-Fulford, 2008). In order to reconfirm this phenomenon both HCT-116 and HepG2 cells were cultured in 2D and 3D cell culture systems. In accordance with previous studies, HCT-116 cells display decreased proliferation rates in 3D than in 2D cell culture system (Fig. 1a). Cell proliferation rates gave similar trends at three different time-points (day 3, 5 and 7). Conversely, HepG2 cells display increased proliferation rates under 3D cell culture system (Fig. 1b).

Next, the impact of metabolic stress—induced by oxygen- and serum-deprivation—on cell proliferation rates in the selected cancer cell lines was assessed. The cell proliferation rates in these metabolically stressed conditions were compared to that in normoxic full-serum (10%) growth conditions. Interestingly, HCT-116 cells—that also displayed better proliferative capacity in 2D system—were less sensitive to metabolic stress under 2D cell culture system (Fig. 2a). However, the growth of HCT-116 tumor spheroids was significantly reduced under serum-deprived conditions but not under hypoxic conditions. HepG2 cells on the other hand were more sensitive to metabolic stress under 2D cell culture system, while the growth of HepG2 tumor spheroids was not significantly affected by serum-deprivation or hypoxia (Fig. 2b). In this study the impact of metabolic stress on diameter of HCT-116 (Fig. 2c) and HepG2 cells (Fig. 2d) was also studied. The data on spheroids diameter mostly followed the similar trends as above i.e. metabolic stress induced reduced growth of HCT-116 spheroids but not of HepG2 spheroids. This data indicated that the

proliferation capacity of the given cell lines was associated with the ability to deal with metabolic stress under 2D or 3D systems. HCT-116 coped better with metabolic stress in 2D system while HepG2 in 3D system. It has been previously reported that cancer cells cultivated under nutrient-deprivation try to up-regulate their metabolic pathways to compensate for reduced supply of nutrients (Daniels *et al.*, 2014; Noreen *et al.*, 2018). Only the cell-types capable of sufficiently compensating for limited nutrient supply—via *de novo* synthesis or activation of alternate pathways—maintain their proliferation rates under metabolic stress.

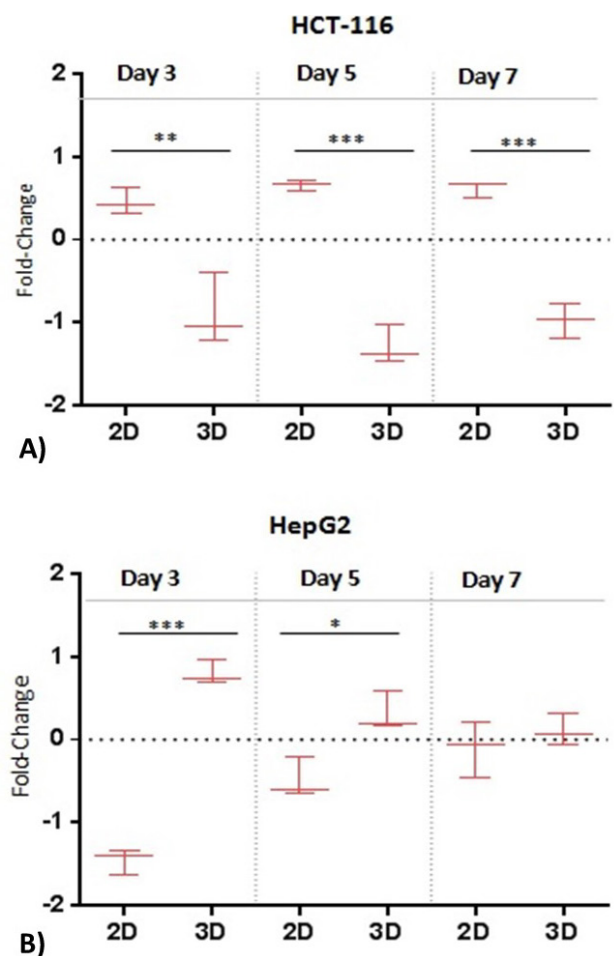


Fig. 1. Comparison of proliferation rates in 2D and 3D cell culture systems. (A) HCT-116 and (B) HepG2 cells were cultivated (seeding density; 3×10^4 cells) in 2D and 3D cell culture systems for 3, 5 or 7 days. At each time point DNA/protein quantification assays were performed to determine cell number. Data were median normalized and LOG2 transformed. Data are representative of three independent experiments. Significance was determined by unpaired t-test. *Significantly different ($p \leq 0.05$; ** $p \leq 0.01$; *** $p \leq 0.001$), n.s not significant ($p > 0.05$).

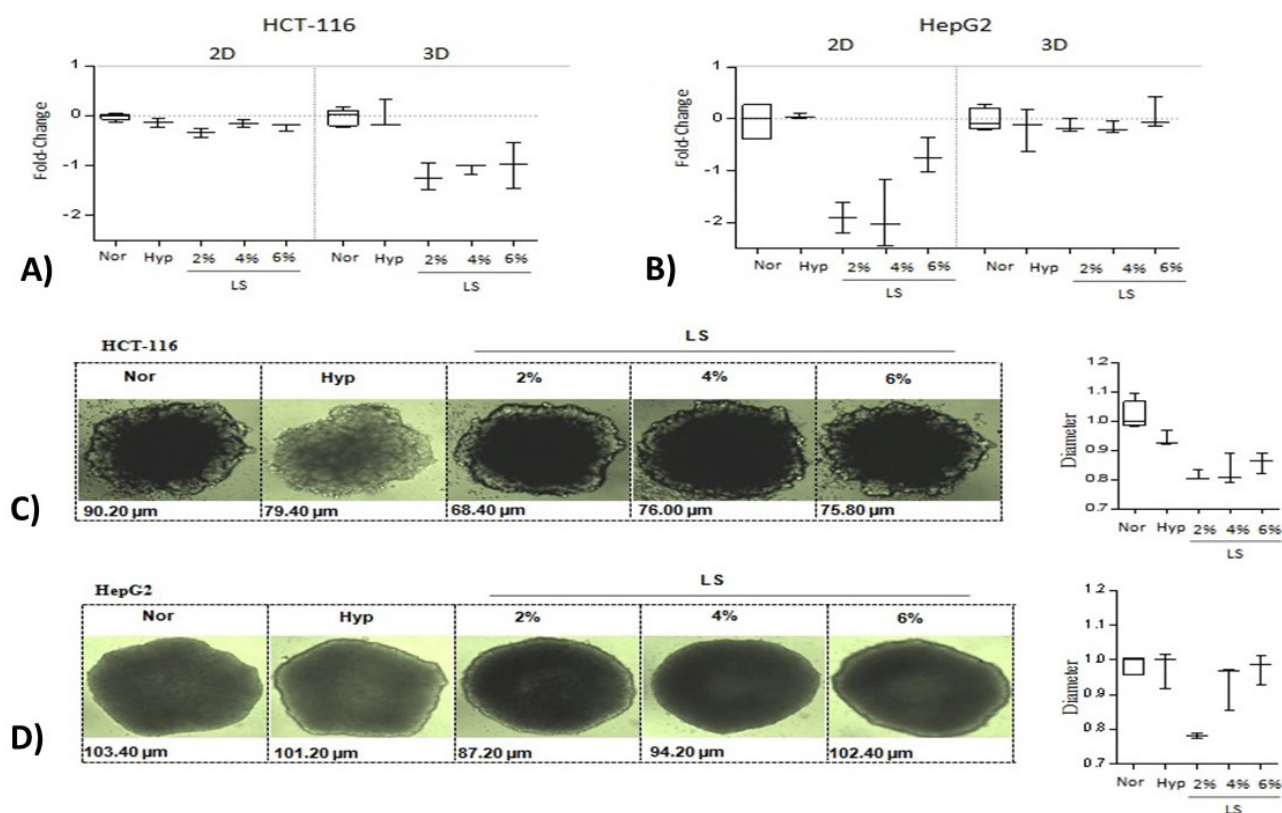


Fig. 2. Impact of metabolic stress on cell proliferation rates in 2D and 3D cell culture systems. (A) HCT-116 and (B) HepG2 cells were cultivated (seeding density; 3×10^4 cells) in 2D and 3D cell culture systems under normal growth conditions (10% FBS; 20% Oxygen), hypoxia (10% FBS; 0% Oxygen) or low-serum growth conditions (2, 4, or 6% FBS; 20% Oxygen). DNA/protein quantification assays were performed to determine cell number. Data were normalized to normal conditions and LOG2 transformed. Data are representative of three independent experiments. Representative microscopy images of (C) HCT-116 and (D) HepG2 spheroids generated in aforementioned culture conditions. The panels on the right compare diameters of the corresponding cell line spheroids. Data were normalized with median of the normal condition. (Abbreviations: Nor, normal growth conditions; Hyp, hypoxia; LS, low-serum growth conditions).

HepG2 cells are known to display high number of lipid-droplets (LD) under normoxic conditions and are frequently used as a model for studying LD biogenesis. When compared to HCT-116 cells the baseline levels of triglycerides—one of the major components of LDs—were six-fold higher in HepG2 cells (Supplementary Fig. 1a). This high lipid-load in HepG2 cells may also affect their proliferation rates in 3D cell culture system. TG-content in HepG2 cells cultivated under 3D and 2D cell culture systems was compared. HepG2 cells in 3D spheroids display significantly lower levels of TG in comparison to 2D-cultured cells (Supplementary Fig. 1b). It has been proposed that LDs support cancer-cell survival/tumor progression under nutrient-deprivation and the nutrient-deprived cancer cells mobilize lipids from intracellular stores (Baenke *et al.*, 2013). Cancer cells across a 3D spheroid have differential access to nutrients including

lipids—that are abundantly required for cancer cell proliferation and progression. Hence, nutrient-deprived cells within the core of spheroids would have been able to mobilize the stored lipids in LD causing decrease in lipid-deposits in 3D-cultured cells. HCT-116 cells—that have lower baseline TGs—displayed higher TG-content in 3D cultured cells in comparison to 2D-cultured cells (Supplementary Fig. 1c). Previous research reports have shown that cancer cells display increased lipid-load under metabolic stress conditions (Daniëls *et al.*, 2014; Kamphorst *et al.*, 2014). This increased lipid-load in HCT-116 spheroids could be attributed to increase metabolic stress in these cells under 3D system.

Conclusion

The presented work shows that cells' capability to deal with metabolic stress affects its proliferative capacity

under 2D or 3D system. Further, studies are required to understand the molecular mechanisms deriving cell proliferation rates under 2D or 3D systems.

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Supplementary material

There is supplementary material associated with this article. Access the material online at: <http://dx.doi.org/10.17582/journal.pjz/2019.51.....>

Statement of conflict of interest

The author declares that they have no conflict of interests.

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Supplementary Material

Short Communication: The Ability to Deal with Metabolic Stress Correlates with Cell Proliferation Rates under 2D or 3D Cell Culture System

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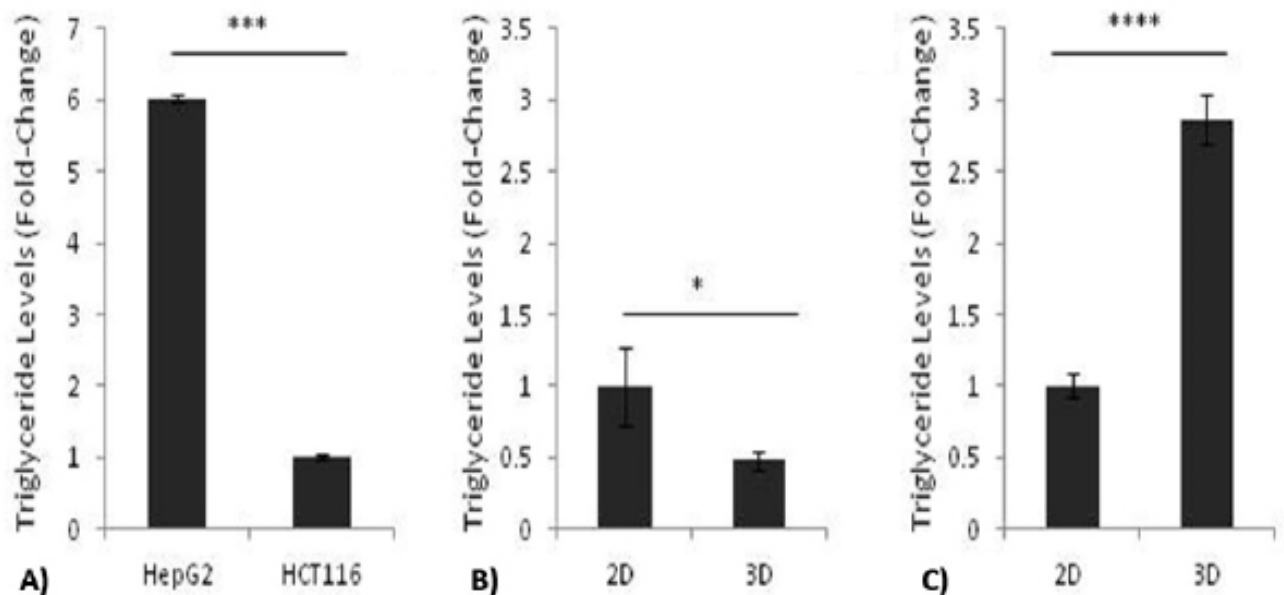
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Supplementary Fig. 1. Triglyceride levels in HepG2 and HCT-116 cells. (A) Comparison of baseline triglyceride-level between HepG2 and HCT-116 cells. Cells were cultured under normal growth conditions for 10 days. (B) Comparison of TG-levels in HepG2 cells cultivated under 2D vs. 3D cell Culture systems (C) Comparison of TG-levels in HCT-116 cells cultivated under 2D vs. 3D cell Culture systems. Data were normalized to protein content. Significance was determined by unpaired t-test. *significantly different (* $p \leq 0.05$; ** $p \leq 0.001$; *** $p \leq 0.001$), n.s not significant ($p > 0.05$).