3D tumor spheroids

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Abstract

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Introduction

PDAC belongs to the top five of cancer-related deaths in the world and the poor prognosis is primarily due to its advanced stage at diagnosis , the progress in its treatment remains too slow as a consequence of the complex physiopathology of this tumor characterized by a heterogeneous cellular composition and the accumulation of a very dense fibrotic tissue [**???**]: Due to that pancreatic cancer is a heterogeneous disease, is often modelled using established cell lines in the laboratory.

In recent years, three-dimensional (3D) culture systems have gained increasing recognition as an effective tool for biological research. One widely used 3D culturing technique is the application of multicellular spheroids (MCS).Cells cultured in 3D more closely mimic the physiological environment compared to conventional monolayer culture systems. Spheroids are three-dimensional spherical cellular aggregates with high cell-density, that more closely simulate conditions existing in solid tumors where hypoxia and alterations related to intracellular metabolism occur due to poor availability of nutrients from blood vessels [2].

Pancreatic tumor microenvironment

In Pancreatic ductal adenocarcinoma (PDA), the major components of the tumor microenvironment are a complex population of fibroblasts forming the bulk of the stroma, vasculature, inflammatory and immune cells [3]. PDA is associated with evolving alterations in the tumor microenvironment, including increasing fibrosis and extracellular matrix deposition (desmoplasia). Increasing desmoplasia accompanies progressive disease and creates intratumoral pressure that compresses the vasculature, resulting in limited blood flow to the tumor and consequent hypoxia and low nutrient delivery [4].

Interactions between the neoplastic and non-neoplastic cells and cellular matrix have been proposed to stimulate the extensive desmoplastic reaction. Stroma production is promoted by the activation of multiple cell signalling pathways [5,6] these signalling cascades lead to secretion of structural matrix components including proteoglycans, collagen and fibronectin and the activation of catalytic enzymes such as proteinases.

Matrix metalloproteinases (MMPs) are a family of zinc-dependent endopeptidases, they are involved in degradation of the extracellular matrix . MMPs support tissue remodeling and stimulate neovascularization and inflammatory response, both in physiological and in pathological conditions, for example, in tumors [7].

MMP-2, MMP-7, and MMP-9 expressions correlate with various morphological features of the PDAC tumor such as inflammation, necrosis, and formation of the new blood vessels [8] and their tissue inhibitors (TIMPs) TIMPs 1–3 compared with normal pancreas [9].

Pancreatic stellate cells (PSC) are responsible for desmoplasic generation [10]. The activation of PSC from a quiescent to an activated state is an intercellular stimuli from tumor-stromal interactions. PCS can be transform into myofibroblast-like cells, which express α-smooth muscle actin (α-SMA) as cancer-associated fibroblasts (CAF) [11,12].

CAF are important components of tumor stroma and affect cancer growth, survival, metastasis, angiogenesis and resistance to chemotherapy or radiotherapy through various cytokines 13, and contribute to a diminished immune function 14.

2D and 3D cell culture

Cell culture is a widely used *in vitro* tool to improve the understanding of cell biology, cellular mechanisms, tissue morphology, drug action, protein production and the development of tissue engineering 15.

Three-dimensional (3D) cell culture is well documented to regain intrinsic metabolic properties and to better mimic the in vivo situation than two-dimensional (2D) cell culture 16.

A comparative molecular analyses from 2D and 3D *in vitro* conditions revealed that cancer cell phenotype is highly influenced by its microenvironment.Through the examination of cancer cell transcriptional behavior, cellular processes were closely linked to functions promoted by different culturing conditions. Cancer cells grown in monolayers favored rapid proliferation, and this behavior was corroborated by up-regulation of cell cycle progression genes in addition to metabolic processes that synthesize DNA, RNA, and proteins. Unlike cancer cells cultured in monolayers, cancer cells in 3D down-regulate proliferative processes, while up-regulating genes involved in ECM organization and cell adhesion 17.

When performing 3D cell culture experiments, the cell environment can be manipulated to mimic that of a cell in vivo and provide more accurate data about cell-to-cell interactions, tumor characteristics, drug discovery, metabolic profiling, stem cell research, and other types of diseases 18.

Alterations in cancer cell behavior under different growth conditions underlie the importance of defining culturing conditions that preserve endogenous tumor behavior. While monolayer cultures promote the most non-native behavior of cancer cells, this model still maintains value due to its ease and scalability towards applications targeting cancer-growth driving pathways. However, analysis and discovery of potential therapeutics targeting stromal interactions, ECM development, or cell signaling may yield erroneous results in this system.The 3D culturing and inclusion of stromal cell types do show increased similarity to in vivo cancer behavior. However, improvements upon ex vivo culture conditions that allow all stromal components to persist will greatly enhance our ability to conduct pre-clinical screens that may more closely recapitulate the biological responses of patients 17.

The structure of multicellular spheroids.

MCSs are cell aggregates with complex cell-to-cell adhesions and cell-to-matrix interactions, which results in gradient generation for nutrients, gases, growth factors and signal factors 19.

It has been reported that MCS formation involves three critical steps. In the first stage, dispersed single-cells are drawn closer to form aggregations where ECM fibers act as a long-chain linker through the binding of integrins. This is followed by a period in which cell aggregates pause in compaction, because of the accumulation of sufficient amounts of E-cadherins. Finally, in the third stage, cells are compacted into solid aggregates to form MCSs due to the homophilic cadherin–cadherin binding 20.

The formation of MCS process initiates with an spontaneous self-assembly and cell-cell interactions. These interactions are regulated by adhesions proteins such as E-cadherin, α-catenin and P-cadherin [21,22] Cell-to-matrix interactions is the base for cell building and is influenced by integrins mainly by β1-integrin. The integration of integrin-ECM facilitates the cell aggregation process that connect a cell with its environment in the context of spheroid formation [**???**,20,20].

The cytoskeleton also plays an important role in spheroid formation. During spheroid formation the actin filaments undergo significant changes and the expanded microfilaments as stress fibres become along the cell periphery. In this step, the cytoskeleton is a force generation structure performing a continuos pre-stressed lattice that keeps structural stability [23; [**???**](200103)48:3<175::AID-CM1007>3.0.CO;2-2].

The internal structure of spheroids comprises different cell layers. MCS include hypoxic, proliferative apoptotic/necrotic areas as a consequence of oxygen and nutrient gradients [**???**]. Small micro-spheroids of <200µm diameter mostly include proliferating and normoxic cells, mimic three-dimensional cell-cell and cell-matrix interactions but they are inappropriate to reflect pathophysiological conditions with hypoxic areas in the spheroid center or to mimic proliferation gradients 24. However, spheroids with diameters of approximately 200-300µm results in a typical zonation, with proliferative zones at the surface co-existing with normoxic quiescent zones in the middle and hypoxic zones in the core 25. Finally, MCS > 500 µm diameter the formation of necrotic areas is observed as described in microregion of tumor *in vivo* 26.

Due to these characteristics, MCS mimic the first avascular stages of tumor formation, and exhibit important tumor aggressiveness features such as enhanced multicellular resistance, migration, and invasion, as well as an enhanced clonogenic capacity 27.

Methods for the generation of MCS

MCS and PDA

Due to that pancreatic cancer is a heterogeneous disease researchers have been developed models capable to reproduce *in vitro* the heterogeneous tumor microenvironment.

Several methods to create 3D tumors *in vitro* have been proposed, with hanging drop technique being the simplest and most frequently used. However, in many cell lines this method has failed to form the desired 3D tumor structures.

A modified hanging drop method for 3D spheroid formation facilitated with methylcellulose yield a straightforward production of spheroids in PDAC cells and form well-rounded spheroids after 5 days in hanging drops. These spheroidshave have high tolerance to mechanical force, thus enabling standard manipulations and display some hallmarks of solid tumors, such as hypoxic zone, proliferating cells, and apoptotic regions 28.

3D pancreatic cancer spheroids, based on pancreatic cancer cells and fibroblast co-culture demonstrate innate desmoplastic properties and stay poorly permeable with relevant diffusion barrier function. Spheroid-based xenografts produced different extracellular matrix (ECM) components with uniformity in terms of ECM architecture recapitulating clinical PDAC tumors. Moreover, establishment of tumors by transplantation of spheroids demonstrate higher expression of pro-fibrotic and pro-survival PDAC hallmarks 29.

In the last years the characterization of a novel 3D tumor model has been in full swing. A triple co-culture of pancreatic cancer cells (PANC-1), fibroblasts (MRC-5) and endothelial cells (HUVEC) to form a heterotype multicellular tumor spheroid (MCTS). The integration of the three cell types enable the presence of a core rich in fibroblasts and fibronectin in which endothelial cells are homogeneously distributed 30.

A microchannel model allow to develop a 3D pancreatic tumor in *vitro* by co-culturing pancreatic tumor spheroids with PSC in a collagen matrix. Under these conditions spheroids and PSCs are

mutually activated when co-cultured. Under co-culture condition, tumor spheroids acquire a migratory phenotype with cancer cell-cell interactions, cell-ECM interactions, and cancer cell-PSC interactions [31].

References

1. **Open collaborative writing with Manubot**
   Daniel S. Himmelstein, Vincent Rubinetti, David R. Slochower, Dongbo Hu, Venkat S. Malladi, Casey S. Greene, Anthony Gitter
   PLOS Computational Biology (2019-06-24) https://doi.org/c7np
   DOI: 10.1371/journal.pcbi.1007128 · PMID: 31233491 · PMCID: PMC6611653

2. **Cancer cell spheroids are a better screen for the photodynamic efficiency of glycosylated photosensitizers**
   Patrícia M. R. Pereira, Naxhije Berisha, N. V. S. Dinesh K. Bhupathiraju, Rosa Fernandes, João P. C. Tomé, Charles Michael Drain
   PLOS ONE (2017-05-17) https://doi.org/f986w7
   DOI: 10.1371/journal.pone.0177737 · PMID: 28545086 · PMCID: PMC5435229

3. **Stromal biology of pancreatic cancer**
   Gerald C. Chu, Alec C. Kimmelman, Aram F. Hezel, Ronald A. DePinho
   Journal of Cellular Biochemistry (2007-07-01) https://doi.org/b8w83q
   DOI: 10.1002/jcb.21209 · PMID: 17266048

4. **Pancreatic Cancer Metabolism: Breaking It Down to Build It Back Up**
   R. M. Perera, N. Bardeesy
   Cancer Discovery (2015-11-03) https://doi.org/ggt22b
   DOI: 10.1158/2159-8290.cd-15-0671 · PMID: 26534901 · PMCID: PMC4687899

5. **Tumor–stromal cell interaction under hypoxia increases the invasiveness of pancreatic cancer cells through the hepatocyte growth factor/c-Met pathway**
   Takao Ide, Yoshihiko Kitajima, Atsushi Miyoshi, Takao Ohtsuka, Mayumi Mitsuno, Kazuma Ohtaka, Yasuo Koga, Kohji Miyazaki
   International Journal of Cancer (2006-12-15) https://doi.org/bcmh42
   DOI: 10.1002/ijc.22178 · PMID: 16998831

6. **Aberrant signaling pathways in pancreatic cancer: A two compartment view**
   Angela L. McCleary-Wheeler, Robert McWilliams, Martin E. Fernandez-Zapico
   Molecular Carcinogenesis (2012-01) https://doi.org/dhx9xm
   DOI: 10.1002/mc.20827 · PMID: 22162229 · PMCID: PMC3253704

7. **ALTERED MATRIX METALLOPROTEINASE EXPRESSION ASSOCIATED WITH ONCOGENE-MEDIATED CELLULAR TRANSFORMATION AND METASTASIS FORMATION**
   R Baruch
   Cell Biology International (2001-05) https://doi.org/fnvccv
   DOI: 10.1006/cbir.2000.0647 · PMID: 11401328

8. **Expressions of Matrix Metalloproteinases 2, 7, and 9 in Carcinogenesis of Pancreatic Ductal Adenocarcinoma**
   Katarzyna Jakubowska, Anna Pryczynicz, Joanna Januszewska, Iwona Sidorkiewicz, Andrzej Kemona, Andrzej Niewiński, Łukasz Lewczuk, Bogusław Kędra, Katarzyna Guzińska-Ustymowicz
   Disease Markers (2016) https://doi.org/f9hdkw
   DOI: 10.1155/2016/9895721 · PMID: 27429508 · PMCID: PMC4939209

9. **Comprehensive Analysis of Matrix Metalloproteinase and Tissue Inhibitor Expression in Pancreatic Cancer: Increased Expression of Matrix Metalloproteinase-7 Predicts Poor**

Survival
L. E. Jones
Clinical Cancer Research (2004-04-15) https://doi.org/c7sfqg
DOI: 10.1158/1078-0432.ccr-1157-03 · PMID: 15102692

10. **Conophylline suppresses pancreatic cancer desmoplasia and cancer-promoting cytokines produced by cancer-associated fibroblasts**
Norihiro Ishii, Kenichiro Araki, Takehiko Yokobori, Kei Hagiwara, Dorgormaa Gantumur, Takahiro Yamanaka, Tadashi Handa, Mariko Tsukagoshi, Takamichi Igarashi, Akira Watanabe, … Ken Shirabe
Cancer Science (2018-12-13) https://doi.org/ggt57m
DOI: 10.1111/cas.13847 · PMID: 30353606 · PMCID: PMC6317962

11. **A Starring Role for Stellate Cells in the Pancreatic Cancer Microenvironment**
Minoti V. Apte, Jeremy S. Wilson, Aurelia Lugea, Stephen J. Pandol
Gastroenterology (2013-05) https://doi.org/f2j2rr
DOI: 10.1053/j.gastro.2012.11.037 · PMID: 23622130 · PMCID: PMC3729446

12. **CD10+ Pancreatic Stellate Cells Enhance the Progression of Pancreatic Cancer**
Naoki Ikenaga, Kenoki Ohuchida, Kazuhiro Mizumoto, Lin Cui, Tadashi Kayashima, Katsuya Morimatsu, Taiki Moriyama, Kohei Nakata, Hayato Fujita, Masao Tanaka
Gastroenterology (2010-09) https://doi.org/cps2bn
DOI: 10.1053/j.gastro.2010.05.084 · PMID: 20685603

13. **Cancer-associated fibroblasts in pancreatic adenocarcinoma**
Boju Pan, Quan Liao, Zheyu Niu, Li Zhou, Yupei Zhao
Future Oncology (2015-09) https://doi.org/f7sxbh
DOI: 10.2217/fon.15.176 · PMID: 26284509

14. **Human Pancreatic Carcinoma-Associated Fibroblasts Promote Expression of Co-inhibitory Markers on CD4+ and CD8+ T-Cells**
Laia Gorchs, Carlos Fernández Moro, Peter Bankhead, Katharina P. Kern, Imrul Sadeak, Qingda Meng, Elena Rangelova, Helen Kaipe
Frontiers in Immunology (2019-04-24) https://doi.org/ggt57p
DOI: 10.3389/fimmu.2019.00847 · PMID: 31068935 · PMCID: PMC6491453

15. **2D and 3D cell cultures – a comparison of different types of cancer cell cultures**
Marta Kapałczyńska, Tomasz Kolenda, Weronika Przybyła, Maria Zajączkowska, Anna Teresiak, Violetta Filas, Matthew Ibbs, Renata Bliźniak, Łukasz Łuczewski, Katarzyna Lamperska
Archives of Medical Science (2016) https://doi.org/ggt3hn
DOI: 10.5114/aoms.2016.63743 · PMID: 30002710 · PMCID: PMC6040128

16. **Oncogenic human herpesvirus hijacks proline metabolism for tumorigenesis**
Un Yung Choi, Jae Jin Lee, Angela Park, Wei Zhu, Hye-Ra Lee, Youn Jung Choi, Ji-Seung Yoo, Claire Yu, Pinghui Feng, Shou-Jiang Gao, … Jae U. Jung
Proceedings of the National Academy of Sciences (2020-04-07) https://doi.org/ggt4fr
DOI: 10.1073/pnas.1918607117 · PMID: 32213586 · PMCID: PMC7149499

17. **Comparative Molecular Analysis of Cancer Behavior Cultured In Vitro, In Vivo, and Ex Vivo**
Nicholas R. Hum, Aimy Sebastian, Sean F. Gilmore, Wei He, Kelly A. Martin, Aubree Hinckley, Karen R. Dubbin, Monica L. Moya, Elizabeth K. Wheeler, Matthew A. Coleman, Gabriela G. Loots
Cancers (2020-03-14) https://doi.org/ggt4ft
DOI: 10.3390/cancers12030690 · PMID: 32183351 · PMCID: PMC7140030

18. **Is It Time to Start Transitioning From 2D to 3D Cell Culture?**
Caleb Jensen, Yong Teng
Frontiers in Molecular Biosciences (2020-03-06) https://doi.org/ggt4fs
DOI: 10.3389/fmolb.2020.00033 · PMID: 32211418 · PMCID: PMC7067892

19. **Advances in multicellular spheroids formation**
X. Cui, Y. Hartanto, H. Zhang
Journal of The Royal Society Interface (2017-02) https://doi.org/ggt8pm
DOI: 10.1098/rsif.2016.0877 · PMID: 28202590 · PMCID: PMC5332573

20. **Dynamic analysis of hepatoma spheroid formation: roles of E-cadherin and β1-integrin**
Ruei-Zeng Lin, Li-Fang Chou, Chi-Chen Michael Chien, Hwan-You Chang
Cell and Tissue Research (2006-02-18) https://doi.org/dx2zbb
DOI: 10.1007/s00441-005-0148-2 · PMID: 16489443

21. **E-cadherin, actin, microtubules and FAK dominate different spheroid formation phases and important elements of tissue integrity**
I. Smyrek, B. Mathew, S. C. Fischer, S. M. Lissek, S. Becker, E. H. K. Stelzer
Biology Open (2019-01-15) https://doi.org/ggt8pn
DOI: 10.1242/bio.037051 · PMID: 30578251 · PMCID: PMC6361217

22. **Diversity of cell-mediated adhesions in breast cancer spheroids**
Andrea Ivascu, Manfred Kubbies
International Journal of Oncology (2007-12-01) https://doi.org/gf8czq
DOI: 10.3892/ijo.31.6.1403

23. **Rapid Enhancement of Cellular Spheroid Assembly by Acoustically Driven Microcentrifugation**
Layla Alhasan, Aisha Qi, Aswan Al-Abboodi, Amgad Rezk, Peggy P. Y. Chan, Ciprian Iliescu, Leslie Y. Yeo
ACS Biomaterials Science & Engineering (2016-05-18) https://doi.org/ggt8pj
DOI: 10.1021/acsbiomaterials.6b00144

24. **Formation of size-controllable tumour spheroids using a microfluidic pillar array (μFPA) device**
Wanyoung Lim, Hong-Hoa Hoang, Daeun You, Jeonghun Han, Jeong Eon Lee, Sangmin Kim, Sungsu Park
The Analyst (2018) https://doi.org/gfnsnm
DOI: 10.1039/c8an01752b · PMID: 30379148

25. **3D tumor spheroid models for in vitro therapeutic screening: a systematic approach to enhance the biological relevance of data obtained**
Michele Zanoni, Filippo Piccinini, Chiara Arienti, Alice Zamagni, Spartaco Santi, Rolando Polico, Alessandro Bevilacqua, Anna Tesei
Scientific Reports (2016-01-11) https://doi.org/ggt8pk
DOI: 10.1038/srep19103 · PMID: 26752500 · PMCID: PMC4707510

26. **Spherical Cancer Models in Tumor Biology**
Louis-Bastien Weiswald, Dominique Bellet, Virginie Dangles-Marie
Neoplasia (2015-01) https://doi.org/f6xf5r
DOI: 10.1016/j.neo.2014.12.004 · PMID: 25622895 · PMCID: PMC4309685

27. **Comparison of 2D- and 3D-culture models as drug-testing platforms in breast cancer**
YOSHINORI IMAMURA, TORU MUKOHARA, YOHEI SHIMONO, YOHEI FUNAKOSHI, NAOKO CHAYAHARA, MASANORI TOYODA, NAOMI KIYOTA, SHINTARO TAKAO, SEISHI KONO, TETSUYA NAKATSURA, HIRONOBU MINAMI
Oncology Reports (2015-04) https://doi.org/ggt8pp
DOI: 10.3892/or.2015.3767 · PMID: 25634491

28. **Generation of Homogenous Three-Dimensional Pancreatic Cancer Cell Spheroids Using an Improved Hanging Drop Technique**
Matthew J. Ware, Kevin Colbert, Vazrik Keshishian, Jason Ho, Stuart J. Corr, Steven A. Curley, Biana Godin
Tissue Engineering Part C: Methods (2016-04) https://doi.org/f8f74k
DOI: 10.1089/ten.tec.2015.0280 · PMID: 26830354 · PMCID: PMC4827286

29. **Subcutaneous Inoculation of 3D Pancreatic Cancer Spheroids Results in Development of Reproducible Stroma-Rich Tumors**
Mikhail Durymanov, Christian Kroll, Anastasia Permyakova, Elizabeth O'Neill, Raed Sulaiman, Michael Person, Joshua Reineke
Translational Oncology (2019-01) https://doi.org/ggt6j8
DOI: 10.1016/j.tranon.2018.10.003 · PMID: 30554606 · PMCID: PMC6295361

30. **Multicellular spheroid based on a triple co-culture: A novel 3D model to mimic pancreatic tumor complexity**
Gianpiero Lazzari, Valérie Nicolas, Michiya Matsusaki, Mitsuru Akashi, Patrick Couvreur, Simona Mura
Acta Biomaterialia (2018-09) https://doi.org/ggt6j7
DOI: 10.1016/j.actbio.2018.08.008 · PMID: 30099198

31. **Microfluidic co-culture of pancreatic tumor spheroids with stellate cells as a novel 3D model for investigation of stroma-mediated cell motility and drug resistance**
Ji-Hyun Lee, Seul-Ki Kim, Iftikhar Ali Khawar, Su-Yeong Jeong, Seok Chung, Hyo-Jeong Kuh
Journal of Experimental & Clinical Cancer Research (2018-01-12) https://doi.org/ggt6j9
DOI: 10.1186/s13046-017-0654-6 · PMID: 29329547 · PMCID: PMC5767067