Tumoroid

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The term “tumoroid” means “tumor-like organoid”: tumoroids typically derive from primary tumors harvested from oncological patients and they can mimic human tumor microenvironment (TME); nowadays, they are considered a promising tool for cost-effective studies on novel anticancer drugs to be used in precision medicine in the field of oncology.

Keywords: translational medicine ; scaffolds ; tissue engineering ; oncology ; organoids ; cell model ; stem cells

1. Introduction

Tumoroids are typically clustered in 3D structures, totally grown in vitro: these structures can organize themselves into 3D organoids similar to the primary tissue where cells have been harvested[1]. Tumoroids can mimic human tumor microenvironment (TME); nowadays, they are considered a promising tool for cost-effective studies on novel anticancer drugs to be used in precision medicine[2].

Over the years, several promising techniques have been used to fabricate 3D scaffolds for cell culture. A well-known strategy was to put cells in “spinner flasks” under continuous stirring: this strategy has allowed the creation of spherical clusters of cells, growing drop-by-drop; however, this technique required huge quantities of culture medium and the availability of specific equipment.

Alternative techniques, called 3D micro-molds, were also investigated to overcome the high culture medium consumption highlighted in the previous method: these techniques were able to produce clusters with different shapes, not only spheroids[3].

2. Tumoroids in Cancer Research

A deep knowledge of the local microenvironment in cancer onset may lead to controlling the fate of cancer cells. Breast cancer, for example, develops into a highly structured ECM, creating complex interactions among cells and ECM through direct contact and signaling molecules[4]. The breakdown of the ECM may create the proper conditions to stimulate specific factors supporting the onset of cancer[2]. To better investigate this aspect, tumoroids have been created using breast cancer cells and ECM: these compounds were able to induce a massive differentiation and growth of stem cells obtained from other organs after their injection into the mammary glands of mice[5]. Tumoroids have been used to understand if the healthy mammary microenvironment was able to induce a physiological behavior in breast cancer cells, and the response of cancer cells to new drugs[6].

In another study, tumoroids deriving from primary and metastatic colorectal adenocarcinoma were used to study how K-RAS mutation (Kirsten rat sarcoma mutation) affects colorectal adenocarcinoma growth. The K-RAS protein contributes to the transmission of growth signals in the nucleus of cells, leading to an increased cell growth[7]. The K-RAS pathway is amplified in colorectal cancer: in fact, abnormal K-RAS mutations can induce the hyperproliferation of the epithelium, resulting in the development of infiltrating adenocarcinomas[8].

In vitro investigations into tumoroids have clearly demonstrated that cancer cells cultured on 3D scaffolds derived from autologous ECM are fully able to reproduce the same microenvironment developed in vivo. Of course, the availability of reliable and complex cancer models working in safe and controlled experimental conditions has created the right conditions to test the effectiveness of novel chemotherapies, anticipating their side effects, and the activation of harmful immune reactions[7]. (Table 1)

Recently, the analysis of tumoroids from patients affected by colorectal cancer (CRC) showed a common molecular pattern in all the samples investigated; moreover, the authors reported that tumoroids were also responding to chemotherapy in the same way observed in the cancers treated in vivo. Small samples of human-derived tumoroids have

been injected into murine models: after a brief engraftment time, tumoroids developed invasive and aggressive colorectal cancers, with metastases in the lungs and liver. These tumoroids engrafted on murine colon mucosa were treated with novel drugs, showing therapeutic effects completely comparable with those reported on humans[9] (Table 1).

Scientists have used tumoroids-based models to perform in-depth investigation into such pathways involving the immune system cells in cancer progression: this strategy allowed assessment of the impact of different chemotherapies on the immune reply, to calibrate the dose-effect percentage on aggressive cancers[10].

Tumoroids have also gained an important role in the study of brain tumors. An in vitro 3D study-model called neoplastic brain organoid (NeoCOR) was performed on this topic: the invasiveness of tumor and the effects of drugs were investigated on cells treated with CRISPR-Cas9 to carry specific mutations. The NeoCOR model achieved the reproduction all the tumor environments related to brain cancers[11].

Researchers have successfully generated “mini-tumoroids” (up to 0.5 mm), to study liver cancers. Thus far, 29 new drugs were tested on liver-cancer mini-tumoroids: a new drug was found to inhibit the activation of the ERK protein, gaining interest for future liver cancer therapies[12]. Following this in vitro study, mini-tumoroids were injected into mice livers, then treated with the newly discovered drug: the in vivo results showed a significant reduction in tumor growth in those mice treated with the new drug, thus confirming the reliability of the in vitro preliminary tests achieved with tumoroids[12]. (Table 1) This reliability has been further confirmed in other studies, such as a clinical trial conducted on oncological patients treated with radiation therapy and with chemotherapy for colorectal cancer: here tumoroids were able to reproduce in vitro the same biological effects reported on cells/tissues of patients[13].

Tumoroids have been strategic to disclosing the interactions among cancer cells, autologous mesenchymal stem cells (MSCs,) and local ECM[14]. Nevertheless, a recent study on pancreatic cancer, has also investigated induced pluripotent stem cells (iPSCs): briefly, the combination of stem cells and iPSCs was able to perfectly reproduce functional pancreatic cells within their stroma, and these cells were used to study their interactions with cancer cells[15].

Stem cells isolated from cancer tissues can themselves differentiate into tumor-like tissues; however, the behavior of such cells seems to be influenced by the local environment. In a study conducted in 2018, researchers investigated the behavior of stem cells from cancer tissues grown in two different experimental conditions: on standard tissue culture plates (TCPs) and NanoCulture Plates (NCPs); stem cells grown on NCPs created tumoroids with tighter intercellular adhesions, and they also showed morphologies and superficial markers exactly reproducing the characteristics of primary tumors. On the contrary, stem cells grown on TCPs expressed genes associated with cell differentiation. The authors also found that stem cells grown on NCPs were able to release exosomes containing epithelial cell adhesion molecules (EpCAM) and heat shock protein 90 (HSP90) that have been closely related to an increased growth of cancers[16].

Table 1. Tumoroids: in vitro and in vivo studies.

Study model	Type of cancer	Therapeutic approach	Main Results	References
Engelbreth-Holm-Swarm mouse sarcoma cells	Breast cancer	Chemotherapy	Tumoroids had similar morphology and gene expression of patients with breast cancer	Lee et al. (2007)
Cells of colorectal adenocarcinoma	Metastatic colorectal adenocarcinoma	Chemotherapy	KRAS protein increases cell growth and mitotic activity.	Mousavi et al. (2019)
Human tumoroids were injected into the murine mucosa	Rectal cancer	Chemotherapy	The grafted tumoroids showed equal sensitivity to therapies administered in patients	Ganesh et al. (2019)

Cells of colorectal carcinoma	Colorectal carcinoma	Chemotherapy	3D-tumoroids represent a valid in vitro approach to validate new drug therapies	Finnberg et al. (2017)
3D model called neoplastic brain organoid	Brain tumors	Chemotherapy	NeoCOR showed better results as a 3D model for clinical studies on brain tumors	Shane t al. (2018)
Cancer cells in different 3D tumoroids	Human Primary Liver Cancer	Chemotherapy	SCH772984 inhibited the activation of the ERK protein and demonstrated to play a crucial role in the tumorigenesis	Broutier et al. (2017)
Cultured tumoroids in mice	Human Primary Liver Cancer	Chemotherapy	SCH772984 reduced tumor growth in mice treated with this drug	Broutier et al. (2017)
Cultured tumoroids	Colorectal and liver cancer	Chemotherapy	Organoids maintain the biological characteristics of their original tumor	Jansen et al. (2019)
ADSCs in tumoroid environment	Different cancers	Chemotherapy	The ADSCs maintain their genetic stability and retain all the physiological characteristics of their original tissue.	Huch et al. (2015)
Tumoroids with ESCs and iPSCs in mice models	Pancreatic Tumor	Chemotherapy	ESC and iPSC tumoroids generated functional pancreatic cells	Hohwieler et al. (2019)
Different cell lines in different tumoroids	Different cancers	Chemotherapy	3D tumoroids were able to release tumor-related exosomes	Takanori Eguchi et al. (2018)
Oral Mucosal Organoids	Oral squamous cell carcinoma	Chemotherapy	Oral cancers tumoroids are a smart platform for personalized therapy	Driehuis et al. (2019)
Cell Carcinoma Organoids	Head and Neck Squamous Cell Carcinoma	Chemotherapy	Tumoroids can reproduce both genetic and molecular characteristics of the primary tumors	Hill et al. (2019)

31 lines of tumoroids derived from squamous cell carcinoma of the head and neck (HNSCC)	Squamous cell carcinoma of the head and neck (HNSCC)	Chemotherapy	Tumoroids improve studies on personalized approaches to HNSCC	Driehuis et al. (2019)
Organoids from healthy tissue and tumor tissue	Squamous cell carcinoma of the head and neck (HNSCC)	Photodynamic therapy	Photodynamic therapy can influence epidermal growth factor (EGFR) and tumor growth	Driehuis et al. (2019)
Organoids from tumor tissue	Esophageal carcinoma, Squamous cell carcinoma of the head and neck (HNSCC)	Chemotherapy	Tumoroids were used to test drugs against the onset of HNSCC by suppression of IL-6	Karakasheva et al. (2018)
Metastatic cells of metastatic colorectal and gastro-esophageal carcinoma	Colorectal and gastro-esophageal carcinoma	Chemotherapy	3D tumoroids were used to test sensitivity and specificity to different drugs	Vlachogiannis et al. (2018)

3. Features of 3D Tumoroids

The organoid is an in vitro 3D cell cluster deriving from stem cells or progenitor organs that behave similarly to the counterpart in vivo both in morphology and in functionality[17]. The main feature of 3D tumoroids is the ability to self-organize and segregate cells to form structures with histogenic properties similar to those in vivo, which is called self-organization[18].

The cultivation of tumoroids in clinical research allows the possibility of cultivating several different cell types belonging to the same organ together. The possibility of directly cultivating different cells allows the researcher to investigate the characteristics of an in vivo system, thus referring to all the cells that compose it and being able to analyze their interactions accordingly[17].

Physiologically the neoplastic cells within a tissue are organized in a complex three-dimensional network, stabilized by nutrient gradients and signal transduction mechanisms determined by cell–cell and cell–extracellular matrix–cell contact. The models generated thanks to the three-dimensional culture systems can satisfy the aforementioned requirements and can therefore be used as preclinical tumor models[19]. Multicellular spheroids can contain an extensive extracellular matrix that determines a network of connections not only cell–cell but also cell–matrix responsible for the penetration and action of drugs. The particular in vitro interaction of the 3D model also affects the distribution and function of biological effectors, such as hormones and growth factors, which regulate the mechanisms of growth, differentiation, and cellular death. These models more accurately reflect the characteristics in vivo not only at a biochemical and mechanical level, but also at the level of gene and protein expression[20].

Three-dimensional systems engineered through nanoimprinted scaffolds, according to Yukie Yoshii et al., in a work published in early 2011, have better reproduced the native tumor microenvironment favoring growth, migration, and intercellular adhesion. The formation of the spheroids starting from the sowing of the MSCs on the scaffolds has also preserved and optimized cell proliferation and viability. The same authors of the experimental study also concluded by stressing how these models are useful for delineating the biological mechanisms that regulate the pathological anomalies observed in cancer. Nanoimprinted scaffolds are used as 3D culture models to facilitate spontaneous tumor cell migration and well-regulated spheroid formation[21]. Furthermore, these 3D tumoroids are characterized by the absence of innervation and vascularization, their three-sheet structure allows for long cellular maintenance, high genetic stability, and possible cryopreservation[22].

The latest studies on tumoroids confirm their use in research on the toxicity of nanomaterials. Through the use of 3D structures, the researchers would seem to have designed the nanoparticles for the specific targeting of tumors avoiding systemic toxicity, the degradation of the NPs within the body, and the accumulation in the internal organs without affecting

the biological processes. In particular, ex vivo organoid models can be used in the early stages of the development of nanopharmaceuticals[23][24].

References

1. Susan Tsai; Laura McOlash; Katie A. Palen; Bryon D. Johnson; Christine Duris; Qiuhui Yang; Michael B. Dwinell; Bryan Hunt; Douglas B. Evans; Jill A. Gershan; et al. Development of primary human pancreatic cancer organoids, matched s tromal and immune cells and 3D tumor microenvironment models. *BMC Cancer* **2018**, *18*, 335, 10.1186/s12885-018-42 38-4.
2. Michael A. Phelan; Peter I. Lelkes; Anand Swaroop; Mini and customized low-cost bioreactors for optimized high-throug hput generation of tissue organoids.. *Stem Cell Investigation* **2018**, *5*, 33-33, 10.21037/sci.2018.09.06.
3. Georgios Vlachogiannis; Somaieh Hedayat; Alexandra Vatsiou; Yann Jamin; Javier Fernández-Mateos; Khurum Khan; Andrea Lampis; Katherine Eason; Ian Huntingford; Rosemary Burke; et al. Patient-derived organoids model treatment r esponse of metastatic gastrointestinal cancers. *Science* **2018**, *359*, 920-926, 10.1126/science.aao2774.
4. Toshio Takahashi; Organoids for Drug Discovery and Personalized Medicine. *Annual Review of Pharmacology and Toxi cology* **2019**, *59*, 447-462, 10.1146/annurev-pharmtox-010818-021108.
5. Donglai Lv; Zongtao Hu; Lin Lu; Husheng Lu; Xiuli Xu; Three-dimensional cell culture: A powerful tool in tumor research and drug discovery. *Oncology Letters* **2017**, *14*, 6999-7010, 10.3892/ol.2017.7134.
6. Robert D. Bruno; Jodie M. Fleming; Andrea L. George; Corinne A. Boulanger; Pepper J. Schedin; Gilbert H. Smith; Ma mmary extracellular matrix directs differentiation of testicular and embryonic stem cells to form functional mammary gla nds in vivo. *Scientific Reports* **2017**, *7*, 40196, 10.1038/srep40196.
7. Nabi Mousavi; Sarah Line Bring Truelsen; Grith Hagel; Lars N. Jørgensen; Henrik Harling; Vera Timmermans; Linea Ce cilie Melchior; Anna Hammerich Thysen; Steffen Heegaard; Jacob Thastrup; et al. KRAS mutations in the parental tum our accelerate in vitro growth of tumoroids established from colorectal adenocarcinoma. *International Journal of Experi mental Pathology* **2019**, *100*, 12-18, 10.1111/iep.12308.
8. Manuel Collado; Jesus Gil; Alejo Efeyan; Carmen Guerra; Alberto J. Schuhmacher; Marta Barradas; Alberto Benguria; Ángel Zaballos; Juana M. Flores; Mariano Barbacid; et al. Senescence in premalignant tumours. *Nature* **2005**, *436*, 64 2-642, 10.1038/436642a.
9. Karuna Ganesh; Chao Wu; Kevin P. O'Rourke; Bryan C. Szeglin; Youyun Zheng; Charles-Etienne Gabriel Sauvé; Moha mmad Adileh; Isaac Wasserman; Michael R. Marco; Amanda S. Kim; et al. A rectal cancer organoid platform to study in dividual responses to chemoradiation. *Nature Medicine* **2019**, *25*, 1607-1614, 10.1038/s41591-019-0584-2.
10. Niklas K. Finnberg; Prashanth Gokare; Avital Lev; Sergei I. Grivennikov; Alexander W. MacFarlane IV; Kerry S. Campb ell; Ryan M. Winters; Karen Kaputa; Jeffrey M. Farma; Abbas El-Sayed Abbas; et al. Application of 3D tumoroid system s to define immune and cytotoxic therapeutic responses based on tumoroid and tissue slice culture molecular signature s. *Oncotarget* **2017**, *8*, 66747-66757, 10.18632/oncotarget.19965.
11. Shan Bian; Marko Repic; Zhenming Guo; Anoop Kavirayani; Thomas R. Burkard; Joshua A. Bagley; Christian Krauditsc h; Juergen A. Knoblich; Genetically engineered cerebral organoids model brain tumor formation.. *Nature Chemical Biol ogy* **2018**, *15*, 631-639, 10.1038/s41592-018-0070-7.
12. Laura Broutier; Gianmarco Mastrogiovanni; Monique M.A. Verstegen; Hayley E. Francies; Lena Morrill Gavarró; Charle s R Bradshaw; George E Allen; Robert Arnes-Benito; Olga Sidorova; Marcia P. Gaspersz; et al. Human primary liver ca ncer–derived organoid cultures for disease modeling and drug screening. *Nature Medicine* **2017**, *23*, 1424-1435, 10.10 38/nm.4438.
13. L.H. Jensen; C. Dam; G. Hagel; C. Vagn-Hansen; H. Harling; B.M. Havelund; A. Jakobsen; J. Lindebjerg; S.R. Rafaels en; O. Thastrup; et al. Factors of importance in procuring tumoroids from colorectal liver metastasis biopsies for precisi on medicine. *Annals of Oncology* **2019**, *30*, v214, 10.1093/annonc/mdz246.045.
14. M. Huch; B. K. Koo; Modeling mouse and human development using organoid cultures. *Development* **2015**, *142*, 3113-3125, 10.1242/dev.118570.
15. Meike Hohwieler; Martin Müller; Pierre-Olivier Frappart; Sandra Heller; Pancreatic Progenitors and Organoids as a Prer equisite to Model Pancreatic Diseases and Cancer. *Stem Cells International* **2019**, *2019*, 1-11, 10.1155/2019/9301382.
16. Taka Eguchi; Chiharu Sogawa; Yuka Okusha; Kenta Uchibe; Ryosuke Iinuma; Kisho Ono; Keisuke Nakano; Jun Murak ami; Manabu Itoh; Kazuya Arai; et al. Organoids with cancer stem cell-like properties secrete exosomes and HSP90 in a 3D nanoenvironment. *PLoS ONE* **2018**, *13*, e0191109, 10.1371/journal.pone.0191109.

17. Francesco Pampaloni; Emmanuel G. Reynaud; Ernst H.K. Stelzer; The third dimension bridges the gap between cell c ulture and live tissue. *Nature Reviews Molecular Cell Biology* **2007**, *8*, 839-845, 10.1038/nrm2236.
18. Anna Birgersdotter; Rickard Sandberg; Ingemar Ernberg; Gene expression perturbation in vitro—A growing case for thr ee-dimensional (3D) culture systems. *Seminars in Cancer Biology* **2005**, *15*, 405-412, 10.1016/j.semcancer.2005.06.00 9.
19. Jong Bin Kim; R. C. Stein; Mike J. O'hare; Three-dimensional in vitro tissue culture models of breast cancer — a revie w. *Breast Cancer Research and Treatment* **2004**, *85*, 281-291, 10.1023/b:brea.0000025418.88785.2b.
20. Brendon M. Baker; Christopher S. Chen; Deconstructing the third dimension – how 3D culture microenvironments alter cellular cues. *Journal of Cell Science* **2012**, *125*, 3015-3024, 10.1242/jcs.079509.
21. Yukie Yoshii; Atsuo Waki; Kaori Yoshida; Anna Kakezuka; Maki Kobayashi; Hideo Namiki; Yusei Kuroda; Yasushi Kiyon o; Hiroshi Yoshii; Takako Furukawa; et al. The use of nanoimprinted scaffolds as 3D culture models to facilitate spontan eous tumor cell migration and well-regulated spheroid formation. *Biomaterials* **2011**, *32*, 6052-6058, 10.1016/j.biomater ials.2011.04.076.
22. Pasquale Marrazzo; Francesco Paduano; Francesca Palmieri; Massimo Marrelli; Marco Tatullo; Highly Efficient In Vitro Reparative Behaviour of Dental Pulp Stem Cells Cultured with Standardised Platelet Lysate Supplementation. *Stem Ce lls International* **2016**, *2016*, 1-16, 10.1155/2016/7230987.
23. Khaitan, D.; Dwarakanath, B. Multicellular spheroids as anin vitromodel in experimental oncology: Applications in transl ational medicine. Expert Opin. Drug Discov. 2006, 1, 663–675.
24. Millard, M.; Yakavets, I.; Zorin, V.; Kulmukhamedova, A.; Marchal, S.; Bezdetnaya, L. Drug delivery to solid tumors: The predictive value of the multicellular tumor spheroid model for nanomedicine screening. Int. J. Nanomed. 2017, 12, 7993 –8007.
