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REVIEW

Relevance of humanized three-dimensional tumor tissue models: a descriptive systematic literature review

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Abstract

Despite numerous advances in tumor screening, diagnosis, and treatment, to date, tumors remain one of the leading causes of death, principally due to metastasis and the physiological damage produced by tumor growth. Among the main limits related to the study of tumor physiology there is the complex and heterogeneity nature of its environment and the absence of relevant, simple and inexpensive models able to mimic the biological processes occurring in patients allowing the correct clinical translation of results. To enhance the understanding of the mechanisms of tumors and to develop and evaluate new therapeutic approaches the set-up of advanced and alternative models is mandatory. One of the more translational approaches seems to be the use of humanized three-dimensional (3D) tissue culture. This model allows to accurately mimic tumor morphology and biology, maintaining the *native* microenvironment without any manipulation. However, little is still known on the real clinical relevance of these models for the study of tumor mechanisms and for the screening of new therapy. The aim of this descriptive systematic literature review was to evaluate and summarize the current knowledge on human 3D tumor tissue culture models. We reviewed the strategies employed by researchers to set-up these systems, also considering the different approaches and culture conditions used. All these aspects greatly contribute to the existing knowledge on tumors, providing a specific link to clinical scenarios and making the humanized 3D tumor tissue models a more attractive tool both for researchers and clinicians.

Keywords Tumor · Metastasis · Tissue culture · Three-dimensional model · Clinical translation · Systematic literature review

Introduction

Tumors represent a type of multifaceted pathological condition, where normal cells begin to be hyper-proliferative and start the expression of a number of factors, i.e., cytokines, chemokines and growth factors, involved in cell proliferation, invasion and metastasis [1]. To date, surgery associated to standard chemotherapeutic protocols and radiotherapy represents the first-line treatment for most tumors, with a significant increase in survival rates [2, 3]. Immunotherapy, i.e., immune checkpoint blockade, cancer vaccines, and adoptive cell therapy, also allows an increase in anti-tumor immune responses and an improvement of the patient's clinical outcomes with minimal toxicity [4]. However, tumors still continue to represent one of the main causes of mortality [5, 6] principally due to metastasis and the physiological damage produced by tumor growth. The most frequent sites for metastatic disease are lung, liver, thyroid, bone and brain [7, 8]. To date, the therapeutic strategies used for metastasis are mostly palliative, with very limited opportunities for complete eradication [6]. Thus, the development and evaluation of new and advanced treatments for both tumor and metastasis are mandatory. Nevertheless, this issue requires not only a great understanding of the cellular and molecular mechanisms leading to tumor and/or metastasis but also the use of clinically relevant models able to strictly recapitulate and mimic the real complexity of the in vivo physiology.

In the last years, to investigate the mechanisms underlying tumor pathogenesis, progression and resistance to treatments, two-dimensional (2D) in vitro models have been widely employed [9–12]. These models are easy to

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handle and quite inexpensive to set-up and have led to a greater understanding in the ability of tumor cells to grow [13]. However, 2D models are for some aspects reductive and do not provide information about the real biological mechanisms; moreover, they are not able to mimic tumor and metastasis macrostructure, cellular heterogeneity and microenvironment complexity [14–16]. In addition, super imposed spatial cues, including substrate depth and cell connectivity, limit the applicability of these models for testing new therapies [17]. These restrictions provide unreliable data for translating results into clinical applications. In vivo animal models overcome many of the limits linked to 2D models, trying to mimic the native microenvironment in which tumors and metastasis reside [9]. There are several established in vivo models that differ in the species used, type of cancer/metastasis and method of cancer/metastasis induction [18]. Generally, xenograft animal models are extensively used to study tumorigenesis process and new therapies, showing several advantageous features, such as short reproductive cycle and ease manipulation [9, 19–21]. Patient derived xenografts, established from small fragments of human tumor tissue samples directly implanted into immunocompromised mice, are also frequently used as preclinical models able to closely resemble tumor/metastasis phenotype and human intra-tumor heterogeneity [21–24]. However, these models are expensive, also in terms of ethical issues, and challenging in the set-up as they show difficulties in tracking tumor growth and in drug screening studies. Moreover, the length of time necessary for implantation, propagation, and drug screening makes this model unsuitable for direct clinical use in patients [25, 26]. Recent studies tried to overcome several of the limits linked to 2D and in vivo animal models with the development of advanced three-dimensional (3D) in vitro models [9, 27–30]. Several types of 3D systems were designed to resemble in vivo tumors, considering both tumor heterogeneity and tumor–stroma interactions [31, 32]. In vitro 3D models include tumor-derived organoids and spheroids that tried to reproduce the tumor microenvironment [33]. However, these approaches mimic the tumor complexity only partially following a mechanical dissociation and enzymatic treatments of the tumor tissue [34–36]. Although the selective growth of tumor cells in an artificial environment can be studied and used in 3D, they recapitulate only few aspects of the tumor complexity, and remain essentially reductionist models [14]. A more translational approach seems to be the use of 3D tissue culture, in particular culture of patient-derived tumor tissue [37–40]. These models allow to accurately mimic tumor morphology and biology, thus maintaining the *native* microenvironment [41–43] without any manipulation [44–48]. However, little is still known on the possible clinical relevance of these 3D tissue models for the study of tumor mechanisms, but also for the evaluation of novel and advanced therapeutic strategies.

This descriptive systematic literature review considered and evaluated humanized 3D tumor tissue models and the strategies employed by researchers to set-up these systems, as well as taking into consideration their advantages and/or disadvantages, thus to understand if these models can be used as clinically relevant, advanced and translational systems for the study of tumor mechanisms and for the evaluation of novel therapeutic strategies.

Methods

Descriptive systematic literature review

Our descriptive literature review involved a systematic search that was carried out, according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) statement, in three databases (www.pubmed.org, www.scopus.com, www.webofknowledge.com). The keywords were: "(tumor OR cancer) AND (tissue culture) AND (ex vivo model OR ex vivo explant)". We sought to identify studies, where 3D models of tumor tissue were employed. Publications from 2009 to 2019 (original articles in English and full text) were included. Exclusion criteria were articles not written in English, reviews and articles in which cell cultures, spheroids, organoids, cancer-on-chip, bioreactors, microfluidic devices and in vivo models were used. Additional studies that were not found by our initial search were identified analyzing the reference lists from the included articles. A public reference manager (www.mendeley.com) was used to delete duplicate articles.

Results

An initial literature search retrieved 1782 articles: 372 articles were identified using PubMed, 1061 articles using Scopus and 349 were found in ISI Web of Knowledge (Fig. 1). Articles were submitted to a public reference manager (Mendeley 1.14, www.mendeley.com) to eliminate duplicate articles ($n = 107$). The resulting articles ($n = 1675$) were evaluated by two independent researchers (DC and FS) by reviewing titles and abstracts. Subsequently, 1607 complete articles were reviewed to establish whether the publication met the inclusion criteria and 59 articles were recognized eligible for this review.

3D tumor tissue models setup

As reported in Table 1, from the 59 articles on 3D human tumor tissue model analyzed in this review 16 articles used

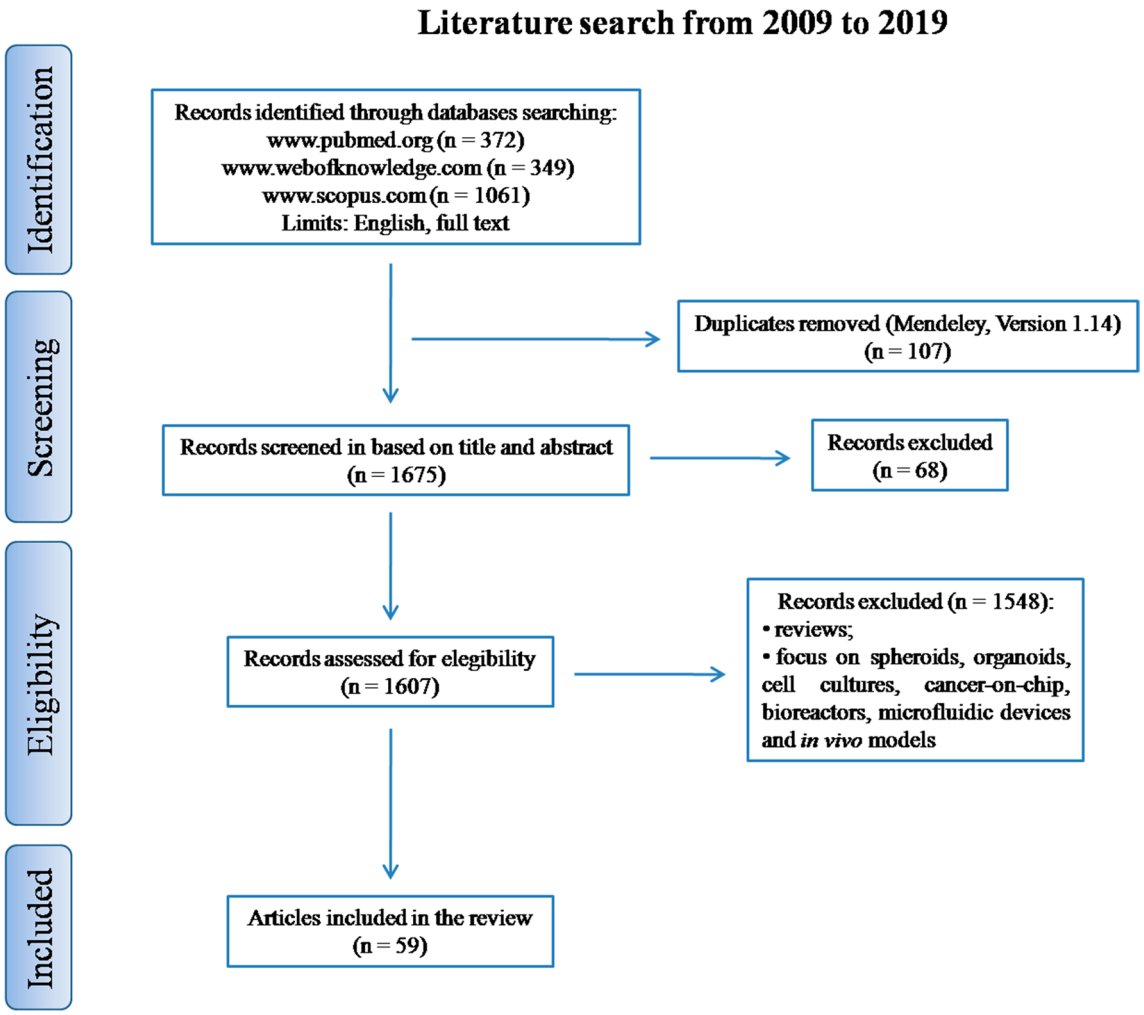

Fig. 1 Systematic literature review flow diagram

tumor tissue from breast [49–64], 14 from prostate [60, 63, 65–76], six from brain [64, 77–81], seven from lung [40, 63, 64, 82–85], eight from colorectal [55, 63, 64, 86–90], five from pancreas [63, 64, 91–93], five from ovary [63, 64, 94–96], one from testicle [97], two from head and neck (oral, oropharyngeal and nasal origin) [98, 99], two from liver [63, 100], two from skin [64, 101], three from uterus [63, 64, 102], two from stomach [63, 103], four from bladder/ureter [63, 64, 76, 104], three from kidney [63, 64, 105], and one from dental apparatus (mandible) [106] (Fig. 2).

Human tissue withdrawal and resection techniques

The analyzed studies retrieved from this review showed that human tumor tissue for the set-up of 3D models is usually obtained and employed within minutes or hours from surgical resection, thus minimizing tissue deterioration and loss of cellular viability [50, 54, 59, 60, 97, 103, 104]. Several studies specifically demonstrated that tumor tissue resection does not interfere with morphology and functional activity of the tissue, ensuring the preservation of cell phenotypes and the heterogeneity of cancer sub-populations [54, 61, 92]. However, it was observed that, though manual manipulation of tumor tissue samples, using choppers, scissors and scalpels, is technically simple and easy to perform it does present drawbacks, specifically the development of isolating fragments of various shapes and thicknesses, obtained from tumor regions of heterogeneous composition [40, 52, 54, 64, 66, 76, 84, 106]. Therefore, several studies showed a further refinement of the cut method involving the use of microtomes and vibratomes to prepare tumor samples with standardized and reproducible characteristics [53, 54, 58, 59, 61–63, 71, 79–81, 83, 87, 92, 94, 95, 98, 102, 105].

Culture media

Concerning the experimental set-up of these models, different culture media were used in the analyzed studies.

Table 1 3D tumor tissue models

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Oral, oropharyngeal and nasal	Therapy evaluation	Tissue fragments: four from oral cavity, four of oropharyngeal origin, one from the nasal cavity Resection method: cuted into slices (300 µm) using a vibratome Medium: DMEM, supplemented with FBS, penicillin/streptomycin Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin) Immunohistochemisty (Ki-67, yH2AX, pERK, p53)	Therapy decrease proliferative activity and p53 levels, remain unchanged or increase slightly DNA damage	Affolter et al. [98]
Breast	Therapy evaluation	Tissue fragments: one from breast Resection method: cuted into slices (200 µm) using a microtome Medium: MEGM supplemented with growth factors and amphotericin B Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and dynamic (shaking platform)	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67)	Therapy reduce tumor cell proliferation and viability, induce cell death in a time-dependent manner	Antoszczak et al. [62]
Mandible	Feasibility of the 3D tissue model	Tissue fragments: three from posterior mandible, one from anterior mandible (two odontogenic myxoma and two cemento-ossifying fibromas) Resection method: cuted into slices (1.5–2 mm) using a scalpel Medium: DMEM supplemented with FBS, penicillin/streptomycin and amphotericin B Microenvironment: normoxic Type of culture: with scaffolds (type I collagen scaffold) and static	Histology (hematoxylin/eosin) Immunohistochemistry (nestin) Invasion assay	Tissue architecture, cell viability, proliferation and migration preserved for more than 30 days, without apoptosis presence	Bastos et al. [106]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Pancreas	Feasibility of the 3D tissue model	Tissue fragments: two from pancreas Resection method: cuted into pieces Medium: DMEM supplemented with FBS and penicillin/streptomycin Microenvironment: normoxic Type of culture: with scaffolds (VTE tissue support) and static	Histology (hematoxylin/eosin) Immunohistochemistry (cleaved caspase 3) Gene expression and ELISA test (KRT19, α-SMA, THBS1, EGFR, PDGFRα, s-FLT1, PLGF, VEGF)	Histo-architecture, viability, growth factors release and gene expression profiles preserved for 10 days with cell death minimal	Bazou et al. [93]
Oral and oropharyngeal	Therapy evaluation	Tissue fragments: 20 from oral and laryngeal origin Resection method: cuted into slices Medium: not specified, supplemented with autologous serum Microenvironment: normoxic Type of culture: without scaffolds (plates coated with tumor matrix proteins) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, cleaved caspase 3, p53, cyclin D1, p16)	Therapy inhibit cell proliferation and induce apoptosis	Bhattacharyya et al. [99]
Ureter	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 55 from bladder Resection method: cuted into Sections (5 mm) Medium: K-SFM supplemented with rEGF, BPE and mezlocillin Microenvironment: normoxic Type of culture: with scaffolds (gelatine matrices, denatured fibrilar type I collagen) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, cleaved caspase 3) Immunofluorescence (TUNEL, pan-CK)	Tissue morphology and cell vitality preserved for 20 days Therapy increase apoptosis	Bolenz et al. [104]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Breast	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: three from breast Resection method: cuted into slices (250–300 µm) using a tissue slicer Medium: DMEM/F12 supplemented with FBS, bovine insulin, gentamicin, insulin-transferrin-selenium and glucose Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and dynamic (in agitation)	Viability assay (Alamar Blue, LDH) Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67)	Morphology, viability and metabolic activity preserved for 96 h without necrosis presence Therapy decrease tumor vitality	Carranza-Torres et al. [61]
Skin	Therapy evaluation	Tissue fragments: five from skin Resection method: sliced in cubes (2 mm^3) Medium: DMEM supplemented with FBS and penicillin/streptomycin Microenvironment: normoxic Type of culture: with scaffolds (culture inserts) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67) Gene expression and western blot (PCNA, NEO1, RGMA, NTN1, PTCH1, GLI1, BCL2)	Therapy decrease proliferation and mRNA and protein levels of NEO1, NTN1, GLI1, PTCH1 and BCL2	Casas et al. [101]
Prostate	Therapy evaluation	Tissue fragments: ten from prostate Resection method: dissected into pieces (1 mm^3) Medium: RPMI 1640 supplemented with FBS, antibiotic/antimycotic solution, hydrocortisone and insulin Microenvironment: normoxic Type of culture: with scaffolds (pre-soaked gelatin sponges) and static	Immunohistochemistry (Ki-67, cleaved caspase 3) Western blot (Hsp70, AR, Akt)	Therapy increase Hsp70 expression levels and apoptotic activity (22–23%), decrease AR and Akt (2 Hsp90 client proteins) expression levels and proliferative activity (0.5–1.8%)	Centenera et al. [65]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Breast and prostate	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 14 from breast and 23 from prostate Resection method: cuted into pieces (1 mm^3) Medium: RPMI 1640 supplemented with FBS, antimycotic/antibiotic solution, hydrocortisone, and insulin Microenvironment: normoxic Type of culture: with scaffolds (soaked gelatin sponges) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, brdU uptake, cleaved caspase 3, HIF1α, AR, PR, PSA, ERα) Gene expression (PSA, PGR)	Tissue morphology, viability and endocrine signaling (epithelial cell positivity for AR and ERα and expression of PSA and PGR) preserved for 6 days and de novo proliferation cell capacity (43%) for 96 h Therapy decrease PGR expression in 6/14 tissues (43%), increase in 5/14 (36%), no effect in 3/14 (21%), reduce PSA in 10/23 tissues (44%), increase in 6/23 (26%), no effect in 7/23 (30%)	Centenera et al. [60]
Colorectal	Feasibility of the 3D tissue model	Tissue fragments: 36 from colon Resection method: cuted in slices (230 µm) using a vibratome Medium: DMEM Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	NIR bio-imaging and western blot (EGFR)	EGF-NIR specifically and strongly label EGFR positive tissues, while adjacent tissue and EGFR negative tissues express weak NIR signals	Cohen et al. [87]
Colorectal	Feasibility of the 3D tissue model	Tissue fragments: 19 from colon Resection method: cuted into slices Medium: DMEM Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	NIR bio-imaging and western blot (EGFR)	EGF-NIR specifically and strongly label EGFR positive tissues, while EGFR negative tissues express weak NIR signals, as well as IRDye800CW binding that provides non-specific absorption	Cohen et al. [86]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Brain	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: six from frontal, one from ganglia, three from corpus callosum, five from temporal, two from parietal, two from temporal parietal Resection method: cuted into slices (250 µm) using a tissue chopper Medium: MEM supplemented with Hank's balanced salt solution, horse serum, l-glutamine, glucose, and penicillin/streptomycin Microenvironment: normoxic Type of culture: with scaffolds (membrane culture inserts to liquid/air interface) and static	Immunohistochemistry (Ki-67, GFAP, cleaved caspase 3, DCF-DA) Gene expression and western blot (FIG-ROS1, p-ALK, p-Met, survivin, Akt, Mcl-1)	Tissue architecture, cell viability and gene-expression profiles preserved for up to 14 days Therapy induce 80% cell death with increase in ROS, apoptosis and DNA fragmention and inhibition of FIG, ROS1, p-ALK, p-Met, survivin, Akt and Mcl-1	Das et al. [78]
Brain	Therapy evaluation	Tissue fragments: not specified Resection method: cuted into slices (250 µm) using a tissue chopper Medium: MEM supplemented with Hank's balanced salt solution, horse serum, l-glutamine, glucose, and penicillin/streptomycin Microenvironment: normoxic Type of culture: with scaffolds (culture inserts, at liquid/air interface) and static	Immunohistochemistry and western blot (cleaved caspase 3, HDAC, SAHA)	Therapy induce 50% cell death and histone H3 and H4 acetylation, with inhibition of HDAC activity	Das et al. [77]
Ovary	Therapy evaluation	Tissue fragments: not specified Resection method: cuted into slices (300 µm) using a microtome Medium: RPMI supplemented with antibiotic Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, pSTAT3 Tyr705, VEGF)	Therapy reduce proliferation and angiogenesis, cause disorganization of the tissue architecture with increase of necrosis	ElNaggar et al. [94]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Breast	Therapy evaluation	Tissue fragments: 33 from breast Resection method: cuted in slices (400 µm) using a vibratome Medium: Ham F-12 supplemented with FBS, penicillin/streptomycin, amphoterycin B and kanamycin Microenvironment: normoxic Type of culture: with scaffolds (membrane inserts) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, cleaved caspase 3, p53) Immunohistochemistry (γH2AX)	Therapy decrease cell proliferation, increase apoptosis, expression of p53 and levels of γH2AX (DNA breakage)	Faversani et al. [59]
Uterus	Feasibility of the 3D tissue model evaluation	Tissue fragments: not specified Resection method: cuted into slices (500 µm) using a vibratome Medium: D-MEM/F12 or MCDB-131 or SMC supplemented with penicillin/streptomycin, amphotericin B, l-Glutamin, FCS or horse serum or serum replacement, estradiol E2 or progesterone or EGF Microenvironment: normoxic and hypoxic Type of culture: with scaffolds (plates with metal grids or membrane inserts) and static	Immunohistochemistry (Ki-67, brdU)	High Proliferation and low apoptosis with metal grids, DMEM/F12, horse serum, estradiol E2 and EGF; no significant difference between the normoxic and hypoxic conditions	Fiebitz et al. [102]
Breast	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 30 from breast Resection method: cuted into slices (400 µm) using a tissue slicer Medium: RPMI 1640 supplemented with FBS, insulin, glutamine, ampicilin, streptomycin, EGF and hydrocortisone Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Viability assay (BrdU uptake) Immunohistochemistry (Ki-67) Gene expression (p-AKT, p-mTOR, p-S6K1, p-4EBP1)	Morphological integrity, viability, proliferation and AKT/mTOR pathway activation preserved for 24 h Therapy reduce gene expression and activity of the pAKT/mTOR pathway decreasing positive tumor cells for the phosphorylated forms AKT, mTOR, S6K1, 4EBP1	Grosso et al. [58]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Prostate	Therapy evaluation	Tissue fragments: 12 from prostate Resection method: cuted into slices (1 mm^3) Medium: 199 with Earle's salts supplemented with FBS penicillin/streptomycin, glutamine, insulin, dexamethasone and DHT Microenvironment: normoxic Type of culture: with scaffolds (stainless steel grids) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Stat5a/b)	Therapy reduce viability, levels of nuclear Stat5a/b expression, and induce apoptosis with DNA fragmentation in 9/12 tissues	Gu et al. [68]
Prostate	Therapy evaluation	Tissue fragments: ten cancerous and ten benign from prostate Resection method: cuted into pieces (0.5-mm) using a razor blade Medium: DMEM/F12 supplemented with FCS, penicillin/streptomycin and glutamax Microenvironment: normoxic Type of culture: with scaffolds (culture inserts, at air/liquid interface) and static	Gene expression (PSA, TMPRSS2, FKBP5)	Therapy reduce AR activity decreasing expression of PSA (56%), TMPRSS2 (39%) and FKBP5 (23%) in benign tissues and in 4/10 cancerous tissues	Handle et al. [66]
Lung	Therapy evaluation	Tissue fragments: three from lung Resection method: cuted into pieces Medium: RPMI 1640 supplemented with FCS Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Immunohistochemistry (Ki-67) Gene expression (COX-2, PGE_2)	Therapy increase tumor cell proliferation and expression of COX-2 and PGE2 levels	Hattar et al. [85]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Liver	Therapy evaluation	Tissue fragments: ten HBV-positive and ten HBV-negative from liver Resection method: cuted into pieces (1-mm^3) Medium: DMEM supplemented with FBS and penicillin/streptomycin Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates), static and dynamic (rotating platform)	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, TUNEL, CCK-8) Western blot (Hbx, Raf, Erk)	Therapy increase apoptosis, inhibit cell growth and levels of HBx, Raf and Erk	Huang et al. [100]
Testicle	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: three from testicle Resection method: cuted into pieces (1 mm^3) Medium: DMEM F12 supplemented with penicillin/streptomycin, insulin, transferrin, selenium and FBS or BSA Microenvironment: normoxic Type of culture: without scaffolds (in suspension) and static	Histology (hematoxylin/eosin, PLAP) Immunohistochemistry (Ki-67, BrdU, cleaved caspase 3, TUNEL) Gene expression and western blot (KIT, AP2γ)	Tissue integrity, proliferation and viability preserved for 14 days without apoptosis increase Therapy decrease KIT transcript at both gene and protein level without affecting the level of AP2γ transcript	Jørgensen et al. [97]
Lung	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 45 from lung Resection method: cuted into fragments (2–3 mm^3) using skin graft blades Medium: DMEM supplemented with glucose, FCS and penicillin/streptomycin Microenvironment: normoxic Type of culture: with scaffolds (culture inserts) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, cleaved PARP)	Tissue architecture preserved for 72 h with low levels of cell death Therapy decrease proliferation and induce cell death with apoptosis and DNA damage	Karekla et al. [40]
Breast	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: not specified Resection method: cuted into pieces (1 mm) Medium: MEGM Microenvironment: normoxic Type of culture: with scaffolds (type I collagen matrix) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, cleaved caspase 3, Rac1, STAT3)	Tissues viability (80–100%) preserved and cellular spread in collagen after 6–8 days Therapy decrease cell proliferation, blocks cell invasion into collagen and STAT3 transcription factor and induce apoptosis	Katz et al. [57]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Breast	Therapy evaluation	Tissue fragments: six from breast Resection method: cuted into Sections (1-mm^3) Medium: not specified, supplemented with hormone-stripped FBS Microenvironment: normoxic Type of culture: with scaffolds (soaked gelatin sponges) and static	Immunohistochemistry (Ki-67, phospho-Ser294 PR, phospho-ERK1/2)	Therapy blocks PR Ser294 phosphorylation and ERK 1/2 activity	Knutson et al. [56]
Prostate	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 36 from prostate Resection method: cuted into slices (300 µm) using a tissue chopper Medium: DMEM supplemented with FCS Microenvironment: normoxic Type of culture: with scaffolds (moisturized membranes) and static	Viability assay (EdU, pimonidazole) Histology (hematoxylin/eosin) Immunohistochemistry (γH2AX, 53BP1)	Cell viability, proliferation and oxygenation preserved for 15 days Therapy increase the amount of DNA-double strand break repair	Kocker et al. [67]
Intestine	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: eight from intestine Resection method: cuted into slices (400 µm) using a tissue chopper Medium: RPMI 1640 supplemented with FCS, l-glutamine, amphotericin B and penicillin/streptomycin Microenvironment: normoxic Type of culture: with scaffolds (membrane inserts) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, CK8, cleaved caspase 3)	Tissue morphology and cellular integrity preserved for 6 days Therapy decrease tumor cellularity and increase apoptotic processes	Koerfer et al. [103]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Brain, breast, lung, colon, rectal, uterus, ovary, pancreas, kidney, skin, bladder	Therapy evaluation	Tissue fragments: five from brain, one from breast, three from lung, six from colon, four from rectal, 12 from uterus, one from ovary, two from pancreas, one from kidney, eight from skin, one from bladder Resection method: cuted into pieces (10-mm^3) using a scalpel Medium: α-medium supplemented with FBS Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin)	Therapy increase cell death	Le Boeuf et al. [64]
Lung	Feasibility of the 3D tissue model evaluation	Tissue fragments: 70 from lung Resection method: cuted into fragments using a razor blade Medium: DMEM/F-12 supplemented with FCS, l-glutamine and penicillin/streptomycin Microenvironment: normoxic and hypoxic Type of culture: without scaffolds (on the bottom of plates) and static	Viability assay (MTT, pimonidazole) Immunohistochemistry (cleaved caspase 3, HIF-1α) Gene expression (CA IX)	Viability preserved for 3 days with minimal apoptosis rates comparable between normoxia and hypoxia; increase in HIF-1α and CA IX under hypoxia	Leithner et al. [84]
Prostate	Therapy evaluation	Tissue fragments: 11 from prostate Resection method: cuted into pieces (1 mm^3) Medium: 199 with Earle's salts supplemented with FBS, penicillin/streptomycin, glutamine, insulin, dexamethasone and DHT Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin) Immunohistochemistry and gene expression (Stat5a/b)	Therapy induce apoptosis with loss of viable epithelium and reduce level of nuclear Stat5a/b expression in 8/11 tissues	Liao et al. [70]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Prostate	Therapy evaluation	Tissue fragments: 11 from prostate Resection method: cuted into pieces (1 mm^3) Medium: not specified Microenvironment: normoxic Type of culture: with scaffolds (gelatine sponges soaked) and static	Immunohistochemistry (cleaved caspase 3) Western blot (pY576-FAK)	Therapy decrease cell viability and FAK phosphorylation at Y576, increase apoptosis	Lin et al. [69]
Prostate	Therapy evaluation	Tissue fragments: four from prostate Resection method: cuted into pieces (1-mm^3) Medium: not specified Microenvironment: normoxic Type of culture: with scaffolds (matrix-covered grids) and static	Immunofluorescence (γH2AX) Western blot and gene expression (Rad51, Stat5a/b)	Therapy inhibit Jak2-Stat5a/b signaling with reduction of Rad51 expression, decrease DNA damage repair with increation of cell death	Maranto et al. [72]
Prostate	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: not specified Resection method: cuted into slices (300 μm) using a tissue slicer Medium: PFMR-4A supplemented with antibiotic/antimycotic and in the presence or absence of androgen Microenvironment: normoxic Type of culture: with scaffolds (titanium mesh inserts) and dynamic (rotating platform)	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, cleaved caspase 3) Gene expression (p63)	Functional viability preserved for 5 days; few proliferating cells and many apoptotic cells in the absence of androgen Therapy cause epithelial degeneration, reduce viability and androgen expression, increase apoptosis	Maund et al. [71]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Pancreas	Feasibility of the 3D tissue model evaluation	Tissue fragments: 12 from pancreas Resection method: cuted into slices (350 µm) using a microtome Medium: CMRL 1066 supplemented with HEPES, sodium pyruvate, zinc sulfate, insulin–transferrin–sodium selenite solution, human serum AB, penicillin/streptomycin and diphenyl diselenide Microenvironment: normoxic and hypoxic Type of culture: with scaffolds (culture inserts) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67) Gene expression (pS6, CAIX)	Viability, structural and morphological integrity, phenotypic characteristics, proliferative and metabolic activity preserved for 96 h, without no effect of normoxic or hypoxic conditions	Misra et al. [92]
Breast and colorectal	Therapy evaluation	Tissue fragments: one from breast and one from colorectal Resection method: cuted into cubes (4 mm) Medium: IMDM supplemented with FBS and antibiotic Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Gene expression (COX-2, IL-10, IDO)	Therapy inhibit induction of immunosuppressive factors (COX-2, IL-10, IDO)	Muthuswamy et al. [55]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Breast	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 15 from breast Resection method: cuted manually into pieces (2 mm) or automatilly (300 µm) using a vibratome Medium: DMEM/HAM's F12 supplemented with FCS, antibiotics, in the presence or absence of hydrocortisone, insulin, transferrin, 3,3′,5 triiodothyronine, EGF, cholera toxin and adenine, or RPMI-1640 supplemented with FCS, antibiotics, in the presence or absence of l-glutamine, hydrocortisone, insulin, cholera toxin and EGF Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates), static and dynamic (mini orbital shaker)	Histology (hematoxylin/eosin) Immunohistochemistry (Cytokeratin, EdU, TUNEL)	Tissue morphology, viability and cell proliferation preserved for 7 days (with automatic cutting, rotation and DMEM/HAM's F12 supplemented with factors) Therapy decrease proliferation rate and induce cell death	Naipal et al. [54]
Prostate	Therapy evaluation	Tissue fragments: 46 from prostate Resection method: cuted into pieces (1 mm^3) Medium: RPMI 1640 supplemented with FBS, antibiotic/antimycotic solution, hydrocortisone and insulin Microenvironment: normoxic Type of culture: with scaffolds (pre-soaked gelatin sponges) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67) Western blot and mass spectrometry (DNAJA1)	Therapy inhibit cell proliferation, induce apoptosis, alters proteins TIMP1, SERPINA3 and CYP51A and increase expression of DNAJA1	Nguyen et al. [73]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Colorectal	Feasibility of the 3D tissue model evaluation	Tissue fragments: seven from colorectal Resection method: cuted into pieces (10–20 mg) Medium: MEM supplemented with L-glutamine, FBS, penicillin/streptomycin, gentamicin and saponin Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and dynamic (mild stirring)	Immunohistochemistry (VDAC, HK-II, DAPI) Oxygraphy (OXPHOS) Metabolic analysis (citrate synthase)	Tissues with mitochondria high respiration rates and metabolic activity	Ounpuu et al. [88]
Brain	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: not specified Resection method: cuted into slices (350 µm) using a vibratome Medium: neurobasal medium supplemented with HEPES, B-27, l-glutamine, glutamax, penicillin/streptomycin and nystatin Microenvironment: normoxic Type of culture: with scaffolds (hydrophilic PTFE inserts) and static	FISH (EGFR)	Cell proliferation and migration preserved for 72 h with minimal apoptosis Therapy decrease cell migration	Parker et al. [80]
Brain	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 50 from brain Resection method: cuted into pieces (300–350 µm) using a vibratome Medium: neuronal medium supplemented with HEPES, B-27, l-glutamine, L-alanyl-L-glutamine dipeptide, penicillin/streptomycin and nystatin Microenvironment: normoxic and hypoxic Type of culture: with scaffolds (PTFE inserts) and static	Histology (hematoxylin/eosin) Immunohistochemistry (CD68) ELISA test (VEGF)	Tissue morphology and cell migration preserved for 15 days; VEGF secretion increase under hypoxia than normoxia Therapy decrease cell migration	Parker et al. [79]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Lung	Therapy evaluation	Tissue fragments: 18 from lung Resection method: cuted into pieces Medium: RPMI 1640 Microenvironment: normoxic Type of culture: without scaffold (on the bottom of plates) and static	Immunohistochemistry and gene expression (EML4)	Therapy increase EML4 expression in 4/18 tissues and decrease in 5/18	Radtke et al. [82]
Breast	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 80 from breast Resection method: cuted into pieces (2000 µg) using a vibrotome Medium: DMEM supplemented with penicillin/streptomycin and fungizone Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67) Bioluminescence imaging	Cell viability (80%) and morphology preserved for 96 h Therapy with transduction efficiency > 50%	Rajendran et al. [53]
Colorectal	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 33 from colorectal Resection method: cuted into slices (2000 µm) using a vibrotome Medium: DMEM supplemented with penicillin/streptomycin, fungizone, gentamicin and in the presence or absence of cobalt Microenvironment: hypoxic Type of culture: without scaffolds (on the bottom of plates) and static	Bioluminescence imaging and flow cytometric Gene expression (HIF-1α, CXCR4)	Viability preserved for 48 h, no detrimental effects on gene expression with cyclical and prolonged hypoxia, decrease gene expression by more than 80% with chronic hypoxia Therapy with transduction efficiency	Rajendran et al. [89]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Breast	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 36 from breast Resection method: cuted into slices (100 µm) using a tissue chopper Medium: DMEM/F-12 supplemented with FCS Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67)	Morphological integrity preserved for 48 h Therapy decrease cell proliferation in 19/19 PRA-H (with ratio PR-A/PR-B ≥ 1.2) tissues and in 3/10 PRB-H (with ratio PR-A/PR-B ≤ 0.83), while increase in 2/10 PRB-H tissues	Rojas et al. [52]
Ovary	Therapy evaluation	Tissue fragments: not specified Resection method: cuted into slices (300 µm) using a microtome Medium: RPMI supplemented with antibiotic Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin) Immunohistochemistry (TUNEL) Gene expression (VEGF, STAT3, BCL2, Cyclin D1, Cyclin D2)	Therapy cause tissue architecture loss and tumor cells necrosis with increase in apoptosis, and decrease in mRNA expression levels for STAT3 (45.5%), VEGF (57.7%), BCl2 (70.9%), Cyclin D1 (64.2%), and Cyclin D2 (77.4%)	Saini et al. [95]
Breast	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 30 from breast Resection method: cuted into pieces (1 mm^3) Medium: not specified supplemented with estrogen Microenvironment: normoxic Type of culture: with scaffolds (type I collagen matrix) and static	Statistical metrics (HER, HER2)	HER2 status provides better stratification of morphological response to therapy than ER status. HER2- tumors being more responsive in terms of morphological change to therapy than HER2 + tumors	Savage et al. [51]
Breast	Therapy evaluation	Tissue fragments: not specified Resection method: dissected into pieces (3 mm^3) Medium: D-MEM/F-12 supplemented with penicillin/streptomycin, insulin, prolactin, transferrin and hydrocortisone Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Immunohistochemistry (Ki-67, GPER)	Therapy decrease cell proliferation	Scaling et al. [50]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Colorectal	Therapy evaluation	Tissues: ten from colorectal Resection method: cuted into slices (300 µm) Medium: DMEM supplemented with penicillin/streptomycin Microenvironmkent: normoxic Type of culture: with scaffolds (filter membrane inserts, at air/liquid interface) and static	Immunohistochemistry (cleaved PARP, Ki-67) ATP assay	Therapy decrease ATP content and increase the amount of apoptotic cells (from 8.9 to 31.5%), without change proliferative capacity	Scherr et al. [90]
Prostate	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 24 from prostate Resection method: cuted into pieces Medium: not specified Microenvironment: normoxic Type of culture: with scaffolds (wound-healing sponges) and static	Histology (hematoxylin/eosin) Immunohistochemistry (Ki-67, BrdU, AR, PSA) Gene expression (PSA, TMPRSS2, FKBP5)	Tissue morphology, viability and endogenous AR signaling preserved for 6 days with de novo proliferative capacity of tumor cells (active DNA replication) Therapy decrease cell proliferation and PSA, TMPRSS2, FKBP5	Shafi et al. [74]
Lung	Therapy evaluation	Tissue fragments: 12 from lung Resection method: cuted into slices (400 µm) using a vibratome Medium: not specified Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Histology (hematoxylin/eosin) Immunohistochemistry (cleaved caspase 3, 5-mC, HDAC) Gene expression (CDH13, CYP1A1, LINE1)	Therapy cause demethylation of LINE1, increase methylation of CYP1A1 and CDH13	Sirchia et al. [83]
Prostate	Therapy evaluation	Tissue fragments: five from prostate Resection method: cuted into pieces (1-mm^3) Medium: 199 with Earle's salts supplemented with FBS, penicillin/streptomycin, l-glutamine, insulin, dexamethasone and DHT Microenvironment: normoxic Type of culture: with scaffolds (lens papers and stainless steel grids) and static	Immunohistochemistry (Stat5a/b, E-cadherin, Twist1)	Therapy suppress Stat5a/b activation, increase E-cadherin and decrease Twist1 levels	Talati et al. [75]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Ovary	Therapy evaluation	Tissue fragments: 14 from ovary Resection method: cuted into cubes (4 mm) using a knife Medium: not specified, supplemented with antibiotic Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Gene expression (IFN, NFkB, RIPK-1) ELISA assay (CXCL10, CCL22, CXCL12, COX2, IDO, IL-10)	Therapy induce tumor-associated suppressive factors and activate the pathways of dsRNA recognition	Theodoraki et al. [96]
Lung, prostate, colon, breast, bladder, liver, kidney, ovary, pancreas, stomach, uterus	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: 32 from lung, 27 from prostate, 53 from colon, 52 from breast, three from bladder, six from liver, ten from kidney, four from ovary, three from pancreas, 14 from stomach, four from uterus Resection method: cuted into slices (400 µm) using a vibratome Medium: Ham F-12 supplemented with FBS, penicillin/streptomycin, amphoterycin B and kanamycin Microenvironment: normoxic Type of culture: with scaffolds (membrane inserts) and static	Viability assay (MTT) Histology (hematoxylin/eosin, TUNEL) Immunohistochemistry (Ki-67) Gene expression (p-AKT, p-S6RP)	Morphology, proliferation, viability and apoptosis minimal preserved for 120 h with functionally active and stable PI3K/Akt signaling pathway both at the transcriptional and protein level Therapy decrease cell viability and proliferation, p-Akt and p-S6RP levels, increase apoptosis	Vaira et al. [63]
Prostate and bladder	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: five from prostate and five from bladder Resection method: cuted into pieces (1 mm^3) using scissors Medium: DMEM supplemented with D-Glucose, pyruvate, FCS and penicillin/streptomycin (prostate); EMEM supplemented with FCS and penicillin/streptomycin (bladder) Microenvironment: hypoxic Type of culture: with scaffolds (nitrocellulose filter inserts) and static	Histology (hematoxylin/eosin) Immunohistochemistry (cleaved caspase 3, KRT18)	Tissue architecture, morphology and viability preserved for 4–5 days Therapy induce apoptosis and decrease cell proliferation	van de Merbel et al. [76]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Pancreas	Feasibility of the 3D tissue model and therapy evaluation	Tissue fragments: six from pancreas Resection method: cuted into slices (250 µm) using a tissue slicer Medium: DMEM supplemented with l-glutamine, penicillin/streptomycin, amphotericin (fungizone), insulin, transferrin, selenium and HEPES Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and dynamic (in shaking)	Viability assay (WST-1) Histology (hematoxylin/eosin)	Tissue viability and morphology (57%) preserved for 3 days Therapy with transduction efficiency	van Geer et al. [91]
Breast	Therapy evaluation	Tissue fragments: 26 from breast Resection method: cuted into pieces (1 mm^3) Medium: MEGM complete Microenvironment: normoxic Type of culture: with scaffolds (collagen type I scaffold) and static	Histology (hematoxylin/eosin, CAIX) Immunohistochemistry (Ki-67, cleaved caspase 3)	Therapy inhibit cell proliferation and invasion, increase cell death and apoptosis	Ward et al. [49]
Kidney	Therapy evaluation	Tissue fragments: six from kidney Resection method: cuted into slices (300 µm) using a microtome Medium: DMEM supplemented with penicillin/streptomycin Microenvironment: normoxic Type of culture: with scaffolds (porous filter membrane inserts) and static	Histology (hematoxilin/eosin) Immunohistochemistry and western blot (DcR3)	Therapy reduce cell proliferation and DcR3 expression	Weissinger et al. [105]

Table 1 (continued)

Tumor tissue	Aim of the study	Experimental set-up	Analyses	Main results	References
Brain	Therapy evaluation	Tissue fragments: two from brain Resection method: cuted into slices (300 µm) using a microtome Medium: DMEM-F12 supplemented with FBS and antibiotic Microenvironment: normoxic Type of culture: without scaffolds (on the bottom of plates) and static	Western blot (acetyl H3, acetyl H4, p-cdk1, cdk1, cyclin B1, p21)	Therapy cause histone hyperacetylation (H3, H4), increase p21 levels, reduce cyclin B1, induce apoptosis and G2 phase cell cycle arrest	Xu et al. [81]

DMEM Dulbecco's Modified Eagle Medium, *FBS* Fetal Bovine Serum, *p-ERK* phospho-extracellular signal-regulated protein kinases, *DNA* DeoxyriboNucleic Acid, *µm* micrometer, *MEGM* Mammary Epithelial Cell Growth Medium, *3D* three-dimensional, *mm* millimeter, *VTE* Vascularized Tumour Explants, *ELISA* Enzyme-Linked Immunosorbent Assay, *KRT19* Keratin 19, *α-SMA* Smooth Muscle Actin, Alpha, *THBS1* Thrombospondin 1, *s-FLT1* Soluble Fms-Like Tyrosine kinase-1, *VEGF* Vascular Endothelial Growth Factor, *EGFR* Epidermal Growth Factor Receptor, *PDGFRα* Platelet-Derived Growth Factor Receptor A, *PLGF* Placental Growth Factor, *K-SFM* Keratinocyte Serum-Free Growth Medium, *EGF* Epidermal Growth Factor, *BPE* Bovine Pituitary Extract, *pan-CK* pan Cytokeratin, *TUNEL* Terminal deoxynucleotidyl transferase dUTP nick end labeling, *DMEM/F12* Dulbecco's Modified Eagle Medium/Nutrient Mixture F-12, *LDH* Lactate Dehydrogenase, *h* hours, *PCNA* Proliferating cell nuclear antigen, *NEO1* Neogenin-1, *RGMA* Repulsive guidance molecule A, *NTN1* Netrin-1, *GLI1* glioma-associated oncogene homolog 1, *BCL2* B-cell lymphoma 2, *PTCH1* protein patched homolog 1, *Hsp70* heat shock protein 70, *Hsp90* heat shock protein 90, *AR* Androgen Receptor, *RPMI* Roswell Park Memorial Institute, *Akt* protein kinase B, *brdU* Bromodeoxyuridine, *HIF1α* hypoxia-inducible factor 1-alpha, *PSA* prostate-specific antigen, *ERα* estrogen receptor alpha, *ER* estrogen receptor, *PGR* Progesterone Receptor, *PR* progesterone receptor, *NIR* near infrared, *GFAP* glial fibrillary acidic protein, *DCF-DA* 2′,7′-dichlorodihydrofluorescein diacetate, *MEM* Minimum Essential Medium, *ROS* reactive oxygen species, *p-ALK* phospho-anaplastic lymphoma kinase, *Mcl-1* myeloid cell leukemia-1, *FIG* fusion kinase in glioblastoma, *ROS1* ros proto-oncogene 1, *HDAC* histone deacetylase, *SAHA* suberoylanilide hydroxamic acid, *FCS* Fetal Calf Serum, *SMC* smooth muscle cell growth media, *p-AKT* phospho-protein kinase B, *p-mTOR* phospho-mammalian target of rapamycin, *p-S6K1* phospho-ribosomal protein S6 kinase beta-1, *p-4EBP1* phospho-eukaryotic translation initiation factor 4E-binding protein 1, *DHT* dihydrotestosterone, *TMPRSS2* transmembrane protease, serine 2, *FKBP5* FK506 binding protein 5, *PGE2* prostaglandin E2, *COX-2* cyclooxygenase 2, *HBV* hepatitis B virus, *Hbx* Hepatitis B Virus X Protein, *CCK-8* Cell Counting Kit-8, *BSA* bovine serum albumin, *PLAP* placental alkaline phosphatase, *AP2γ* Activating Protein 2γ, *STAT3* signal transducer and activator of transcription 3, *p-ERK1/2* phospho-extracellular signal-regulated protein kinases 1 and 2, *EdU* 5-Ethynyl-2′-deoxyuridine, *53BP1* p53-binding protein 1, *CK8* Keratin 8, *CA IX* carbonic anhydrase IX, *MTT* 3-(4,5-Dimethylthiazol-2-yl)-2,5-diphenyltetrazolium bromide, *Rac1* ras-related C3 botulinum toxin substrate 1, *FAK* focal adhesion kinase, *HEPES* 4-(2-hydroxyethyl)-1-piperazineethanesulfonic acid, *CMRL* Connaught Medical Research Laboratories, *IMDM* Iscove's Modified Dulbecco's Media, *IL-10* Interleukin 10, *IDO* Indoleamine 2,3-dioxygenase, *TIMP1* tissue inhibitors of metalloproteinases-1, *SERPINA3* serpin peptidase inhibitor clade A member 3, *DNAJA1* DnaJ homolog subfamily A member 1, *mg* milligram, *OXPHOS* oxidative phosphorylation, *DAPI* 4′,6-diamidino-2-phenylindole, *HK-II* mitochondrial hexokinase II, *VDAC* voltage-dependent anion-selective canne, *B-27* human leukocyte antigen, *FISH* fluorescent in situ hybridization, *PTFE* polytetrafluoroethylene, *CD68* Cluster of Differentiation 68, *EML4* echinoderm microtubule-associated protein-like 4, *CXCR4* C-X-C chemokine receptor type 4, *PR-A* progesterone receptor isoform A, *PR-B* progesterone receptor isoform B, *PRA-H* high progesterone receptor isoform A, *PRB-H* high progesterone receptor isoform B, *HER2* human epidermal growth factor receptor 2, *HER* human epidermal growth factor receptor, *GPER* G protein-coupled estrogen receptor 1, *PARP* poli ADP-ribosio polimerasi, *ATP* adenosine triphosphate, *5-mC* 5-methylcytosine, *CDH13* Cadherin-13, *LINE1* long interspersed element-1, *Twist1* twist-related protein 1, *IFN* Interferon, *NFkB* nuclear factor kappa-light-chain-enhancer of activated B cells, *RIPK-1* receptor-interacting serine/threonine-protein kinase 1, *dsRNA* Double-stranded RiboNucleic Acid, *CXCL10* chemokine interferon-γ inducible protein 10, *CCL22* C–C motif chemokine 22, *CXCL12* C-X-C Motif Chemokine Ligand 12, *KRT18* Keratin 18, *WST-1* water-soluble tetrazolium salt-1, *DcR3* decoy receptor 3, *PI3K* phosphoinositide 3-kinase, *p-cdk1* phospho-cyclin-dependent kinase 1, *cdk1* cyclin-dependent kinase 1, *EMEM* Eagle's Minimum Essential Medium

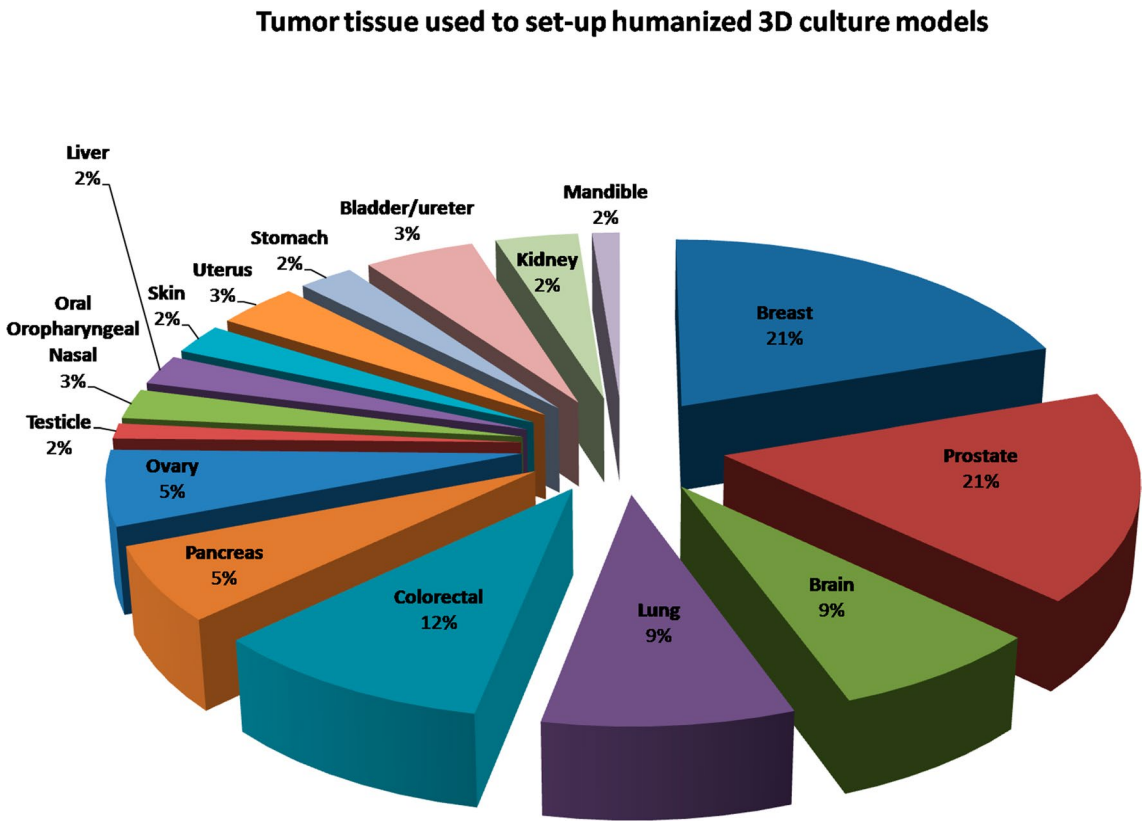

Fig. 2 Tumors' tissue employed to set-up humanized 3D culture models

Generally, commercially available media, i.e., Dulbecco's Modified Eagle Medium (DMEM), DMEM/F12, RPMI, supplemented with fetal bovine serum (FBS) and antibiotics (penicillin/streptomycin) were employed. For some tumors the basic culture medium was added with nutrients and growth factors to optimize the culture conditions [50, 54, 58, 60, 61, 65, 68, 70, 73, 75, 79, 80]. For instance, in breast and prostate cancer tissue culture the medium was often supplemented with insulin [50, 54, 58, 60, 61, 65, 68, 70, 73, 75], while in glioblastoma tissue culture with B-27 factor [79, 80]. Some authors supplemented the culture medium also with estrogens or androgens to further maintain the endocrine signaling, as in breast and prostate cancer tissue cultures [50, 56, 59, 60, 71, 74].

Microenvironment

Another important issue for 3D tumor tissue models set-up is the microenvironment, since hypoxia is typically present in solid tumors and is known to enhance tumor progression and therapy resistance. Results from the reviewed studies showed that 4/59 studies [79, 84, 92, 102] used a hypoxic environment to culture tumor tissue (oxygen 1–2%), but none of them found clear advantage in the hypoxic microenvironment over the normoxic ones (oxygen 20%) [92, 102]. In fact, despite different oxygen levels, the tissues apoptosis rates were comparable among normoxic and hypoxic cultures, suggesting the adaptation of tumor tissue to the microenvironment [84]. Leithner et al. [84] also observed that tissues cultured under hypoxia were entirely hypoxic, while only a core of hypoxia was found in tissues cultured under normoxia. However, it was observed that major hypoxia-markers were significantly increased in hypoxic tissues culture, i.e., hypoxia-inducible factor 1-alpha (HIF-1α) and carbonic anhydrase IX (CA IX) [84]. It was also observed that HIF-2α, which is known to be stabilized by hypoxia, was expressed only at low levels, both in normoxia and hypoxia, and was not elevated in hypoxic tissues. This indicates that the difference in oxygen concentration was preserved inside the tumor tissues [84]. Contrarily, Parker et al. [79] demonstrated that by culturing the tissues under hypoxia, they showed a rapid physiologic response, inducing the release of vascular endothelial growth factor (VEGF) that has not been observed under normoxia [79].

Culture methods

In addition to different techniques for tumor tissue resection, culture medium and microenvironment (normoxic and hypoxic), to set-up the 3D tumor tissue models also

different culture methods were used. In most of the examined studies tumor tissues were cultured on the bottom of the plates and submerged in medium [50, 52–55, 58, 61, 62, 64, 70, 81–89, 91, 94–100]. In these experimental setups, to avoid loss of tissue integrity and viability, the incorporation of a continuous rotational movement seems to be critical for the perfusion of oxygen to the tumor tissue and for the nutrient exchange [54, 61, 62, 71, 88, 91, 100]. In fact, Naipal et al. [54] showed that breast cancer tissues cultured under dynamic conditions, i.e., subjected to rotation using a mini orbital shaker placed in the incubator, maintained its viability for 7 days and showed more proliferating cells compared to the same tissue cultured under static conditions [54]. Several studies also cultured the tumor tissue on different types of scaffolds [40, 49, 51, 56, 57, 59, 60, 63, 65–69, 71–80, 90, 92, 93, 101–106]. Some authors used a lens paper supported by a mesh grid made of titanium or stainless steel for prostate tumor tissues culture, showing the maintain of their viability for up to 5–7 days [68, 71, 72, 75], while uterine leiomyoma tissues cultured on metal grids preserved their viability and proliferation for 48 h [102]. As alternative to titanium grid, many authors utilized also media pre-soaked gelatin sponge, collagen type I matrix, tissue support (VTEs) or different type of inserts (PTFE, porous filter membranes or moisturized) as alternative scaffold for tumor tissue culture [40, 49, 51, 56, 57, 59, 60, 63, 65–67, 69, 73, 74, 76–80, 90, 92, 93, 101, 103–106]. In some studies the tumor tissue was cultured on the top of the sponge, matrix or inserted at the air/liquid interface, acting as point of exchange for nutrients, thus allowing the preservation of the 3D tissue structure and giving an efficient oxygenation and a good viability to the tumor tissues [40, 49, 51, 56, 57, 59, 60, 63, 65–67, 69, 73, 74, 76–80, 90, 92, 93, 101, 103–106]. Bastos et al. [106] cultured ectomesenchymal odontogenic tumor tissue on type I collagen scaffolds obtaining a good reproduction of the growth pattern including cell proliferation and migration into the collagen matrix, preservation of the tissue architecture and maintenance of cell viability for more than 30 days [106]. Similarly, also urothelial carcinoma tissues cultured on gelatine matrices preserved its morphology and cell vitality over 20 day culture [104]. Pancreatic cancer tissues cultured on a supporting tissue bed containing stromal cells, matrix and vasculature (VTEs) maintained its histo-architecture, viability and genomic status of the primary tumor up to 10 days when compared to tumor tissues cultured without support [93]. Finally, some studies showed that breast and prostate cancer tissues, cultured on gelatine sponge, maintained tissue morphology and viability up to 6 days also showed the capacity for de novo cells proliferation [60, 74]. In addition to the roles of matrices in the maintenance of structure and morphology of tumor tissues, it was seen that they also improve the expression of steroid receptors during culture, thus delaying the loss of stromal cells [56, 59, 74].

Evaluation measurement tools

3D tumor tissue systems, with and/or without scaffolds, also allows performing histological, biochemical and molecular analyses directly on the tissue to measure tumor cell proliferation, detect the occurrence of genomic lesions and cell death and to examine the activation of oncogenic signal transduction cascades. Histopathological analysis allowed detecting and/or confirm the absence of significant change in tissue morphology and cell density; cells showed complete integrity and no areas of degeneration and/or necrosis [40, 52–54, 57, 60, 61, 63, 67, 71, 74, 76, 79, 91–93, 97, 103, 104, 106]. Immunohistochemical analyses (Ki-67, cleaved caspase-3, CK8, brdU uptake, TUNEL) revealed significant levels of ongoing proliferation of tumor cells [40, 53, 54, 57, 60, 61, 63, 71, 74, 76, 84, 92, 97, 102–104], confirming the ability of tumor tissues to maintain 80–90% of original viability [53, 54, 57, 58, 60, 63, 71, 74, 76, 79, 84, 92, 97, 102–104] without significant increase in cell apoptosis [40, 63, 84, 93, 97, 102]. Finally, also molecular (RT-PCR) and biochemical analyses (western blot, ELISA assay) on tissues and/or culture medium confirmed the maintenance of gene expression profile (p-AKT, p-S6RP, p-mTOR, p-S6K1 and p-4EBP1 of PI3K/AKT/TOR pathway; GLI1, NEO1, NTN1 and RGMA of SHH/GLI pathway; AR target genes PSA, TMPRSS2, FKBP5; hypoxia markers CAIX, HIF-1α; KIT, AP2γ, FIG, ROS1, p-ALK, p-Met, survivin, Akt, Mcl-1, KRT19) and protein levels (VEGF, EGFR, PLGF, s-FLT1, KIT, AP2γ, FIG, ROS1, p-ALK, p-Met, survivin, Akt, Mcl-1, NEO1, NTN1, RGMA, pS6, α-SMA, THBS1, MET, EGFR, PDGFRα) of primary tumor [58, 60, 63, 74, 78, 79, 84, 86, 87, 92, 93, 97, 101].

New treatment evaluation

The ability of these systems to strictly recapitulate the real complexity of the tumor physiological microenvironment, allowed to use these models also as preclinical tool to evaluate the response to novel drugs, alternative chemotherapeutics and small molecule inhibitors [40, 52, 54, 57, 58, 60, 61, 63, 67, 71, 74, 76, 78–80, 97, 103, 104]. In fact, in many studies the ability to culture and preserve tumor tissue for long periods of times (up to 3–7 days) consented a more adequate exposure and response to chemotherapeutic agents and/or targeted therapies [40, 53, 54, 60, 61, 63, 74, 76, 92, 103]. For instance, Affolter et al. [98] showed that the treatment with a MEK inhibitor associated to irradiation lead to an extensive DNA damage in head and neck squamous cell carcinoma (tissues derived from the oral and nasal cavity),

with decrease of p53 phosphorylation and with a strong γH2AX staining, indicative of a DNA repair response and of a decrease of proliferative activity [98]. Several authors in 3D prostate cancer tissue cultures demonstrated an anti-proliferative and pro-apoptotic responses, determined by low levels of Ki67 and high levels of cleaved caspase-3, to novel heat shock protein 90 (Hsp90) inhibitors [65, 73], PARP-1 inhibitors [67] and Jack2 inhibitors [68, 70, 72, 75]. Always using prostate cancer 3D culture it was demonstrated that the co-treatment with focal adhesion kinase (FAK) inhibitor (a cytoplasmic tyrosine kinase able to regulate a plethora of downstream signaling pathways involved in cell migration, proliferation and death) and docetaxel (an anti-mitotic chemotherapy drug), and treatment with piperlongumine decreased tumor cell viability and induced apoptosis [69, 71]. Using breast cancer tissue, it was also demonstrated that the nitroimidazole-based sulfonamide, carbonic anhydrase IX (CAIX) inhibitors, salinomycin and its modified derivative and paclitaxel (synthetic compound) decrease cancer cells proliferation, inhibit invasion and increase cell death and apoptosis [49, 61, 62]. In addition, paclitaxel revealed an antineoplastic synergistic effect in combination to natural bioactive compounds as caffeic acid, ursolic acid and rosmarinic acid [61]. Combination treatments with crizotinib and temozolomide drugs induced ~ 80% of cell death with an increase in reactive oxygen species (ROS) production [78] and the arrest of G2 phase cell cycle, in presence of histone deacetylase (HDAC) inhibitors [81], in glioblastoma cultures. Finally, several chemotherapeutic agents as cisplatin, 5-FU (5-fluorouracil), docetaxel, FAC (fluorouracil, adriamycin, cytoxan), carboplatin and gemcitabine, were also used and evaluated in 3D tumor tissues cultures [40, 54, 69, 74, 76, 82, 95, 100, 103]. In detail, gemcitabine and docetaxel induced apoptosis and decreased cell proliferation in bladder and prostate cancer tissue cultures, respectively [69, 76]. Carboplatin increased echinoderm microtubule-associated protein-like 4 (EML4) expressions [82], while cisplatin decreased proliferation and induced cell death with apoptosis and DNA damage in non-small cell lung cancer tissues [40]. Using breast cancer tissue culture, it was also demonstrated that FAC decreased proliferation rate and induced cell death [54]. Finally, Koerferet al. [103] showed that the combination cisplatin and 5-FU (5-fluorouracil) decrease tumor cellularity and increase the apoptotic processes in gastric and esophagogastric tumor tissues [103]. Some studies demonstrated that these models are also suitable for the validation of alternative anti-cancer approaches such as oncolytic viral infection or gene therapy [53, 64, 89, 91]. Using a pancreatic adenocarcinoma 3D tissues culture van Geer et al. [91] compared different viral vectors, i.e., lentiviral vectors, adenovirus (Ad) and adeno associated virus (AAV) expressing the reporter genes green fluorescent protein (GFP), to study their transduction efficiency. Reporter genes expression indicated that the pancreatic tissues was infected and transduced efficiently by Ad and AAV, whereas transduction with lentiviral vectors was limited [91]. Results obtained from Ad delivery of the firefly luciferase (FLuc) reporter gene indicated that colon tumor tissue was more amenable to Ad transduction than other tumor histologic types examined (i.e., breast and ovary). Ad transduction levels were significantly higher than a range of standard gene delivery viral and non-viral methods examined in colon tissue [89]. Also, breast cancer tissue was used to evaluated gene delivery for several vectors (Ad, AVV, lipofection, ultrasound, electroporation and naked DNA), confirming that Ad was the most efficient gene delivery vector with transduction efficiency > 50%, while ultrasound proved the optimal non-viral gene delivery method in tumor tissues [53]. Concerning oncolytic therapy, two genetically distinct viruses, vesicular stomatitis virus (VSV) and vaccinia virus (VV), were combined and used to infect tumor tissues of rectum, colon, brain and endometrium, showing that VV synergistically enhanced VSV antitumor activity, dependent in large part on the activity of the VV B18R gene product. A recombinant version of VSV expressing the fusion-associated small-transmembrane (p14FAST) protein also further enhanced the ability of VV to spread through an infected monolayer, resulting in an oncolytic effect, where in each virus enhanced the ability of the other to replicate and/or spread in tumor cells [64].

Discussion and future direction

In contrast with more simplistic models, the humanized 3D tumor tissue culture represents an attractive physiological approach able to better mimic the real in vivo tumor complexity. The great advantage of this model is that the tumor environment is the same of the clinical *scenario.* Furthermore, obtaining tumor tissue from patients is fast, simple, ethically correct (after Ethical Committee approval and patients' consent), economically sound and does not require any enzymatic treatments that can alter the 'normal' physiological conditions of the tumor. The use of this model also allows to obtain many tissue samples from the same patient permitting an accurate control of all culture factors, which consent to have reproducible results. In addition, it is important to emphasize that the use of tumor tissue models follow the 3R (Replacement, Reduction, Refinement) principles related to a more ethical use of animals for scientific purposes. Many stimulating ideas about the use and improvement of these models emerged from this review (specific culture media, oxygen intake, dynamic conditions, support/inserts/scaffolds). In fact, it was observed that tissue culture provides the opportunity to study the tumor in the context of a natural and intact microenvironment, including

all cell types as well as the native extracellular matrix, thus maintaining the naturally occurring interactions between tumor and stroma [14]. In addition, given the influence of the tumor environment and other aspects of tumor biology on drug sensitivity, the 3D tumor tissue cultures evaluated in this review appear also suitable to study and evaluate novel therapeutic strategies, also considering patient individual characteristics and specific tumors subtypes. This aspect is of fundamental importance, as it would allow to evaluate drug response for tumors and/or metastases considering a personalized approach. However, comparing the ex vivo 3D tumor tissue culture data on therapeutic strategies to what is known from clinical trials, it was observed that only few clinical data are currently available. To date, few clinical trials on small-molecule inhibitors that block the conversion of ERK to its activated form via inhibition of MEK1/2, alone or in combination with therapeutic agents, are present and showed preliminary antitumor activity in patients with different solid tumors type, i.e., ovarian cancer (NCT01663857), biliary tract cancers (NCT01943864), metastatic melanoma (NCT01584648; NCT01037127; NCT01597908; NCT01072175; NCT01271803; NCT01689519; NCT01245062) and non-small-cell lung cancer (NCT01362296). However, in two clinical trials the combination of these inhibitors with therapeutic agents did not show significant clinical activity (NCT01941927; NCT01231581) [107] and there are no clinical trials on the effects of the association of irradiation and MEK inhibitors in solid tumors. Moreover, Hsp90 inhibitors have been tested in metastatic castration-resistant prostate cancer and lung cancer showing partial responses due to their toxicities without meet primary endpoint (NCT01259089) [65]. Phase I and II trials are present and also evaluated the effectiveness, safety, tolerability, pharmacokinetics, and clinical anti-cancer activity of FAK, HDAC and CAIX inhibitors, alone or in combination with cytotoxic agents, showing a good tolerability and acceptable security profiles in patients with advanced solid tumors (NCT02915523; NCT00926640; NCT00741234; NCT02909452; NCT02032810; NCT00496444; NCT00098891; NCT02780804; NCT02805660; NCT01023737) [108–110]. However, at present, several clinical trials on these inhibitors alone or combined with immunotherapeutic agents are still ongoing, others have been completed and two of them did not improve the clinical outcomes in patients with thyroid cancer (NCT00134043; NCT00437957) [111]. Despite numerous clinical trials being carried out, at present, it is not possible to draw any definitive conclusions about the clinical benefit of these new and advanced therapeutic strategies evaluated through ex vivo 3D tumor tissue culture. This is principally due to the fact that the majority of clinical trials are still ongoing or have only recently completed and have yet to post results (NCT03543969; NCT02216669). Thus, currently it is not yet possible to determine the real predictive value of this ex vivo 3D tumor tissue cultures. In addition, several methodological challenges and limitations still remain including difficulty in maintenance of culture for a long period of time and currently limited developments for the translational research. The realization and production of these tissue culture models are dependent on the starting tissues availability and amount received following surgery, and on the risk of possible contamination of the tissues (even when antibiotics and antimycotics are used). Moreover, tumor tissue samples are often collected from patients with advanced disease or patients that are already been subjected to different therapeutic intervention, and this could interfere with the response of the therapy to be tested by the 3D model. Another important issue is that it is always necessary to consider that during surgery and 3D culture set-up, the vascular system of the tumor is disconnected from systemic blood flow, and this aspect may interfere with drug penetration. Hypoxia, which is present in all solid tumors, if not considered, could also represent a potential problem for the cultures. Another key point is that none of the examined studies developed and evaluated humanized 3D cultures from metastatic tissue. This is probably due to the difficulty of managing such a heterogeneous tissue, where numerous cells type and factors, i.e., cytokines, chemokines and growth factors, play different roles. In addition, in many cases, it is difficult to obtain metastatic tissues, since in specific circumstance, it is not possible to perform surgery because of the advanced disease, extensive or localized tumor in complex position, where the surgical procedure could damage critical adjacent organs and tissues. The set-up of 3D metastasis models will be of fundamental importance, as they could reproduce a realistic and controllable microenvironment that better clarifies the molecular mechanisms that support metastasis growth and colonization, and for identifying strategies able to minimize their development.

In conclusion, the use of humanized 3D tumor tissue culture provides an interesting tool that could bridge the gap between results based on monolayer culture of cancer cell lines and the reality in human solid tumors. This model reproduces overall tumor tissue viability as well as maintenance of structural integrity, both at the microscopic and ultra-structural level allowing to investigate therapy responses. Importantly, using these models, the tumor cells retain proliferative activity and morphological phenotypes. However, since each tumor has different characteristics, the set-up of tumor culture and the choice of the culture conditions should be carefully evaluated.

What remains to be proven over the longer term is whether this approach can indeed predict the correct clinical response to specific therapeutic strategies, allowing also a better quantification of drug responses or resistance in patients, thus providing high-throughput analysis and

leading to new more effective tumor treatments. Future clinical studies, comparing pre- and post-treatment tissues to include parallel ex vivo cultures of pre-treatment tumor tissues, will validate the model using clinically relevant end points, correlating tissue culture parameters with patient clinical outcomes [60]. This will provide an opportunity to investigate novel mechanisms of treatment resistance and identify biomarkers of treatment response in the preclinical phase that can then be integrated into clinical studies, and are essential for the realization of personalized cancer medicine, reducing damage and increasing patients benefits [60, 112]. In addition, it would also be important to set-up a tissue bank to collect and store patient tumor tissue samples for advancing translational biomedical research to allow study of genes, RNA and proteins, and to explore the biological mechanisms that support tumors etiology and biology, and the development of novel treatments, thus to facilitate a personalized approach for tumors and metastases [113].

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Compliance with ethical standards

Conflict of interest The authors declare that they have no conflict of interest.

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