Pharmacology & Therapeutics 163 (2016) 94–108

Contents lists available at ScienceDirect

Pharmacology & Therapeutics

journal homepage: www.elsevier.com/locate/pharmthera

Three-dimensional culture systems in cancer research: Focus on tumor spheroid model

Sritama Nath [a], Gayathri R. Devi [a,b,*]

[a] Division of Surgical Sciences, Department of Surgery, Duke University School of Medicine, Durham, NC 27710, United States
[b] Duke Cancer Institute, Women's Cancer Program, Duke University School of Medicine, Durham, NC 27710, United States

ARTICLE INFO

Available online 8 April 2016

Keywords:
Tumor emboli
Apoptosis
High-throughput screening
Inflammatory breast cancer
Oxidative stress
Invasion

ABSTRACT

Cancer cells propagated in three-dimensional (3D) culture systems exhibit physiologically relevant cell–cell and cell–matrix interactions, gene expression and signaling pathway profiles, heterogeneity and structural complexity that reflect in vivo tumors. In recent years, development of various 3D models has improved the study of host–tumor interaction and use of high-throughput screening platforms for anti-cancer drug discovery and development. This review attempts to summarize the various 3D culture systems, with an emphasis on the most well characterized and widely applied model — multicellular tumor spheroids. This review also highlights the various techniques to generate tumor spheroids, methods to characterize them, and its applicability in cancer research.

Contents

1. Introduction

Compelling evidence from two decades of research has revealed the critical role of tumor microenvironment (TME) in cancer development and progression (Mbeunkui & Johann, 2009; Quail & Joyce, 2013). The cellular components of the TME (transformed epithelial cells, cancer-associated fibroblasts (CAFs), tumor infiltrating mesenchymal stem cells (MSCs), tumor infiltrating lymphocytes (TILs), and endothelial cells) interact with tumor cells and impact various biological characteristics such as proliferation, migration, and therapeutic resistance (Joyce & Pollard, 2009; Loebinger et al., 2009; Zhu et al., 2009; Wong & Wang, 2000; Baker & Chen, 2012; Quail & Joyce, 2013; Kyurkchiev et al., 2014; Smith & Kang, 2014; Fedorenko & Smalley, 2015; Karakasheva et al., 2015; Yulyana et al., 2015). The non-cellular components of the TME (extracellular matrix (ECM), growth factors, cytokines, and chemokines) play an equally significant role in cancer progression, by presenting cues that affect fundamental aspects of tumor cell biology (Paszek et al., 2005; Levental et al., 2009; Lu et al., 2012). Dynamic changes in ECM architecture are detected and transduced through transmembrane cell adhesion molecules like integrin, which in turn can activate signaling pathways, causing changes in tumor cell behavior (Fiorilli et al., 2008).

In two-dimensional (2D) culture systems, cells are grown as monolayers on flat solid surface, lacking cell–cell and cell–matrix interactions that are present in native tumors. Additionally, 2D-cultured cells are

Abbreviations: 2D, two-dimensional; 3D, three-dimensional; TME, tumor microenvironment; IBC, inflammatory breast cancer; MTCS, multicellular tumor spheroids; ECM, extracellular matrix; O_2, oxygen; CSC, cancer stem cells.

* Corresponding author at: Department of Surgery, Duke University Medical Center, 2606 DUMC, Durham, NC 27710. Tel.: 919 668 0410.
E-mail address: gayathri.devi@duke.edu (G.R. Devi).

http://dx.doi.org/10.1016/j.pharmthera.2016.03.013
0163-7258/

stretched and undergo cytoskeletal rearrangements acquiring artificial polarity, which in turn causes aberrant gene and protein expression (Cukierman et al., 2001; Nickerson et al., 2001; Kelm et al., 2003; Delarue et al., 2014). In contrast, three-dimensional (3D) culture systems offer the unique opportunity to culture cancer cells alone or with various cell types in a spatially relevant manner, encouraging cell–cell and cell–matrix interactions that closely mimic the native environment of tumors (Baal et al., 2009). These interactions cause the 3D-cultured cells to acquire morphological and cellular characteristics relevant to in vivo tumors (Ma et al., 2012). Some examples include breast cancer cells co-cultured with luminal cells, myoepithelial cells and stromal fibroblasts in 3D exhibit features reflective of ductal carcinoma in situ (Holliday et al., 2009); Ewing tumor MCTS closely resemble patient tumors in context of ERK1/2 MAPK and PI3K ± AKT pathway activation, cell–cell junctions and proliferative index (Lawlor et al., 2002). Comparison of gene and protein expression reveals that metabolic, cell stress-response, structural, signal transduction, and cellular transport proteins are expressed at elevated levels in spheroids compared to 2D-cultured cells (Hickey et al., 2008; Weigelt & Bissell, 2008). Moreover, cell adhesion and junction proteins that influence cell aggregation and compaction can be upregulated in spheroids compared to cells in monolayer (Kang et al., 2007; Oktem et al., 2014). Taken together these studies demonstrate the advantages of using 3D culture systems for in vitro oncology studies, as they allow evaluation of TME's effect on tumor, bridging the gap between 2D culture models and in vivo whole animal systems.

2. Various three-dimensional culture models of tumor

The predominant 3D culture models of cancer include: a) tumor tissue explant, b) "tumor on a chip", and c) multicellular tumor spheroids (MCTS) (Fig. 1, Table 1).

2.1. Tumor tissue explant

"Tumor tissue explant" is one of the earliest 3D models of cancer and involves culturing excised human tumors in tissue culture plates (Ritter et al., 2007). This model has been used mainly for in vitro testing of drug efficacy. In this method, tumor tissue collected after biopsy is cleared of necrotic tissue and is placed on collagen-coated surface, where it adheres to or gets embedded within the collagen (Fig. 1A). Media is added and the tumor is cultured for a desired period of time, followed by intratumoral injection with test compounds (Freeman & Hoffman, 1986). Preservation of the original tumor tissue architecture, including

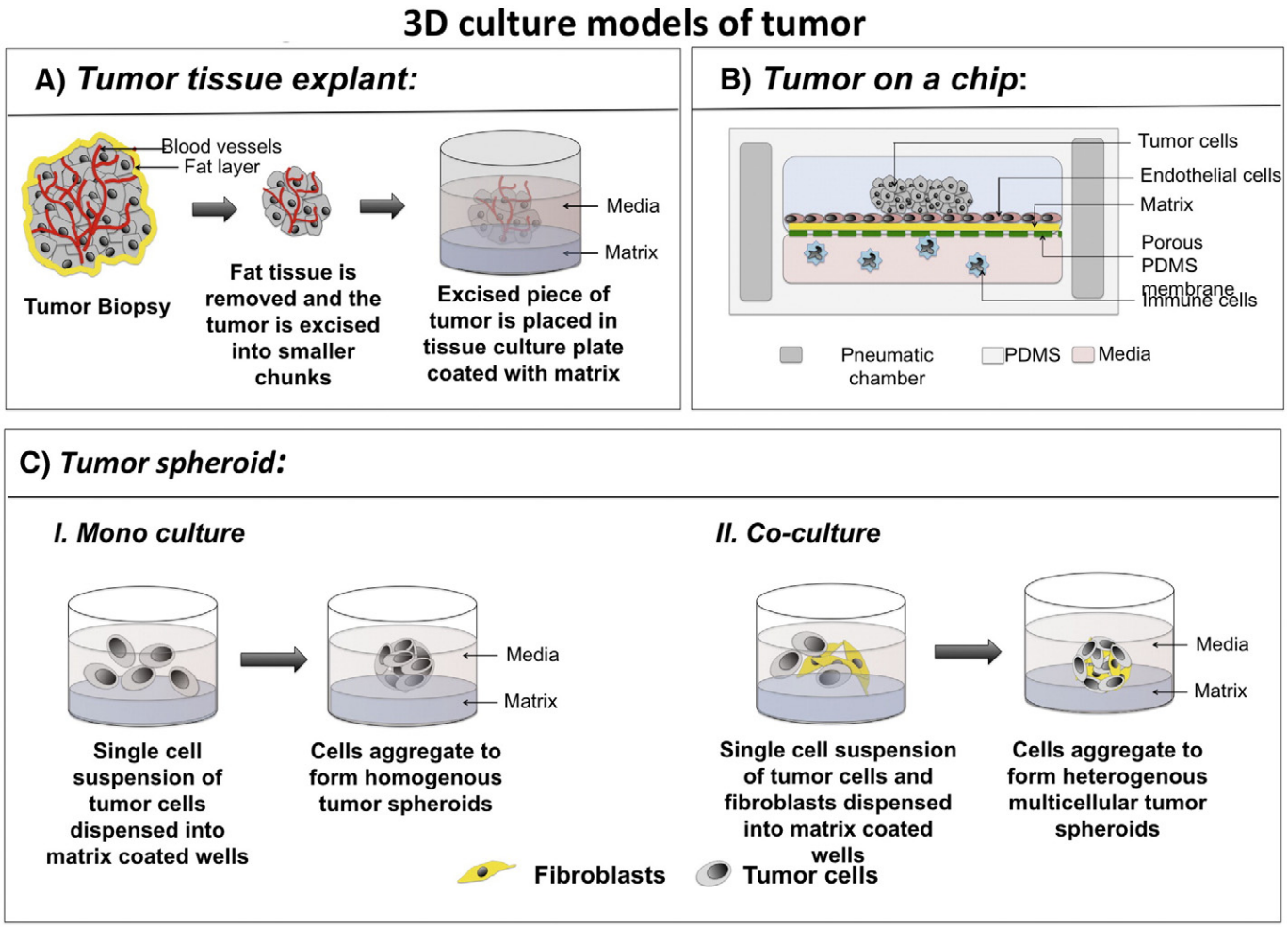

Fig. 1. Schematic representing the various 3D models of cancer. **A.** Excised tumor biopsy is processed to remove the excess fat and necrotic cells, and cut into small pieces. After washing the tumor in PBS, it is placed on a tissue culture plate that has been coated with a matrix, such as Matrigel of methylcellulose, to which the tumor sits atop firmly or is embedded. Media is added and the tumor is cultured for the duration of the experiment. **B.** "Tumor on a chip" represents a vasculature mimicking microfluidic device consisting of PDMS chambers with highly organized microchannels and pneumatic chamber (dark gray) on either sides. The microchannels (pink) contain media, in which immune cells and circulating tumor cells navigate. The top chamber contains matrix coated (yellow) porous membrane (green), with a monolayer of endothelial cells on top. The tumor cells are loaded through an inlet into the top chamber. Cells that have been genetically modified to express fluorescent protein can be observed in real time to monitor their functional changes, such as invasion and migration. **C.** Schematic depicting tumor spheroid formation where tumor spheroids have been generated by culturing tumor cells alone or in combination with fibroblasts.