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Review

Tumor spheroid assembly on hyaluronic acid-based structures: A review

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ABSTRACT

Two-dimensional (2D) cell culture is the main methodology used for screening anticancer therapeutics. However, these 2D cellular models misrepresent the architecture of native tumors, leading, in some cases, to unsuccessful prediction of cancer cell response to drugs. To overcome such limitations, cell growth in three dimensions (3D) arises as an alternative to reproduce in vitro the cellular arrangement found in tumors. Among the 3D cancer models developed so far, spheroids are the most attractive since these are cellular aggregates that broadly mimic many features of solid tumors affecting humans, like cell–cell interactions. One of the most applied techniques for producing spheroids is the liquid overlay technique, in which cells aggregate due to their limited adhesion to certain biomaterials, usually agarose or agar. Recently, the suitability of hyaluronic acid (HA) for spheroids assembly and HA-cell surface receptor interactions has been investigated. Ergo, this review gathers a summary of different studies where HA-based structures were developed and used for tumor spheroids production in order to be used in vitro as reliable 3D tumor models for therapeutic screening purposes.

Contents

1. Introduction

Conventional cancer treatments (e.g., chemotherapy, radiotherapy and surgery) are known for triggering side effects and, in some types of cancer, for displaying a limited therapeutic outcome (Mross & Kratz, 2011). Such limitations demand the development of new therapeutic approaches. To accomplish such an objective, it is pivotal to develop new, accurate in vitro tumor models that can provide reliable experimental evidence on drug screening in a short period of time and with reduced expense. Nowadays, two-dimensional (2D) cell culture is still the standard procedure used to evaluate the effectiveness and safety of new pharmaceutical compounds during pre-clinical assays, since these types of cell culture

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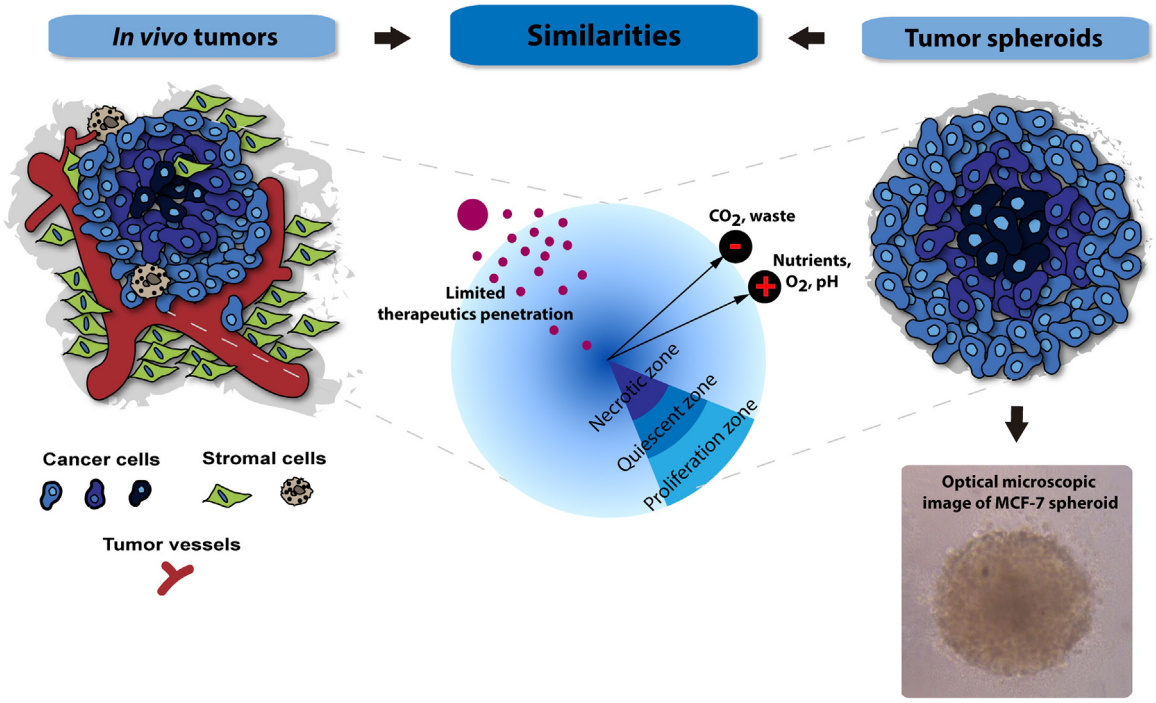

Fig. 1. Common structural features displayed by tumor spheroids and solid tumors. Tumor spheroids are organized in three distinct layers of cells (necrotic, quiescent and proliferative) as in real tumors. Such a structural arrangement is a consequence of nutrients, gases, pH and waste gradients. Additionally, spheroids also display a limited penetration for therapeutic molecules.

are easy to handle, fast to grow and cost-effective. Nevertheless, cell growth on flat surfaces does not completely represent the cell–cell and cell-extracellular matrix (ECM) interactions that occur in real tumors, nor the proliferation, survival, migration or invasion capacity exhibited by cancer cells (Yamada & Cukierman, 2007). Furthermore, the US National Cancer Institute (NCI) considers that various cells of NCI-60 (a group of various cell lines recurrently used by researchers around the world for drug-screening purposes) are adapted to grow on plastic cell culture materials in different conditions from that of their native environment, leading to altered cellular behaviors and expression profiles (Ledford, 2016). As a consequence, some 2D cell culture assays provide inaccurate and wrong predictive data about the activity of bioactive molecules when compared to their in vivo counterpart (Bhadriraju & Chen, 2002).

Due to that, the NCI is developing newer tumor models, like patient-derived xenografts (PDX) that are obtained by implanting pieces of human tumors into mice (Ledford, 2016). Notwithstanding that, the development of other platforms for therapeutic evaluation should avoid ethical and legal issues associated with animal experimentation. Accordingly, researchers are currently developing three-dimensional (3D) cell culture models like spheroids that are able to better reproduce the structural organization presented by solid tumors. The similarities found between them include growth kinetic rates, gene expression profiles and cell layer arrangement (including proliferative, quiescence and necrotic strata) (reviewed in (Fennema, Rivron, Rouwkema, van Blitterswijk, & de Boer, 2013; Mehta, Hsiao, Ingram, Luker, & Takayama, 2012)) (Fig. 1). Moreover, like solid tumors, these 3D cellular aggregates also display nutrients, gases (O_2 and CO_2) and pH gradients. Furthermore, spheroids display higher resistance to therapeutics due to their limited penetration (Fig. 1), as well as due to upregulated survival and anti-apoptotic protein expression (e.g., B-cell lymphoma 2 (Bcl-2) and survivin) (Kim, Ho, & Wu, 2011).

Up to now, gyratory rotation (Sasaki, Yamamoto, Yamaguchi, & Sugiyama, 1984), hanging drop (Timmins & Nielsen, 2007), liquid overlay technique (LOT) (Costa, Gaspar, Coutinho, & Correia, 2014) and microfluidics (Wu, Di Carlo, & Lee, 2008) have been used to produce spheroids in a quick and reproducible way for high throughput screening (HTS). All these techniques allow the production of 3D cellular aggregates constituted by cancer cells or other cell types (e.g., fibroblasts, hepatocytes and stem cells). However, spheroids produced by these techniques display a low percentage of some ECM components as well as cell-ECM interactions. Therefore, a huge effort is currently being made for these 3D models to reproduce the complex tumor ECM, since the mechanisms that regulate the metabolism of cancer cells and also their response to therapeutic molecules can be influenced by the ECM constituents and cells-ECM cross talk (Lu, Weaver, & Werb, 2012).

Hyaluronic acid (HA), also known as hyaluronan or hyaluronate, is a non-sulfated glycosaminoglycan of the proteoglycan complex found in the ECM (Fig. 2) (Toole, 2004). The higher content of HA present in the cancer microenvironment favors tumor progression, leading to a reduced patient life expectancy (Auvinen et al., 2000). The role of HA in cancer progression results from the interaction of this molecule with cell surface receptors that promote transduction of intracellular signals involved in cellular differentiation, survival, proliferation, migration, angiogenesis and resistance to therapeutic molecules (as will be discussed hereafter) (Ahrens et al., 2001; Anttila et al., 2000; Auvinen et al., 2000; David et al., 2004; Kim et al., 2004; Kouvidi et al., 2011; Laurich et al., 2004; Misra, Ghatak, Zoltan-Jones, & Toole, 2003; Toole, 2004; Vincent, Jourdan, Sy, Klein, & Mechti, 2001; Zhang, Underhill, & Chen, 1995; Zhang et al., 2002).

In addition, cells display a reduced adhesion to HA (Khademhosseini et al., 2004; Li et al., 2012; Pavesio, Renier, Cassinelli, & Morra, 1997). This property favors tumor spheroids assembly, considering that when cells are seeded on poorly adhesive biomaterials, the establishment of few cell-biomaterial physical interactions results in the formation of cellular aggregates (Fennema et al., 2013).