NARRATIVE REVIEW

Reconstructing an ovary cancer microenvironment for in vitro 3D drug testing: A new avenue for ovary cancer research

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Abstract

Objective: To elaborate on in vitro 3D ovary cancer models that can mimic the in vivo condition of an ovary cancer microenvironment for anticancer drug discovery.

Methods: Literature research was conducted in NCBI, ScienceDirect, and Pubchem databases. A total of 19 articles relevant to the search terms were included in this review.

Results: The ovary cancer niche has been described as the home of cancer stem cells, multipotent stromal cells, fibroblasts, blood and lymphatic vessels, growth factors, extracellular matrix protein (ECM). Cell cancer behaviour toward anticancer drugs can also be altered by deposition of ECM. A human ovary decellularized protocol generates ovary scaffold. Incorporation of cancer stem cells (CSC) in the ovary cancer niche thus yield 3D reconstructed microenvironment for further anticancer drug testing.

Conclusion: The 3D reconstructed microenvironment provides a platform for further anticancer drug testing.

Keywords: Cancer microenvironment, Scaffold, Ovary cancer, CSC, ECM.

Introduction

Cancer can be treated in many ways, such as by surgery, radiation therapy, and anticancer drugs, for certain period of time. Anticancer drug discovery, however, has shown a low success rate at the preclinical and clinical development phases.[1] Most of these trials failed to give information about the further prediction of the drug efficacy and safety.2 Therefore, models that can mimic the in vivo condition of human tumour microenvironments are needed.[2]

Ovary cancer is one of the silent killers, among other types of cancer, with 152,000 deaths a year.[3] One way to improve the success rate for anticancer drugs, specifically for ovary cancer, is to develop precise models for preclinical development that can be used for drug screening and in vitro disease modelling. 3D in vitro models can mimic human tumour microenvironments in a certain way. They have the ability to form and maintain interactions between cells and cell-ECM, which suggests a better in vivo environment representation. Furthermore, 3D in vitro models overcome 2D in vitro limitations, as 3D models are found to be suitable for observing cell viability, proliferation, signalling and migrations.[4]

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Methods

A literature search was performed from April-July 2019 in three databases, NCBI, ScienceDirect, and Pubchem. The combination of terms used for the search included "cancer ovary niche", "native scaffold", "decellularization", "CSC ovary", and "ECM". Inclusion criteria were: (i) studies published in the last 5 years, (ii) review articles and original articles, and (iii) full text articles open access. A total of 19 articles relevant to the search terms were included in this review. Search strategy is defined in the Table.

Table: Literature search strategy.

Source	Keywords	Counts	Counts-Related	Total
PubMed	Ca ovary niche	15	4	19
	Native scaffold	1480	2	
	Ovary decellularization	7	3	
	CSC ovary	24	6	
Science direct	Cancer ovary niche	708	4	

Result and Discussion

Cancer ovary vary niche components

A cancer microenvironment, or niche — a complex network around cancer cells — is comprised of multipotent stromal cells/mesenchymal stem cells, fibroblasts, blood and lymphatic vessels, growth factors (e.g., VEGF, TGF-β, and PDGF), cytokines, immune cells that produce inflammatory mediators, and an extracellular matrix (ECM). Cancer vessels is important for

cancer cells activity i.e. proliferation, migration, invasion of ECM and metastasize to distant organs. ECM and cancer vessels are supported by Cancer-Associated-Fibroblast (CAF).[6] A cancer alternative therapeutic approach targeting cancer niche may provide a more effective cancer treatment by working against the tumour cells, the ECM, cancer vessels and CAF.[6,7]

Cancer cells invade through dense ECM by degrading the surrounding tissue and creating paths to escape with the use of proteases. Matrix metalloproteinase (MMPs) is the most significant group of enzymes involved in ECM remodelling and collagen along with other proteins in ECM degradation. In epithelial ovarian cancer (EOC), ECM is actively modulated by MMPs leading to tumour progression, whereas further dysregulation is inhibited by tissue inhibitors of metalloproteinases (TIMPs).[8] Several MMPs were highly expressed in eight ovarian cancer cell lines.[9]

MMP-1, which has dominant roles in the degradation of types I, II, and III collagen, has been found to be positively correlated with tumour cell migration and invasion. Ovary cancer cells secrete angiogenic factors and growth-regulated oncogene-alpha. These factors act on CXCR1/2 endothelial receptors which in turn induce protease-activated receptor-1 (PAR1). PAR1 subsequently activates MMP-1.[9]

The alteration of MMPs expression and the modulation of cell-ECM interaction are regulated by transforming growth factor beta induced (TGFBI) and secreted protein acid rich in cysteine (SPARC). TGFBI is the secreted ECM protein which stabilizes microtubule activity during ovarian cancer cells proliferation to the paclitaxel treatment. The sensitization occurs via integrin-mediated FAK and RhoA activation which stabilizes microtubule activity. SPARC is an upstream regulator of TGFBI. SPARC can also interact with collagen type I. This interaction is necessary for collagen fibrillogenesis. SPARC directly interacts with and organizes TGFBI into a mature fibrillar from within the ECM. Since SPARC is secreted into the extracellular tumour microenvironment and is required for TGFBI fibrillar deposition, its expression may indirectly influence a chemotherapy response. Interestingly, both normal and cancer cells elicit growth inhibition when exposed to extracellular-derived SPARC, but only cancer cells undergo increased apoptosis.[10]

ECM composition effect in cancer progression

The composition of ECM can predict the prognosis of the tumour, as ECM acts as a reservoir of growth factors. Under normal conditions, ECM is divided into the interstitial matrix, the connective tissue (stroma)-formed by stromal cells, and the basal membrane — a specialized layer at the base of the epithelial and/or endothelial cells. ECM composition, biomechanics and anisotropy are different in each tissue and organ. In cancer conditions, changes in growth factors and ECM components provide tumour cells and the microenvironment with signals for sustained proliferation, growth promotion, cell death resistance, replicative immortality, angiogenesis induction and invasive progression toward metastasis.[11,12]

Ovarian cancer cells need to aggregate and attach to the mesothelial lining to establish metastasis. These initial steps in ovarian cancer progression, as well as the molecules involved in ovarian cancer cell adhesion, are still poorly understood. There is increasing evidence that molecules in the extracellular matrix (ECM) displayed adhesive properties as shown in the tumour stroma. The tumour cells altered specific tumour niches to facilitate metastasis. Overexpression of Chondroitin-Sulfate-E, a 4,6 sulfated glycosaminoglycan produced by ovarian cancer cells, increases their adhesive properties and are associated with metastatic lesions.[12]

3D in vitro models for anticancer drug discovery

In recent years, 3D in vitro models for anticancer drug discovery have been commonly used in comparison with animal models and 2D in vitro models. Animal models (e.g. tumour-xenograft-mice) are used to evaluate tumour growth, cellular transformation, metastasis, and other tumour cell activities. However, a high cost, standardized animal lab facility and animal handling, in addition to ethical considerations, restricted the use of animal models. 2D in vitro models are quicker to set up, inexpensive, and simple. However, even with these benefits, the 2D in vitro models failed to illustrate a human tumour microenvironment.[5] An in vivo environment, for example a human tumour microenvironment, has interaction between cells and extracellular matrices (ECMs), which 2D in vitro models do not have due to the growth condition of 2D cells. Cancer cells that are grown on this 2D surface lose the signalling pathway that makes the responses for cell proliferation, migration, differentiation and other cell regulations different from the in vivo condition. It can be said that 2D in vitro models are not biologically representative of human tumour microenvironments, and do not represent the growing tumour cells.[1]

Synthetic 3D microenvironments could revert the tumour microenvironment, thus providing a boost in antitumour response. In immunotherapy studies, T cell therapy

showed long-lasting survival benefits limited to a small population of patients in various solid cancer types. The most important limiting factor is the systemic delivery which delivered only small proportions of T cells to the tumour microenvironment. Local immunomodulation using 3D biomaterial-based scaffolds reversed the microenvironment of the tumour. This 3D in vitro model using biomaterial-based scaffolds could be used for sustained delivery of immunomodulator and boosted the antitumour response in tumour mincroenvironment.[13] These synthetic immune niches have been developed rapidly and used to modulate the immune response. 3D in vitro models, like VITVO, have the in - vivo environment that acts as a bridge between animal models and 2D in vitro models. The variety of these 3D in vitro models enable toxicology and preclinical testing or preclinical anticancer drug testing.[4] Another synthetic immune niche to develop is the tumour-draining lymph nodes (TDLNs) which is a main site for proliferation of anticancer T cells. Relatively, this synthetic immune niche can be built with a 3D scaffold model. These 3D in vitro models are similar to the native cancer's immune niches.[4,13]

3D in vitro models were divided into with scaffold and without scaffold (non-scaffold).[5] Scaffold based culture established a structural support for cellular attachment and tissue development. The scaffold was also able to function as mold so that a cells' characteristics and functions appeared in native condition. Scaffold based 3D cultures are derived from natural and synthetic materials. Commonly used natural materials are derived from ECM components such as collagen, fibronectin, gelatin and laminine. Polymers, titanium and peptides are some of the synthetic materials that are used.[14]

The 3D spheroid culture, hanging drop method, low adhesion plates and bioreactors are methods of non-scaffold based cultures. In 3D spheroid culture, there are multicellular aggregates by which cells form their own extracellular matrix components. An appropriate technique to produce 3D spheroid cultures fulfil the following criteria: the efficiency of 3D spheroid formation, spheroid sizes and shapes, cellular physiology and assessment.[5]

Ovary cancer tissue decellularisation as 3D cancer niche model

Ideal scaffolds that mimic cancer microenvironments originate from the decellularisation of cancer tissue, and are referred to as native scaffolds. Ovary scaffold literature encompasses work related to the preservation of fertility by the decellularisation of ovaries and further establishment of ovary tissue engineering. Methods of decellularisation are physical, chemical, and enzymatic, and solubilize nucleic and cellular components.[15] Decellularisation methods are used to eliminate the cellular component from ECM with less damage to the structure and chemical technique. Physical decellularisation techniques comprises of freeze-thawed organ or tissue, sonication of organ or tissue, and grinding of organ or tissue. The physical technique minimizes the use of toxic chemicals in the decellularisation process. However, it is more disruptive to ECM structure. Enzymatic decellularisation techniques used a combination of enzymes which digested the ECM structure. Each of decellularisation methods have advantages and disadvantages. Optimized decellularisation protocol required a combination of physical, chemical and enzymatic techniques. Example of the protocol begins with repetitive freeze-thawed which is then followed by immersion in 1% Triton-X and 0.5% SDS and ends with DNase I digestion. Liu et al. in 2017 demonstrated that this combination is able to decellularise porcine ovary tissue while preserving the function of ECM for further ovary function after in vivo transplantation. Another decellularisation protocol on mouse ovary tissue was used in combination with chemical decellularisation.[15,16]

Chemical decellularisation uses detergents such as sodium lauryl ester sulfate (SLES), Sodium deoxycholate solution (SDC), ionic detergent sodium deoxy sulfate (SDS), and Triton-X. Sodium deoxy sulfate (SDS) and Triton-X are the most frequently used detergents. SDS is more effective in removing nuclear membranes and intracytoplasmic components, but becomes cytotoxic when used for a longer period of time and is disruptive to ECM structures.[15] Triton-X is less disruptive to ECM microstructures than SDS. However, the application of Triton-X combined with DMSO was not effective for the decellularisation of mouse ovary tissue. SDC is a milder detergent compared to SDS, with superior retention of ECM protein (e.g. glycosaminoglycan, collagen and elastic fibres).[16] SLES is another type of detergent that is milder than SDS. Hassanpour et al in 2018 showed that 1% SLES decellularisation for 48 hours followed by DNase I digestion preserved natural ovarian niches. This decellularised ovary scaffold was able to support the ovarian stromal cells and oocytes, thus facilitating folliculogenesis.[17]

Sodium hydroxide (NaOH) was found to be a more suitable agent for decellularisation and preserving ECM in comparison to SDS.[18] Eivazkhani et al 2019 developed a decellularisation protocol by NaOH in mouse, sheep and human ovaries and compared the results with SDS

decellularisation. Ovary bioscaffolds by NaOH decellularisation have a comparable ECM composition with SDS decellularisation. The recellularisation was more efficient in NaOH decellularised ovaries. This difference translates further in favour of follicular reconstruction and maintaining cell viability superior than SDS.[18]

CSC in 3D cancer ovary niche for new drug testing

Cancer stem cells (CSC) have been identified as the cell source of cancer relapse in chemo/radio-resistant cells in heterogeneous cancer cell populations. Incorporating CSC in 3D reconstructed microenvironments could reveal novel anti-cancer drug discovery. CSC niches are composed of physical environments and chemical environments such as Matrigel, laminine and cells such as stromal cells. Hypoxia induces the activation of HIF-1 and HIF-2 signalling pathways via inhibition of DNA binding 1 (ID2)-dependent-Von Hippel Lindau (VHL) inactivation. HIF signalling is required for maintenance of CSC. Matrigel provides a complete environment for the differentiation of CSC. Laminine increased CSC proliferation, self-renewal and tumorigenic capacity. Co-culture CSC with stromal cell components created the molecular composition closed with the in vivo cancer condition.[19]

3D CSC niches can be reconstructed in vitro with or without scaffolds. The scaffold approach uses natural or synthetic scaffolds. Natural scaffolds, such as collagen or gelatin-hydrolysis (product of collagen) function as structural support for CSC or as a carrier for CSC. Another natural scaffold (e.g. Elastin) is more flexible than collagen and has been used for CSC 3D culture. Fibrinogen or fibrin coated the CSCs for superior matrix-cell interaction by binding to integrin. Silk which consists of sericin has the capacity to bind fibrillar components together. Polysacharide based natural scaffolds (e.g. agarose, alginate, HA and chitosan) have been applied as well in 3D CSC culture and assays. The combination of natural scaffolds or mixed scaffolds have shown enhanced strength, cell adherence, compatibility, low immunogenicity and are biodegradable. The limitations of natural scaffolds are nutrient diffusion and the variability of culture results. Synthetic scaffolds overcome those limitations by an engineered-design matrix, such as L-lactic acid, poly(lactide-co-glycolide) (PLGA), polycaprolactone (PCL) or PEG-hydrogel.19

Creating 3D CSC niches without scaffolds uses bioengineering with the usage of stirred tank bioreactors, ultra-low attachment plates, and hanging drop plates. Biomimetic nano-cilia and microfluidics have included the 3D culture of ovarian cancer cell lines. This microenvironment enables real time monitoring of cancer cell differentiation and epigenetic reprogramming.[19]

Conclusion

The 3D ovary cancer niche provides a platform for further anticancer drug and immunotherapy testing. Incorporation of cancer stem cells in the decellularized ovary cancer niche enables real time monitoring of cancer cells behaviour and activity. This microenvironment mimic in vivo conditions in ovarian cancer patients.

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