Published in final edited form as:

Adv Drug Deliv Rev. 2014 December 15; 0: 184–192. doi:10.1016/j.addr.2014.07.003.

Three-dimensional modeling of ovarian cancer

White Erin, Kenny Hilary, and Lengyel Ernst

The University of Chicago

Abstract

New models for epithelial ovarian cancer initiation and metastasis are required to obtain a mechanistic understanding of the disease and to develop new therapeutics. Modeling ovarian cancer however is challenging as a result of the genetic heterogeneity of the malignancy, the diverse pathology, the limited availability of human tissue for research, the atypical mechanisms of metastasis, and because the origin is unclear. Insights into the origin of high-grade serous ovarian carcinomas and mechanisms of metastasis have resulted in the generation of novel three-dimensional (3D) culture models that better approximate the behavior of the tumor cells in vivo than prior two-dimensional models. The 3D models aim to recapitulate the tumor microenvironment, which has a critical role in the pathogenesis of ovarian cancer. Ultimately, findings using models that accurately reflect human ovarian cancer biology are likely to translate into improved clinical outcomes. In this review we discuss the design of new 3D culture models of ovarian cancer primarily using human cells, key studies in which these models have been applied, current limitations, and future applications.

Keywords

Tumor microenvironment; 3D models; Metastasis; Ovarian cancer; Breast cancer; Melanoma; Fallopian tube

1. Introduction

1.1. Ovarian cancer pathogenesis

A mechanistic understanding of the pathogenesis of ovarian cancer (OvCa) requires the development of new experimental models that recapitulate the unique biology of OvCa initiation and progression. However, modeling OvCa is challenging as a consequence of the genetic complexity and diverse pathology of the disease, the limited availability of human tissue for research, the unique mechanisms of metastasis, and because the origin has not yet been determined [1], [2], [3] and [4]. Though the disease is predominantly referred to as OvCa, the term has evolved to reflect the possibility of additional sites of origin including the fallopian tube epithelium and the abdominal peritoneum [5], [6] and [7].

The majority (80%) of OvCa is epithelial in origin [8]. Four subtypes of epithelial OvCa have been defined (serous, endometrioid, clear cell, and mucinous) that are distinguished by

their genetic characteristics and histopathologies [4], [9] and [10]. High-grade serous OvCa is the most prevalent histological subtype and accounts for the high incidence of mortality (5-year survival rate of 30%) associated with the malignancy [10], [11] and [12]. Patients diagnosed with high-grade serous OvCa typically present with advanced-stage metastatic carcinoma as evidenced by extensive intra-abdominal metastasis [4] and [9]. Discovery of OvCa at an advanced stage can be attributed to the non-specific symptoms associated with OvCa, the lack of accurate OvCa biomarkers and screening tools, and the limitations associated with current imaging modalities [3] and [4].

1.2. Origin(s) of ovarian cancer

A significant improvement in our understanding of high-grade serous OvCa pathogenesis requires a better understanding of the possible origins, which include the ovarian surface epithelium, the fallopian tube epithelium, and the abdominal peritoneum [5], [13] and [14]. The discovery of in situ lesions called serous tubal intraepithelial carcinomas (STICs) in the fallopian tube fimbriae of women with BRCA1/2 mutations provided evidence for the fallopian tube as the dominant site of high-grade serous carcinogenesis [15], [16], [17], [18] and [19]. The in situ carcinomas showed similar genetic and morphological characteristics to advanced high-grade serous OvCa tumors, suggesting that the in situ tumors may be precursors to high-grade serous carcinomas [16], [19], [20] and [21]. Based on these observations, a new conceptual model for high-grade serous carcinogenesis has evolved. The current understanding of high-grade serous carcinogenesis from the fallopian tube includes the early acquisition of TP53 mutations by fallopian tube epithelial cells, progression into STICs, and eventual evolution into an invasive tumor that could directly disseminate to the ovarian and peritoneal surfaces [3]. Several new genetic mouse models have emerged that mimic high-grade serous OvCa arising from the fallopian tube. For example, Perets et al. generated a model for high-grade serous OvCa that originates in fallopian tube secretory epithelial cells (FTSECs) and recapitulates the genetic changes (TP53, BRCA, and PTEN mutations) discovered in human high-grade serous OvCa tumors, the preinvasive STIC lesions, and the typical progression to advanced-stage disease [22]. Additionally, Sherman-Baust et al. generated a transgenic mouse model that develops tumors in the fallopian tubes that are highly reminiscent of human STICs [23], [24] and [25]. These models will be important tools for resolving the site and cell of origin of high-grade serous carcinomas and for studying the early events in OvCa progression.

Insights into the genetics of high-grade serous carcinomas have emerged from The Cancer Genome Atlas, which performed a high-resolution, large-scale genomic analysis of high-grade serous OvCa tumors (clinical stages II–IV) [26]. A narrow spectrum of genetic alterations in advanced-stage carcinomas was identified that included a high frequency (96%) of mutations in TP53 and a lower prevalence (< 10%) of somatic mutations in BRCA1, CSMD3, NF1, CDK12, FAT3, GABRA6, BRCA2, and RB1 [26]. Importantly, tumors were also characterized by extensive somatic copy number changes, and mutations and promoter methylation in genes involved in DNA repair [26] and [27]. Despite this cutting-edge sequencing analysis, the key genetic alterations that drive high-grade serous carcinogenesis are only beginning to be understood.

1.3. Mechanisms of ovarian cancer metastasis

Metastasis of OvCa cells is thought to be initiated by detachment of single OvCa cells or multicellular clusters from the primary tumor and transport of these cells throughout the peritoneal cavity through natural peritoneal fluid flow [4]. OvCa cells adhere to the mesothelium that covers the peritoneal surfaces where they initiate metastatic colonies [4] and [28]. The OvCa cells preferentially metastasize to the contralateral ovary, omentum, and abdominal peritoneum [4]. Transformation of the normally soft, adipose-rich omentum into a solid tumor can result in an obstruction of the stomach and bowel [4]. The establishment of the secondary metastatic tumors is often responsible for patient death [4] and [29].

1.4. Principles of three-dimensional models

Three-dimensional (3D) culture models that aim to recapitulate carcinogenesis and metastasis are emerging for multiple epithelial cancers [30] and [31]. Modeling cancer cell behavior in the context of the complex tumor microenvironment requires the generation of organotypic cultures that consist of a variety of distinct cell types and ECM proteins, in a 3D arrangement that is physiologically accurate. These models are frequently established using primary cells that are isolated from human tissue and then assembled into an organized 3D structure that replicates the epithelium from which they originate. Alternatively, entire organs can be transiently cultured in vitro. An ideal model will be the structural and functional equivalent of the tissue, and will retain the genotypic and phenotypic characteristics of each individual cell type. 3D models are advantageous because they have the ability to recapitulate the cross-talk between cancer cells and stromal cells and their interactions with the ECM [32]. The development of these models has led to a better understanding of epithelial cancer biology and to new platforms for testing future therapies.

2. Advances in three-dimensional modeling of epithelial cancers

Insights into the design of 3D culture models of OvCa can be obtained from a critical evaluation of the 3D models that exist for other epithelial cancers including breast cancer and melanoma [33] and [34]. These models have advanced translational research in both fields and have provided a foundation for the development of models in the OvCa field. Therefore, we briefly discuss several of these models and high impact studies in which they have been applied in the following sections.

2.1. Three-dimensional modeling of breast cancer

The development of 3D culture models of breast cancer is attributed in large part to Mina Bissell, Joan Brugge, and colleagues [30], [35], [36], [37] and [38]. In a particularly influential study from the Bissell lab, Weaver et al. investigated the molecular mechanism by which normal and malignant mammary epithelial cells (MECs) can acquire resistance to apoptosis induced by chemotherapeutic agents [39]. Using the HMT-3522 reversible mammary epithelial tumor progression model [39] and [40] and 3D assays in which the MECs were embedded in either reconstituted basement membrane (rBM) or collagen type I gels, they determined that interactions between the MECs and the basement membrane control the response of the MECs to apoptosis [39]. When normal MECs were embedded within the rBM, they formed 3D polarized, non-proliferative structures that mimicked breast

acini [39] and [41]. In contrast, malignant MECs formed non-polarized, proliferative aggregates [39] and [41]. Intriguingly, the rBM conferred resistance to apoptosis to the normal, but not the malignant MECs [39]. When malignant MECs were grown in rBM in the presence of an antibody inhibitor of $\beta 1$ -integrin, the cells formed polarized, reverted mammary structures that were resistant to apoptosis-induction by multiple apoptotic agents. In contrast, when non-malignant MECs were grown in rBM in the presence of an antibody inhibitor of E-cadherin, polarity was significantly perturbed and the cells became highly sensitive to apoptosis. They further demonstrated that resistance to apoptosis was modulated by integrin–cytoskeleton interactions and NF κ B activation. In sum, these studies emphasized how the tissue polarity and microenvironment of the breast contribute to drug resistance and highlight the importance of using 3D models that can recapitulate the cellular environment in vivo in studies of cancer cell behavior.

More recently, Leung and Brugge used a 3D human mammary acinar culture to investigate how constitutive expression of specific oncogenes in single cells within the acini could drive clonal outgrowth of the mutant cell in within a tightly controlled epithelial environment [42]. Human non-transformed mammary MCF10A cells were cultured on rBM to generate polarized, growth-arrested 3D acini that consisted of an organized layer of epithelial cells surrounding an empty lumen. Using a lentiviral infection system, they overexpressed single oncogenes (Myc, myrAKT1, ERBB2, HPV16-E7, cyclin D1T286A) in sporadic cells within the mammary acini along with a fluorescent reporter (GFP) to identify the oncogene-expressing cells. The behavior of the oncogenically-transduced cells was then visualized over 56–85 h by time-lapse confocal microscopy. Overexpression of the receptor tyrosine kinase ERBB2 resulted in "luminal filling", a phenomenon indicative of translocation and outgrowth of the oncogene-expressing cell. This clonal outgrowth of the mutant cell was highly reminiscent of early, in situ breast carcinomas. Overall, this 3D model of mammary acini recapitulates the in vivo organization and cell dynamics of breast epithelial tissue, enabling analysis of the dynamic behavior of single transformed cells during the early stages of breast carcinogenesis in the context of the native tissue. This is an ability that is unique to 3D and not to 2D in vitro culture models.

2.2. Modeling melanoma using human skin reconstructs

Robust 3D reconstructions of human skin have been developed primarily by Meenhard Herlyn and colleagues to investigate the biology of both normal melanocytes and malignant melanoma cells [43], [44] and [45]. Studies using this model have provided critical insights into the mechanisms underlying melanoma initiation and progression. Reconstruction of human skin in vitro was accomplished by isolating primary fibroblasts, keratinocytes, and melanocytes from human tissue (neonatal foreskins) and sequentially assembling them into a stratified epithelium in culture [46]. Tissue culture inserts were first coated with rat tail collagen type I, and then with fibroblasts embedded in collagen to reconstruct the dermis. Once this layer was assembled, keratinocytes and either normal melanocytes or melanoma cells (human cell lines) were added to reconstruct the epidermis [44] and [45]. A basement membrane synthesized in vitro by the keratinocytes and fibroblasts divided the reconstructed epidermis and dermis [45]. In the 3D culture, the individual cell types displayed key phenotypic characteristics that distinguish these cells in vivo, but were not observed when

the cells were cultured as a monolayer. Normal melanocytes remained localized near the basement membrane, while melanoma cells derived from different stage primary melanomas either proliferated within the epidermis (radial growth phase) or invaded the dermis (vertical growth phase), consistent with the stage-specific behaviors that distinguish metastatic melanomas in vivo.

The skin reconstructs have been used to investigate the molecular changes that are responsible for the different stages in melanoma progression [33]. For example, Hsu et al. investigated the critical transition of primary melanomas from radial to vertical growth phase [44]. They determined that overexpression of the $\beta 3$ subunit of integrin $\alpha V\beta 3$ in radial growth phase primary melanoma cells induced the cells to switch from radial to vertical growth phase, triggering invasive melanoma growth and promoting survival in the 3D skin reconstructs. The skin reconstructs have also been used to study mechanisms of melanocyte transformation. Yu et al. demonstrated that a fraction of melanocytes that constitutively expressed mutant BRAFV600E survived rather than underwent oncogene-induced senescence as expected [47]. Additional disruption of p53 in these melanocytes using short-hairpin RNA resulted in immortalization of the oncogene-expressing cells and promoted transformation both in vitro and in vivo. Finally, the skin reconstructs have also been used as preclinical models for drug testing. For example, Lee et al. characterized PLX4032, a high-specificity inhibitor of the BRAFV600E oncogene that exhibits potent anti-melanoma activity [48]. In the 3D skin reconstruct, the inhibitor decreased proliferation and induced apoptosis of mutant BRAFV600E melanoma cells while non-transformed cells were not affected.

3. Three-dimensional modeling of ovarian cancer

In part owing to the development of sophisticated models for breast cancer and melanoma, significant advances have been made towards modeling OvCa. In this section, we highlight multiple intriguing 3D culture models of high-grade serous OvCa initiation and progression. We discuss their advantages and disadvantages, describe high-impact studies in which they have been utilized, and note potential future applications.

3.1. Modeling high-grade serous ovarian carcinogenesis arising from the fallopian tube and ovarian surface epithelia

Given the compelling evidence that high-grade serous OvCa can arise from the fallopian tube epithelium, new models are required that can recapitulate the genetic and molecular events responsible for the neoplastic transformation of this epithelium [3] and [5]. Several new models have emerged that have already provided important insights into the mechanisms of high-grade serous carcinogenesis from the fallopian tube as well as from the ovarian surface epithelium where carcinogenesis has previously been thought to originate.

3.1.1. Ex vivo fallopian tube model—The epithelium of the fallopian tube consists of both secretory and ciliated cells which both contribute to fertility [49]. The ciliated epithelial cells are important for the transport of oocytes from the ovary to the uterus, while the secretory cells promote oocyte and embryo survival [49]. To study mechanisms of transformation fallopian tube epithelial cells, Levanon et al. established a primary ex vivo

model of the human fallopian tube epithelium that recapitulates several structural and functional characteristics of the epithelium in vivo [50]. Secretory and ciliated fallopian tube epithelial cells were dissociated from fresh human fallopian tube fimbriae and co-cultured in vitro on a collagen type IV-coated, porous polyester membrane at the air/liquid boundary (Fig. 1A).

Ultrastructural analysis established the presence of both the secretory and the ciliated cells in a polarized arrangement in the co-culture [50]. Immunophenotypic analysis demonstrated the expression of lineage-specific markers of both fallopian tube secretory and ciliated epithelial cells. The dynamic nature and functionality of the culture were supported by the proliferative response of the cells to wounding and by the beating of the cilia on the ciliated epithelial cells. Additionally, mass spectrometry analysis of the secretome of the culture confirmed the presence of proteins secreted by the secretory cells in vivo.

Levanon et al. used the ex vivo fallopian tube model to investigate the kinetics of DNA repair by fallopian tube epithelial cells in response to genotoxic stress [50]. Interestingly, the FTSECs were less able to repair the DNA damage than the ciliated epithelial cells, suggesting that the secretory cells were more likely to accumulate damage resulting in genomic instability. Collectively, the experimental data from this detailed characterization of the culture indicated that it could recapitulate in vivo cell behavior with high fidelity.

Because of the limited proliferative ability of the FTSECs in culture and because the ex vivo model is small-scale and relatively short-term (viable for several weeks), and requires fresh primary human cells, the Drapkin lab immortalized the FTSECs and established an in vitro model [51] and [52]. While the in vitro model lacks the tissue architecture, polarity, and the ciliated cells compared to the ex vivo model, it enables long-term, large-scale experiments including transformation and xenograft-based tumorigenic studies [5] and [51]. Taken together, the ex vivo and in vitro fallopian tube models are two powerful new tools that can be used to ask critical questions including, what genetic alterations are required to transform the cells and initiate high-grade serous carcinogenesis from the fallopian tube?

3.1.2. In vitro fallopian tube spheroid model—Lawrenson et al. developed a spheroid-based in vitro culture model of primary human FTSECs (Fig. 1B) [53]. FTSECs were isolated from fallopian tubes immediately after surgery and spheroid formation induced by seeding the FTSECs onto poly-2-hydroxyethyl methacrylate (polyHEMA)-coated (non-adhesive) tissue culture dishes. Spheroids consisted of a monolayer of epithelial cells surrounding a hyaline matrix that was reminiscent of the ECM of the fallopian tube epithelium in vivo. When cultured as spheroids, FTSECs could survive and proliferate for 34–60 days. Gene expression analysis revealed changes in the expression > 1000 genes when FTSECs were cultured in 3D compared to 2D. These included genes involved in DNA replication and cell cycle control. The gene expression profiles and behavior of FTSECs cultured in 3D were more consistent with those of normal FTSECs in vivo than the profiles of FTSECs grown in 2D, indicating the 3D spheroid culture better modeled FTSECs in vivo.

Lawrenson et al. previously established a 3D spheroid culture model of immortalized, transformed primary ovarian epithelial cells using a similar culture strategy to that used to

model the fallopian tube epithelium [54]. This 3D model of OvCa initiation from the ovarian surface epithelium in addition to the in vitro fallopian tube spheroid model can be used to further investigate the genetic and molecular requirements for neoplastic transformation of the fallopian tube and ovarian epithelial cells, which are possible cells-of-origin of high-grade serous OvCa [53] and [54]. These two models may also be applicable to high-throughput screening to identify new OvCa therapeutic agents [54] and [55]. A comparison of the genetic changes that initiate transformation of the fallopian tube and ovarian surface epithelia may provide substantial insight into the origin(s) of OvCa and could have important implications for detection of nascent tumors and for treatment.

3.1.3. Organ cultures using alginate hydrogels—Progress has also been made in modeling initiation of high-grade serous OvCa using non-human ex vivo ovarian and fallopian tube organ cultures. For example, Jackson et al. developed an ex vivo organ culture to investigate mechanisms of repair of the ovarian surface epithelium following ovulation, given the potential contribution of ovulation to serous carcinogenesis [56] and [57]. They embedded artificially wounded mouse ovarian tissue in alginate hydrogels for transient ex vivo culture (Fig. 1C) [57]. Immunohistochemical analysis indicated that the ovarian surface epithelial cells expressed specific markers in the ex vivo culture that are characteristic of the cells in vivo (N-cadherin, vimentin, cytokeratin 8). Normal ovarian surface epithelial cells were proliferative after wounding and exhibited motility near the wound surface, allowing changes in the epithelium in response to wounding to be analyzed in 3D and demonstrating the dynamics of the culture. A similar approach was used to study the response of the ovarian surface epithelium to oxidative stress [58] and to investigate the effects of ovulation and DNA damage on the baboon fallopian tube epithelium [59]. It is thus an interesting alternative system for modeling OvCa arising from the fallopian tube and/or the ovarian surface epithelia and may be applied to studies using human tissue.

3.2. Three-dimensional models of high-grade serous ovarian cancer metastasis

3.2.1. Modeling the interactions between ovarian cancer cells and the extracellular matrix—Initially, many in vitro culture models of OvCa metastasis were designed to study the interactions between OvCa cells and the ECM. OvCa cells were typically cultured on a matrix consisting of purified ECM proteins such as collagen and fibronectin or on rBM. Barbolina et al. developed a 3D model of intraperitoneal metastasis in which DOV13 OvCa cells were cultured on 3D collagen type I gels to determine whether OvCa cell adhesion to collagen was mechanistically linked to the expression of MT1-matrix metalloproteinase (MMP) [60]. Using this approach, they determined that integrin signaling initiated by collagen binding resulted in expression of EGR1, a transcription factor that induces MT1-MMP expression and promotes proteolytic degradation of collagen and invasion of the peritoneal tissue during metastatic progression [60] and [61].

Mitra et al. used a related strategy to examine the role of the fibronectin receptor, $\alpha 5 \beta 1$ -integrin, in OvCa metastasis [62]. Using a system in which OvCa cells (cell lines) were cultured on fibronectin-coated surfaces, they uncovered a novel, fibronectin-dependent interaction between $\alpha 5 \beta 1$ -integrin and the receptor tyrosine kinase c-Met. This interaction resulted in the phosphorylation and activation of c-Met by $\alpha 5 \beta 1$ -integrin independent of

hepatocyte growth factor/scatter factor (HGF/SF), the native ligand for c-Met. Analysis of invasion of the OvCa cells through rBM demonstrated that inhibition of $\alpha 5$ -integrin, depletion of c-Met by siRNA, or both simultaneously, resulted in a dramatic decrease in tumor cell invasion. Importantly, the invasive behavior could be recovered by the activation of c-Met by HGF/SF. Collectively, their results indicated that the fibronectin-dependent interaction between $\alpha 5\beta 1$ -integrin and c-Met activates focal adhesion and SRC kinases, linking OvCa cell matrix adhesion to c-Met-directed mitogenic signaling.

Loessner et al. developed a bioengineered 3D platform to model interactions between OvCa cells and the ECM, and to analyze mechanisms of drug resistance [63]. They embedded OvCa cells (OV-MZ-6 and SKOV3 cell lines) in synthetic, polyethylene glycol-based hydrogels in which key biochemical and biophysical properties of the matrices could be modulated including stiffness, integrin binding sites, and protease activity. The synthetic hydrogels were stable for approximately 28 days allowing longer-term experiments to be performed [63] and [64]. Using this platform, they analyzed the ability of the OvCa cells to proliferate and form multicellular spheroids within the matrix and evaluated OvCa cell behavior in response to specific changes in the matrix properties. Their results indicated that OvCa proliferation in the 3D environment was dependent on cell–integrin interactions and on proteolytic remodeling of the ECM. They also evaluated the response of the spheroids to therapeutic drugs in comparison to the behavior of OvCa cells grown as 2D monolayers, to gain insight into mechanisms of chemoresistance. This analysis revealed a higher cell survival rate after treatment with paclitaxel when the cells were cultured in 3D compared to 2D and suggested that the behavior of the cells cultured in the 3D environment was more indicative of their response to chemotherapeutic agents in vivo [63].

3.2.2. The three-dimensional organotypic model of ovarian cancer metastasis

—OvCa metastasizes within the peritoneal cavity, predominantly to the omentum and the peritoneum [4]. The omentum is a visceral fold of the peritoneum that primarily consists of an adipose-rich region referred to as the greater omentum, which covers and protects the abdominal organ, prevents infections, promotes wound healing, stores lipids, and regulates fluid exchange [65] and [66]. The mesothelium covers the surfaces of the omentum and peritoneum [67] and consists of a single layer of mesothelial cells positioned on a basement membrane comprised of collagen (types I and IV), fibronectin, vitronectin, and laminin [66] and [68]. The tissue stroma, consisting of fibroblasts, immune cells, endothelial cells, and ECM proteins, lies directly beneath the basement membrane. The omental tissue is unique in that it contains a vast number of adipocytes upon which the other cell types exist.

In order to study the initial events in OvCa metastasis, Kenny et al. developed a 3D organotypic culture model that aimed to recapitulate the OvCa omental and peritoneal tumor microenvironment [69]. The 3D organotypic culture was assembled from primary human mesothelial cells and fibroblasts isolated from non-diseased human omental tissue. The omental fibroblasts were first mixed with either patient-derived or purified ECM proteins (collagen type I and/or fibronectin) [69] and [70] and seeded as a confluent monolayer on tissue culture dishes. A confluent layer of non-passaged, primary human mesothelial cells was then layered onto the culture using a ratio of 1 fibroblast: 8 mesothelial cells — an approximation based on histological analysis of the cellular composition of human omentum

(Fig. 1D) [69]. Finally, fluorescently labeled OvCa cells (cell lines) were added to the surface of the 3D culture for analysis of cancer cell adhesion, proliferation, and invasion [24] and [69].

Using the 3D organotypic model of metastasis, the functions of mesothelial cells and fibroblasts in OvCa adhesion and invasion were investigated [67] and [69]. OvCa cell adhesion to the 3D culture and the subsequent retraction of mesothelial cells were visualized over time by phase-contrast microscopy [69]. Interestingly, while 60–80% of the OvCa cells that were added to the 3D culture could adhere, only a fraction was capable of invading (18–25%). OvCa cells exhibited increased invasion through collagen specifically compared to fibronectin, vitronectin, and laminin. Analysis of adhesion and invasion of OvCa cells to primary mesothelial cells or fibroblasts alone and in the 3D model revealed that they exerted opposing effects on OvCa cells. Mesothelial cells inhibited OvCa cell adhesion and invasion whereas omental fibroblasts promoted both processes [28] and [69]. More recent analysis demonstrated that OvCa adhesion resulted in the activation of MMP-2 and cleavage of the ECM proteins fibronectin, vitronectin, and collagen type I [71]. Remodeling of these ECM proteins through proteolytic degradation promoted adhesion and invasion of the 3D epithelium by the OvCa cells during initial metastasis.

The 3D organotypic model can be modified to include other human cell types such as adipocytes, macrophages, and/or endothelial cells, which are all components of the OvCa metastatic tumor microenvironment in vivo. For example, Lee et al. incorporated endothelial cells into a 3D endothelial-tumor cell bilayer model of OvCa to investigate folic acid-conjugated fluorescent nanoparticles as potential therapeutics [72]. In another study, Nieman et al. co-cultured OvCa cells with primary human adipocytes that were pre-loaded with fluorescently labeled lipids [73]. Remarkably, co-culture resulted in lipid transfer from adipocytes to cancer cells, which enhanced tumor growth both in vitro and in vivo in a mouse model. The primary human omental adipocytes also promoted homing, migration, and invasion of OvCa cells in an adipokine-mediated process. They speculated that the fatty acids provided by the adipocytes function as an energy source for the OvCa cells that supports rapid tumor growth [73] and [74].

In summary, the 3D organotypic model of OvCa metastasis recapitulates OvCa cell proliferation, adhesion, and invasion of the omentum and abdominal peritoneum, three pivotal events in early OvCa metastasis [4], [28] and [69]. It was specifically designed to mimic the tissue organization of the human omentum and peritoneum and allowed investigation of the contributions of the cells in the OvCa microenvironment as well as the ECM to OvCa metastasis [24], [67] and [69]. Because the cancer cells can be fluorescently labeled, they can be separated from the other cell types in the 3D culture using fluorescence activated cell sorting. This makes it possible to assay changes in gene expression, mRNA or miRNA levels, and/or protein levels and modifications in the OvCa cells specifically [67] and [69]. The principal limitations of the model include the availability of primary human tissue from which the various cell types can be isolated, the viability of the 3D culture (approximately 1 week), patient variability, and the absence of vasculature, immune cells, and additional ECM proteins present in the OvCa microenvironment in vivo.

3.2.3. Ex vivo model of omental metastasis—An alternative strategy to model OvCa metastasis to the omentum was developed by Khan et al. [75]. They established an ex vivo model of the mouse omentum in which the intact tissue could be maintained in culture for approximately 10 days [75] and [76]. The omentum was isolated from a mouse (either immune competent or immunodeficient mice) and placed on a cell culture insert positioned within a single well of a 12-well tissue culture plate. After immobilization of the omentum in culture using a tissue adhesive, fluorescent OvCa cells (SKOV3ip.1, HeyA8, ID8, or CaOV3 cell lines) were added in culture media to recapitulate the initial events in omental metastasis including adhesion, proliferation, and invasion (Fig. 1E) [75]. OvCa cell attachment to the omentum and subsequent formation of microcolonies could be visualized by fluorescence microscopy and by histological analysis, and was reminiscent of high-grade serous carcinomas in vivo [75] and [76]. This approach has several advantages: it maintains the cellular composition and tissue architecture of the omentum, it contains vasculature (though not functional) unlike many other 3D models, and it does not require primary human cells.

3.2.4. In vitro spheroid models of metastasis—OvCa cells may metastasize as spheroids; clusters of cancer cells (30–200 μm in size) that have detached from the primary tumor together with stromal cells or mesothelial cells and ECM proteins [4], [24] and [77]. These spheroids can travel within the peritoneal fluid or ascites, and subsequently attach to mesothelial cells within the peritoneal cavity [78], [79] and [80]. The exact composition and functional significance of OvCa spheroids in peritoneal metastasis, however, are not yet clear. Several new 3D spheroid culture models have emerged to study OvCa progression, a subset of which we review here.

Iwanicki et al. investigated the mechanisms by which OvCa cells invade the mesothelial cell monolayers using in vitro live cell imaging of fluorescently-labeled mesothelial and OvCa cells (Fig. 1F) [81]. To generate fluorescent OvCa spheroids, OVCA433 cells were labeled with CMTPIX-red membrane dye and spheroid formation was induced by plating the cells on polyHEMA-coated tissue culture surfaces. Spheroids were then added to a confluent monolayer of immortalized, GFP-labeled lung mesothelial cells cultured on glass-bottom dishes coated with ECM proteins (fibronectin and/or collagen). The behavior of the mesothelial cells as the OvCa cells attached to the surface was visualized using time-lapse fluorescence microscopy. Interestingly, they determined that the OvCa spheroids drive mesothelial cell displacement (clearance) using an actin- and myosin-dependent mechanism of force generation, and that this promoted attachment to the underlying ECM.

Davidowitz et al. provided more detailed insight into the functional consequences of mesothelial cell clearance [82]. The authors analyzed the ability of spheroids generated from 20 different OvCa cell lines and 21 primary OvCa cell samples to induce mesothelial cell clearance using a 3D in vitro assay that modeled metastatic progression. The cell lines that exhibited strong mesothelial cell clearance activity had higher expression of genes associated with a mesenchymal program (an epithelial-mesenchymal (EMT) core signature) whereas cell lines that displayed weak or undetectable clearance activity had a higher expression of epithelial genes. Manipulation of EMT transcription factor expression could

modulate the clearance activities of the cell lines, suggesting that therapeutics that inhibit pathways that drive EMT may be one strategy to prevent invasion of the peritoneal tissue.

Muranen et al. used a 3D spheroid culture model of both ovarian and breast cancer cells to investigate mechanisms by which cancer cells can become resistant to treatment with small-molecule inhibitors of the PI3K/mTOR pathway [83]. They focused on the PI3K/mTOR signaling cascade because it is frequently activated by genetic alterations in epithelial cancers and is therefore a logical target for cancer therapeutics. Previous studies of PI3K pathway inhibitors were limited by the lack of a 3D model that could allow investigation of the effects of targeted therapeutics on tumor cell behavior (i.e. loss of polarity and survival without ECM adhesion) within a physiologically relevant epithelium. Treatment of OvCa spheroids in rBM with BEZ235, a small-molecule PI3K/mTOR dual-specificity inhibitor, revealed a substantial difference in the responses of the outer rBM-attached OvCa cells in the spheroids compared to the inner spheroid, rBM-detached cells. The matrix-attached cells were resistant to drug-induced apoptosis while the cells that lacked rBM-attachment were observed to undergo apoptosis as evidenced by cleaved caspase-3. The authors extended these results using a reverse phase protein array and determined that the expression of multiple pro-survival signaling proteins was increased in the rBM-attached cells upon treatment with BEZ235, an adaptive response that drives the resistance of the ECM-attached cells to PI3K/mTOR inhibition [83]. Overall, this 3D spheroid culture model will likely be a powerful platform for evaluating mechanisms of resistance by cancer cells to targeted therapeutics and for testing the effectiveness of new therapeutic agents in a more physiological context.

Spheroid cultures have been reported for 31 different OvCa cell lines derived from primary tumors, metastases, or ascites, by culturing cells on polyHEMA-coated tissue culture plates [55]. A comparison of cell lines grown in 2D and 3D indicated that the histological features that are characteristic of primary tumors were recapitulated in the 3D system but not in 2D. They also observed differences in the expression of specific OvCa biomarkers between 2D and 3D cultures. Finally, they determined that the cells grown in 3D exhibited different rates of proliferation, and importantly, were more resistant to two chemotherapeutics (cisplatin and paclitaxel). All three results indicated that the 3D cultures were more accurate models of OvCa biology, and it is likely that this model will be very useful as a new high-throughput drug discovery platform.

Rizvi et al. engineered a unique 3D model of OvCa micronodules to explore the effects of fluidic forces on OvCa growth and metastasis [84]. They developed a customizable microfluidic platform in which OvCa cells were floated over stromal matrices that consisted of growth factor-reduced rBM. Under these conditions, the OvCa cells formed 3D micronodules that were distributed throughout the micro-channel. Unattached cells flowed out of the chamber while the population of OvCa cells that adhered to the rBM was grown under continuous fluid flow for 7 days. A direct comparison of OvCa micronodules grown under flow versus non-flow conditions revealed a striking difference in cell behavior. The cells grown under flow exhibited increased motility and changes in morphology that were consistent with EMT.

The microfluidic system allowed immunofluorescence staining, mRNA isolation and quantitative real-time PCR, and protein isolation for biochemical analysis to be performed on the chip [84]. Thus, this is a new experimental platform to analyze the effects of fluidic forces on tumor cell behavior during metastasis, with the potential for broad application to studies of other malignancies. It will be a useful tool for the evaluation of new therapeutics for ovarian and/or other peritoneal cancers, and can be employed to investigate the evolution of OvCa cells under hydrodynamic forces during the process of dissemination throughout the peritoneal cavity via peritoneal fluid flow.

4. Conclusions and outlook

The uncertainty of the origin of high-grade serous carcinomas combined with the heterogeneous nature of OvCa has prompted the development of a variety of 3D in vitro models. An approach that integrates these individual models may accurately reconstruct the initiation and progression of the disease. Overall, new and complementary models may significantly advance our understanding of the complex biology of the disease and provide new platforms for therapeutic development.

Current 3D OvCa culture models are limited by several factors. First, they do not contain functional vasculature, a key feature of tumors in vivo that promotes metastasis [85], [86] and [87]. Incorporation of this component of the OvCa tumor microenvironment may have important implications for elucidating mechanisms of metastasis and also chemoresistance. Second, these models lack the cells that mediate the human adaptive immune response [88] and [89]. Third, the models are limited in terms of the time frame in which they can be used in experiments. Typically, the models are viable for several weeks at most, and therefore cannot be applied to long-term studies. Finally, the models that require primary human cells are limited by the availability of human tissue for research. To address these limitations, 3D in vitro and ex vivo culture models of OvCa initiation and metastasis have been developed using non-human tissue [58] and [75]. However, there are structural and functional differences between the mouse and human reproductive systems, and this underscores the potential impact of models using human cells.

Ultimately, 3D culture models of OvCa aim to accurately recapitulate the mechanisms that drive neoplastic transformation and metastasis by creating a 3D environment in vitro that mimics human tissue. Although these models are artificial, efforts to maximize their physiological relevance may improve the clinical impact of OvCa research. By integrating the knowledge gained from 3D culture models, genetic mouse models, cell lines, and human tissue, we will continue to unravel the secrets of OvCa biology.

Acknowledgments

We thank G. Isenberg at the University of Chicago for editing the manuscript. This work was supported by the National Cancer Institute (R01 CA111882 and CA169604) grants and an Ovarian Cancer Research Fund Program Project Development Grant (PPD/UC/01.12) to E. Lengyel.

Abbreviations

ECM	Extracellular matrix
EMT	Epithelial–mesenchymal transition
FTSEC	Fallopian tube secretory epithelial cell
HGF/SF	Hepatocyte growth factor/scatter factor
MEC	Mammary epithelial cell
MMP	Matrix metalloproteinase
OvCa	Ovarian cancer
polyHEMA	Poly-2-hydroxyethyl methacrylate
rBM	Reconstituted basement membrane
STIC	Serous tubal intraepithelial carcinoma
3D	Three-dimensional
2D	Two-dimensional

References

1. Landen CN, Birrer MJ, Sood AK. Early events in the pathogenesis of epithelial ovarian cancer. *J. Clin. Oncol.* 2008; 26:995–1005. [PubMed: 18195328]
2. Bowtell DD. The genesis and evolution of high-grade serous ovarian cancer. *Nat. Rev. Cancer.* 2010; 10:803–808. [PubMed: 20944665]
3. Karst AM, Drapkin R. Ovarian cancer pathogenesis: a model in evolution. *J. Oncol.* 2013; 2010:1–13.
4. Lengyel E. Ovarian cancer development and metastasis. *Am. J. Pathol.* 2010; 177:1053–1064. [PubMed: 20651229]
5. Dubeau L, Drapkin R. Coming into focus: the nonovarian origins of ovarian cancer. *Ann. Oncol.* 2013; 24
6. Levanon K, Crum CP, Drapkin R. New insights into the pathogenesis of serous ovarian cancer and its clinical impact. *J. Clin. Oncol.* 2008; 26:5284–5293. [PubMed: 18854563]
7. Nik NN, Vang R, Shih Ie M, Kurman RJ. Origin and pathogenesis of pelvic (ovarian, tubal, and primary peritoneal) serous carcinoma. *Annu. Rev. Pathol.* 2014; 9:27–45. [PubMed: 23937438]
8. Tavassoli, FA.; Devilee, P. Tumors of the ovary and peritoneum. In: Kleihues, P.; Sobin, L., editors. *Tumors of the Breast and Female Genital Organs*. Lyon: IARC Press; 2003. p. 113-203.
9. Bast RC, Hennessy B, Mills GB. The biology of ovarian cancer: new opportunities for translation. *Nat. Rev. Cancer.* 2009; 9:415–428. [PubMed: 19461667]
10. Vaughan S, Coward JI, Bast RC, Berchuck A, Berek JS, Brenton JD, Coukos G, Crum CP, Drapkin R, Etemadmoghadam D, Friedlander M, Gabra H, Kaye SB, Lord CJ, Lengyel E, Levine DA, McNeish IA, Menon U, Mills GB, Nephew KP, Oza A, Sood AK, Stronach EA, Walczak H, Balkwill FR. Rethinking ovarian cancer: recommendations for improving outcomes. *Nat. Rev. Cancer.* 2011; 11:719–725. [PubMed: 21941283]
11. Berns EMJJ, Bowtell DD. The changing view of high-grade serous ovarian cancer. *Cancer Res.* 2012; 72:2701–2704. [PubMed: 22593197]
12. Siegal R, Naishadham D, Jemal A. Cancer statistics. *CA Cancer J. Clin.* 2013; 63:11–30. [PubMed: 23335087]
13. Erickson BK, Conner MG, Landen CN. The role of the fallopian tube in the origin of ovarian cancer. *Am. J. Obstet. Gynecol.* 2013; 1–6.

14. Kurman RJ, Shih IM. The origin and pathogenesis of epithelial ovarian cancer: a proposed unifying theory. *Am. J. Surg. Pathol.* 2010; 34:433–443. [PubMed: 20154587]
15. Piek JM, van Diest PJ, Zweemer RP, Jansen JW, Poort-Keesom RJ, Menko FH, Gille JJ, Jongsma AP, Pals G, Kenemans P, Verheijen RH. Dysplastic changes in prophylactically removed fallopian tubes of women predisposed to developing ovarian cancer. *J. Pathol.* 2001; 195:451–456. [PubMed: 11745677]
16. Kindelberger DW, Lee Y, Miron A, Hirsch MS, Feltmate C, Medeiros F, Callahan MJ, Garner EO, Gordon RW, Birch C, Berkowitz RS, Muto MG, Crum CP. Intraepithelial carcinoma of the fimbria and pelvic serous carcinoma: evidence for a causal relationship. *Am. J. Surg. Pathol.* 2007; 31:161–169. [PubMed: 17255760]
17. Colgan TJ, Murphy J, Cole D, Narod SA, Rosen B. Occult carcinoma in prophylactic oophorectomy specimens: prevalence and association with BRCA germline mutation status. *Am. J. Surg. Pathol.* 2001; 25:1283–1289. [PubMed: 11688463]
18. Medeiros F, Muto MG, Lee Y, Elvin J, Callahan MJ, Feltmate C, Garber J, Cramer D, Crum CP. The tubal fimbria is a preferred site for early adenocarcinoma in women with familial ovarian cancer syndrome. *Am. J. Surg. Pathol.* 2006; 30:230–236. [PubMed: 16434898]
19. Lee Y, Miron A, Drapkin R, Nucci MR, Medeiros F, Saleemuddin A, Garber J, Birch C, Mou H, Gordon RW, Cramer DW, McKeon FD, Crum CP. A candidate precursor to serous carcinoma that originates in the distal fallopian tube. *J. Pathol.* 2007; 211:26–35. [PubMed: 17117391]
20. Kuhn E, Kurman RJ, Vang R, Sehdev AS, Han G, Soslow R, Want TL, Shih LM. TP53 mutations in serous tubal intraepithelial carcinoma and concurrent pelvic high-grade serous carcinoma—evidence supporting the clonal relationship of the two lesions. *J. Pathol.* 2012; 226:421–426. [PubMed: 21990067]
21. Chen EY, Mehra K, Mehrad M, Ning G, Miron A, Mutter GL, Monte N, Quade B, McKeon FD, Yassin Y, Crum CP. Secretory cell outgrowth, PAX2 and serous carcinogenesis in the fallopian tube. *J. Pathol.* 2010; 222:110–116. [PubMed: 20597068]
22. Perets R, Wyant GA, Muto KW, Bijron JG, Poole BB, Chin KT, Chen JYH, Ohman AW, Stekpule CD, Kwak S, Karst AM, Hirsch MS, Setlur SR, Crum CP, Dinulescu DM, Drapkin R. Transformation of the fallopian tube secretory epithelium leads to high-grade serous ovarian cancer in Brca; Tp53; Pten models. *Cancer Cell.* 2013; 24:751–765. [PubMed: 24332043]
23. Sherman-Baust CA, Kuhn E, Valle BL, Shih IM, Kurman RJ, Wang TL, Amano T, Ko MS, Miyoshi I, Araki Y, Lehmann E, Zhang Y, Becker KG, Morin PJ. A genetically engineered ovarian cancer mouse model based on fallopian tube transformation mimics human high-grade serous carcinoma development. *J. Pathol.* 2014; 233:228–237. [PubMed: 24652535]
24. Lengyel E, Burdette JE, Kenny HA, Matei D, Pilrose J, Haluska P, Nephew KP, Hales DB, Stack MS. Epithelial ovarian cancer experimental models. *Oncogene.* 2014; 33:3619–3633. [PubMed: 23934194]
25. Karst AM, Drapkin R. The new face of ovarian cancer modeling: better prospects for detection and treatment. *F1000 Med. Rep.* 2011; 3:1–7. [PubMed: 21399758]
26. The Cancer Genome Atlas Research Network. Integrated genomic analyses of ovarian carcinoma. *Nature.* 2011; 474:609–615. [PubMed: 21720365]
27. Goringe KL, George J, Anglesio MS, Ramakrishna M, Etemadmoghadam D, Cowin P, Sridhar A, Williams LH, Boyle SE, Yanaihara N, Okamoto A, Urashima M, Australian Ovarian Cancer Study. Smyth GK, Campbell IG, Bowtell DD. Copy number analysis identifies novel interactions between genomic loci in ovarian cancer. *PLoS One.* 2010; 5
28. Kenny HA, Nieman KM, Mitra AK, Lengyel E. The first line of intra-abdominal metastatic attack: breaching the mesothelial cell layer. *Cancer Discov.* 2011; 1:100–102. [PubMed: 22013555]
29. Fleming, GF.; Seidman, JD.; Lengyel, E. Epithelial ovarian cancer. In: Barakat, RR.; Berchuck, A.; Markmann, M.; Randall, ME., editors. *Principles and Practice of Gynecologic Oncology.* Philadelphia: Lippincott Williams & Wilkins; 2013. p. 757-848.
30. Debnath J, Brugge JS. Modelling glandular epithelial cancers in three-dimensional cultures. *Nat. Rev. Cancer.* 2005; 5:675–688. [PubMed: 16148884]
31. Yamada KM, Cukierman E. Modeling tissue morphogenesis and cancer in 3D. *Cell.* 2007; 130:601–610. [PubMed: 17719539]

32. Bissell MJ, Radisky D. Putting tumours in context. *Nat. Rev. Cancer*. 2001; 1:46–54. [PubMed: 11900251]
33. Berking C, Herlyn M. Human skin reconstruct models: a new application for studies of melanocyte and melanoma biology. *Histol. Histopathol*. 2001; 16:669–674. [PubMed: 11332722]
34. Weigelt B, Ghajar CM, Bissell MJ. The need for complex 3D culture models to unravel novel pathways and identify accurate biomarkers in breast cancer. *Adv. Drug Deliv. Rev.* 2014; 69–70:42–51.
35. Weaver VM, Howlett AR, Langton-Webster B, Petersen OW, Bissell MJ. The development of a functionally relevant cell culture model of progressive human breast cancer. *Semin. Cancer Biol.* 1995; 6:175–184. [PubMed: 7495986]
36. Weaver VM, Fischer AH, Peterson OW, Bissell MJ. The importance of the microenvironment in breast cancer progression: recapitulation of mammary tumorigenesis using a unique human mammary epithelial cell model and a three-dimensional culture assay. *Biochem. Cell Biol.* 1996; 74:833–851. [PubMed: 9164652]
37. Schmeichel KL, Bissell MJ. Modeling tissue-specific signaling and organ function in three dimensions. *J. Cell Sci.* 2003; 116:2377–2388. [PubMed: 12766184]
38. Lee GY, Kenny PA, Lee EH, Bissell MJ. Three-dimensional culture models of normal and malignant breast epithelial cells. *Nat. Methods*. 2007; 4:359–365. [PubMed: 17396127]
39. Weaver VM, Lelièvre S, Lakins J, Chrenek MA, Jones CR, Giancotti FG, Werb Z, Bissell MJ. $\beta 4$ integrin-dependent formation of polarized three-dimensional architecture confers resistance to apoptosis in normal and malignant mammary epithelium. *Cancer Cell*. 2002; 2:205–216. [PubMed: 12242153]
40. Briand P, Nielsen KV, Madsen MW, Petersen OW. Trisomy 7p and malignant transformation of human breast epithelial cells following epidermal growth factor withdrawal. *Cancer Res.* 1996; 56:2039–2044. [PubMed: 8616848]
41. Peterson OW, Ronnov-Jessen L, Bissell MJ. Interaction with basement membrane serves to rapidly distinguish growth and differentiation pattern of normal and malignant breast epithelial cells. *Proc. Natl. Acad. Sci. U. S. A.* 1992; 89:9064–9068. [PubMed: 1384042]
42. Leung CT, Brugge JS. Outgrowth of single oncogene-expressing cells from suppressive epithelial environments. *Nature*. 2012; 482:410–413. [PubMed: 22318515]
43. Smalley KS, Lioni M, Herlyn M. Life isn't flat: taking cancer biology to the next dimension. *In Vitro Cell. Dev. Biol. Anim.* 2006; 42:242–247. [PubMed: 17163781]
44. Hsu M, Shih D, Meier FE, van Belle PA, Hse J, Elder DE, Buck C, Herlyn M. Adenoviral gene transfer of $\beta 3$ integrin subunit induces conversion from radial to vertical growth phase in primary human melanoma. *Am. J. Pathol.* 1998; 153:1435–1441. [PubMed: 9811334]
45. Meier FE, Nesland M, Hsu M, Martin B, van Belle PA, Elder DE, Schaumburg-Lever G, Garbe C, Walz TM, Donatien P, Crombleholme T, Herlyn M. Human melanoma progression in skin reconstructs. *Am. J. Pathol.* 2000; 156:193–200. [PubMed: 10623667]
46. Li L, Fukunaga-Kalabis M, Herlyn M. The three-dimensional human skin reconstruct model: a tool to study normal skin and melanoma progression. *J. Vis. Exp.* 2011; 54:1–5.
47. Yu H, McDaid R, Lee J, Possik P, Li L, Kumar SM, Elder DE, Van Belle P, Gimotty P, Guerra M, Hammond R, Nathanson KL, Dalla Palma M, Herlyn M, Xu X. The role of BRAF mutation and p53 inactivation during transformation of a subpopulation of primary human melanocytes. *Am. J. Pathol.* 2009; 174:2367–2377. [PubMed: 19389934]
48. Lee JT, Li L, Brafford PA, van den Eijnden M, Halloran MB, Sproesser K, Haass NK, Smalley KS, Tsai J, Bollag G, Herlyn M. PLX4032, a potent inhibitor of the B-Raf V600E oncogene, selectively inhibits V600E-positive melanomas. *Pigment Cell Melanoma Res.* 2010; 23:820–827. [PubMed: 20973932]
49. Stewart C, Behringer RR. Mouse oviduct development. *Results Probl. Cell Differ.* 2012; 55:247–262. [PubMed: 22918811]
50. Levanon K, Ng V, Piao H, Zhang Y, Chang MC, Roh MH, Kindelberger DW, Hirsch MS, Crum CP, Marto JA, Drapkin R. Primary ex vivo cultures of human fallopian tube epithelium as a model for serous ovarian carcinogenesis. *Oncogene*. 2009; 1–11.

51. Karst AM, Levanon K, Drapkin R. Modeling high-grade serous ovarian carcinogenesis from the fallopian tube. *Proc. Natl. Acad. Sci. U. S. A.* 2011; 108:7547–7552. [PubMed: 21502498]
52. Karst AM, Drapkin R. Primary culture and immortalization of human fallopian tube secretory epithelial cells. *Nat. Protoc.* 2012; 7:1755–1764. [PubMed: 22936217]
53. Lawrenson K, Notaridou M, Lee N, Benjamin E, Jacobs IJ, Jones C, Gayther SA. In vitro three-dimensional modeling of fallopian tube secretory epithelial cells. *BMC Cell Biol.* 2013; 27:1–15.
54. Lawrenson K, Sproul D, Grun B, Notaridou M, Benjamin E, Jacobs IJ. Modelling genetic and clinical heterogeneity in epithelial ovarian cancers. *Carcinogenesis.* 2011; 32:1540–1549. [PubMed: 21859834]
55. Lee JM, Mhawech-Fauceglia P, Lee N, Parsanian LC, Lin YG, Gayther SA, Lawrenson K. A three-dimensional microenvironment alters protein expression and chemosensitivity of epithelial ovarian cancer cells in vitro. *Lab. Invest.* 2013; 93:528–542. [PubMed: 23459371]
56. King SM, Quartuccio S, Hillard TS, Inoue K, Burdette JE. Alginate hydrogels for three-dimensional organ culture of ovaries and oviducts. *J. Vis. Exp.* 2011; 52:1–6.
57. Jackson KS, Inoue K, Davis DA, Hillard TS, Burdette JE. Three-dimensional ovarian organ culture as a tool to study normal ovarian surface epithelial wound repair. *Endocrinology.* 2009; 150:3921–3926. [PubMed: 19423762]
58. King SM, Quartuccio SM, Vanderhyden BC, Burdette JE. Early transformative changes in normal ovarian surface epithelium induced by oxidative stress require Akt upregulation, DNA damage and epithelial–stromal interaction. *Carcinogenesis.* 2013; 34:1125–1133. [PubMed: 23299406]
59. King SM, Hilliard TS, Wu LY, Jaffe RC, Fazleabas AT, Burdette JE. The impact of ovulation on fallopian tube epithelial cells: evaluating three hypotheses connecting ovulation and serous ovarian cancer. *Endocr. Relat. Cancer.* 2011; 18:627–642. [PubMed: 21813729]
60. Barbolina MV, Adley BP, Ariztia EV, Liu Y, Stack MS. Microenvironmental regulation of membrane type 1 matrix metalloproteinase activity in ovarian carcinoma cells via collagen-induced EGR1 expression. *J. Biol. Chem.* 2007; 282:4924–4931. [PubMed: 17158885]
61. Barbolina MV, Moss NM, Westfall SD, Liu Y, Burkhalter RJ, Marga F, Forgacs G, Hudson LG, Stack MS. Microenvironmental regulation of ovarian cancer metastasis. *Cancer Treat. Res.* 2009; 149:319–334. [PubMed: 19763443]
62. Mitra AK, Sawada K, Tiwari P, Mui K, Gwin K, Lengyel E. Ligand-independent activation of c-Met by fibronectin and $\alpha 5 \beta 1$ -integrin regulates ovarian cancer invasion and metastasis. *Oncogene.* 2011; 30:1566–1576. [PubMed: 21119598]
63. Loessner D, Stok KS, Lutolf MP, Huttmacher DW, Clements JA, Rizzi SC. Bioengineered 3D platform to explore cell–ECM interactions and drug resistance of epithelial ovarian cancer cells. *Biomaterials.* 2010; 31:8494–8506. [PubMed: 20709389]
64. Loessner D, Rizzi SC, Stok KS, Fuehrmann T, Hollier B, Magdolen V, Huttmacher DW, Clements JA. A bioengineered 3D ovarian cancer model for the assessment of peptidase-mediated enhancement of spheroid growth and intraperitoneal spread. *Biomaterials.* 2013; 34:7389–7400. [PubMed: 23827191]
65. Wilkox S, Ireland G, Khwaja N, Walker M, Butt R, Giorgio-Miller A, Herrick S. A comparative study of the structure of human and murine greater omentum. *Anat. Embryol.* 2005; 209:251–261. [PubMed: 15662530]
66. Liebermann-Meffert D. The greater omentum, anatomy, embryology, and surgical applications. *Surg. Clin. N. Am.* 2000; 80:275–293. [PubMed: 10685153]
67. Kenny HA, Dogan S, Zillhardt M, Mitra AB, Yamada SD, Krausz T, Lengyel E. Organotypic models of metastasis: a three dimensional culture mimicking the human peritoneum and omentum for the study of the early steps of ovarian cancer metastasis. *Cancer Treat. Res.* 2009; 149:335–351. [PubMed: 19763444]
68. Witz CA, Montoya-Rodriguez IA, Cho S, Centonze VE, Bonewald L, Schenken RS. Composition of the extracellular matrix of the peritoneum. *J. Soc. Gynecol. Investig.* 2001; 8:299–304.
69. Kenny HA, Krausz T, Yamada SD, Lengyel E. Use of a novel 3D culture model to elucidate the role of mesothelial cells, fibroblasts and extra-cellular matrices on adhesion and invasion of ovarian cancer cells. *Int. J. Cancer.* 2007; 121:1463–1472. [PubMed: 17546601]

70. Kenny HA, Leonhardt P, Ladanyi A, Yamada SD, Montag AG, Im HK, Jagadeeswaran S, Shaw DE, Mazar AP, Lengyel E. Targeting the urokinase plasminogen activator receptor inhibits ovarian cancer metastasis. *Clin. Cancer Res.* 2011; 17:459–471. [PubMed: 21149615]
71. Kenny HA, Kaur S, Coussens LM, Lengyel E. The initial steps of ovarian cancer cell metastasis are mediated by MMP-2 cleavage of vitronectin and fibronectin. *J. Clin. Invest.* 2008; 118:1367–1379. [PubMed: 18340378]
72. Lee KY, Seow E, Zhang Y, Lim YC. Targeting CCL21-folic acid-upconversion nanoparticles conjugates to folate receptor- α expressing tumor cells in an endothelial-tumor cell bilayer model. *Biomaterials.* 2013; 34:4860–4871. [PubMed: 23562047]
73. Nieman KM, Kenny HA, Penicka CV, Ladanyi A, Buell-Gutbrod R, Zillhardt M, Romero IL, Carey MS, Mills GB, Hotamisligil GS, Yamada SD, Peter ME, Gwin K, Lengyel E. Adipocytes promote ovarian cancer metastasis and provide energy for rapid tumor growth. *Nat. Med.* 2011; 17:1498–1503. [PubMed: 22037646]
74. Nieman KM, Romero IL, Van Houten B, Lengyel E. Adipocyte tissue and adipocytes support tumorigenesis and metastasis. *Biochim. Biophys. Acta.* 2013; 1831:1533–1541. [PubMed: 23500888]
75. Khan SM, Funk HM, Thiolloy S, Lotan TL, Hickson JA, Prins G, Drew AF, Rinker-Schaeffer CW. In vitro metastatic colonization of human ovarian cancer cells to the omentum. *Clin. Exp. Metastasis.* 2010; 27:185–196. [PubMed: 20229256]
76. Clark R, Krishnan V, Schoof M, Rodriguez I, Theriault B, Chekmareva M, Rinker-Schaeffer C. Milky spots promote ovarian cancer metastatic colonization of peritoneal adipose in experimental models. *Am. J. Pathol.* 2013; 183:576–591. [PubMed: 23885715]
77. Allen HJ, Porter C, Gamarra M, Piver MS, Johnson EA. Isolation and morphologic characterization of human ovarian carcinoma cell clusters present in effusions. *Exp. Cell Biol.* 1987; 55:194–208. [PubMed: 3678576]
78. Burleson KM, Hansen LK, Skubitz AP. Ovarian carcinoma spheroids disaggregate on type I collagen and invade live human mesothelial cell monolayers. *Clin. Exp. Metastasis.* 2004; 21:685–697. [PubMed: 16035613]
79. Burleson KM, Casey RC, Skubitz KM, Pambuccian SE, Oegema TR, Skubitz AP. Ovarian carcinoma ascites spheroids adhere to extracellular matrix components and mesothelial cell monolayers. *Gynecol. Oncol.* 2004; 93:170–181. [PubMed: 15047232]
80. Burleson KM, Boente MP, Pambuccian SE, Skubitz AP. Dissaggregation and invasion of ovarian carcinoma ascites spheroids. *J. Transl. Med.* 2006; 24:4–6.
81. Iwanicki M, Davidowitz RA, Ng MR, Besser A, Muranen T, Merritt M, Danuser G, Ince TA, Brugge JS. Ovarian cancer spheroids use myosin-generated force to clear the mesothelium. *Cancer Discov.* 2011; 1:144–157. [PubMed: 22303516]
82. Davidowitz RA, Selfors LM, Iwanicki MP, Elias KM, Karst A, Piao H, Ince TA, Drage MG, Dering J, Konecny GE, Matulonis U, Mills GB, Slamon DJ, Drapkin R, Brugge JS. Mesenchymal gene program-expressing ovarian cancer spheroids exhibit enhanced mesothelial clearance. *J. Clin. Invest.* 2014; 124:2611–2625. [PubMed: 24762435]
83. Muranen T, Selfors LM, Worster DT, Iwanicki MP, Song L, Morales FC, Gao S, Mills GB, Brugge JS. Inhibition of P13K/mTOR leads to adaptive resistance in matrix-attached cancer cells. *Cancer Cell.* 2012; 21:227–239. [PubMed: 22340595]
84. Rizvi I, Gurkan UA, Tasoglu S, Alagic N, Celli JP, Mensah LB, Mai Z, Demirci U, Hasan T. Flow induces epithelial–mesenchymal transition, cellular heterogeneity and biomarker modulation in 3D ovarian cancer nodules. *Proc. Natl. Acad. Sci. U. S. A.* 2013:E1974–E1983. [PubMed: 23645635]
85. Butler JM, Kobayashi H, Rafii S. Instructive role of the vascular niche in promoting tumour growth and tissue repair by angiocrine factors. *Nat. Rev. Cancer.* 2010; 10:138–146. [PubMed: 20094048]
86. Hanahan D, Weinberg RA. Hallmarks of cancer: the next generation. *Cell.* 2011; 144:646–674. [PubMed: 21376230]
87. Schmitt J, Matei D. Targeting angiogenesis in ovarian cancer. *Cancer Treat. Rev.* 2012; 38:272–283. [PubMed: 21764518]

88. Elinav E, Nowarski R, Thaiss CA, Hu B, Chengcheng J, Flavell RA. Inflammation-induced cancer: crosstalk between tumours, immune cells and microorganisms. *Nat. Rev. Cancer*. 2013; 13:759–771. [PubMed: 24154716]
89. Robins HS, Ericson NG, Guenthoer J, O'Briant KC, Tewari M, Drescher CW, Bielas JH. Digital genomic quantification of tumor-infiltrating lymphocytes. *Sci. Transl. Med.* 2014; 5:1–7.

Author Manuscript

Author Manuscript

Author Manuscript

Author Manuscript

Modeling ovarian cancer biology in three dimensions

Figure 1.

Three-dimensional (3D) models to study ovarian cancer. A. *Ex vivo* fallopian tube epithelium model [50]. Fallopian tube secretory and ciliated epithelial cells are isolated from human fallopian tube fimbriae and cultured on a collagen type IV-coated Transwell® tissue culture insert at the interface between the air and culture media. B. *In vitro* fallopian tube spheroid model [53]. Primary human fallopian tube secretory epithelial cells are plated on polyHEMA-coated (non-adhesive) plastic culture dishes to induce spheroid formation. C. *Ex vivo* culture of ovarian tissue fragments [57]. Mouse ovarian tissue fragments are isolated and embedded in an alginate hydrogel and cultured *ex vivo*. D. *In vitro* 3D organotypic model of metastasis to the omentum and abdominal peritoneum [69]. Primary human mesothelial cells and fibroblasts are isolated from non-diseased human omental tissue and assembled into a 3D culture to which fluorescently labeled ovarian cancer cells can be added. E. *Ex vivo* model of omental metastasis [75]. The omentum is isolated from a mouse and immobilized in tissue culture inserts using cell adhesive. Ovarian cancer cells are subsequently added to the culture for experimental analysis. F. *In vitro* spheroid model of metastasis [81]. Fluorescently labeled ovarian cancer cells are added to a confluent monolayer of immortalized, fluorescent mesothelial cells and cell behavior monitored by fluorescence microscopy.