Screening of three-dimensional spheroids of ovarian cancer: identification of novel therapeutics targeting stemness and chemoresistance

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Epithelial ovarian cancer (OvCa) is the leading cause of death from gynecologic malignancies in the United States (1). More than 75% of patients are diagnosed at late stages due to the insipient protracted nature of the disease and lack of specific diagnostic symptoms and/or biomarkers (2). Despite aggressive surgical debulking and cytoreduction, 80% patients experience recurrence of a chemo-resistant disease with limited treatment options (1,3). The standard of care (SOC) treatment is aggressive surgery followed by platinum-taxane chemotherapy, or neo-adjuvant chemotherapy followed by surgery (2). However, cisplatin-resistance and recurrence develop in 25% of patients as early as 6 months, with the overall 5-year survival hovering around 30–40% (1,2).

Despite recent advances in developing chemotherapeutics, treatment options for patients with ovarian cancer are still limited. This limitation is attributed in part to initial testing and validation of cancer cells grown in 2D monolayers in tissue culture plates that do not faithfully recapitulate the human disease as these cells are presented with an artificial growth surface (plastic) and a 2D architecture instead of 3D normal tissue. These conditions impose a selective pressure on cells, which could substantially alter their phenotype and molecular properties and would provide misleading and non-predictive data for in vivo responses (4,5). Therefore, there is unmet need for development of novel preclinical models for evaluation of therapeutics that target OvCa cells and faithfully recapitulate the in vivo milieu. As a consequence, few compounds (~10%) progress to clinical development and many of them fail during clinical trials, due to either lack of clinical efficacy and/or unacceptable toxicity (6). To overcome these limitations, and bridge the critical gap between the need for predictive in vitro platforms, and the availability of bio-tools and techniques, a recent study by Hirst and colleagues (7) from Dr. Andrew K. Godwin group developed a novel approach for drug discovery based on screening their activity in 3D multicellular tumor spheroids (MCTSs) to identify novel candidates, that are often missed by traditional screening in 2D cultures.

They reported that growing ovarian cancer cell lines in MCTSs phenocopied the characteristics of the in vivo tumors with a gradient of Ki67-positive cells being highest at the surface and negative in the center as well as a central hypoxic core. Hypoxia-inducible factor-α (HIF1α) was stabilized in spheroids under normoxic conditions. In contrast, cancer cells grown in 2D cultures exhibited ubiquitous Ki67-staining and HIF1α was stabilization only under hypoxic conditions. Consistently, hypoxia-regulated genes and markers of cancer stem cells (CSCs) and well as canonical stem cell markers were significantly increased in MCTSs relative to their 2D-cultures. MCTSs exhibited a robust paclitaxel-resistance with upregulation of hypoxia-regulated stem cell markers and decreased proliferation following paclitaxel treatment compared with 2D cultures. Investigators confirmed that paclitaxel-resistance and
stemness are not driven by reduced drug penetration, but are rather due to stable phenotypic changes in ovarian cancer cells in 3D MCTSs. They presented evidence that paclitaxel-resistance, and increased markers of stemness persisted after dissociation of cells from MCTSs and growing them in 2D culture. Furthermore, they showed that drugs with limited clinical activity despite their robust *in vitro* activity in 2D-cultures as dasatinib, nilotinib, and ganetespib, induced extensive expression of stem-like genes in 3D MCTSs. The investigators then concluded that the 3D MCTSs induces a chemo-resistant stem-like phenotype that warrants their use for first-line drug screening.

The authors carried further their hypothesis to identify FDA-approved drugs that can be repurposed to accelerate a path to the clinic. They developed a novel approach integrating RNAsseq with *in silico* analysis to identify the regulatory pathways enriched in 3D MCTSs compared to 2D. With stringent cutoffs of top up- and down-regulated genes and *in silico* pathway prediction, they discovered that the top upstream regulators were related to hypoxia and the top drug hits were a nonsteroidal anti-inflammatory drugs (NSAID), fluticasone, and an antidiabetic, rosiglitazone. Thus, they constructed a focused drug library that included known cancer therapeutics, anti-inflammatory and compounds that target metabolic disorders. They reported that common hits in 2D and 3D were predominantly from the current cancer therapeutics that would be identified and validated by traditional screening approaches. Interestingly, the top unique 3D two hits were NSAID drugs, licofelone and glafenine, that have not been evaluated in preclinical or clinical studies for EOC.

Licofelone is a dual inhibitor of cyclooxygenase-2 and 5-lipoxygenase (COX-2/5-LOX) that was initially developed for the treatment of arthritis (8), whereas, glafenine, is a broad spectrum pain killer and an NSAID derivative of anthranilic acid (9). Both drugs reversed the stem-like properties and markers of ovarian cancer cells grown in 3D spheroids, and their effect was cell line-specific though independent of their mutational profile. The investigators selected licofelone for further investigation due its safer clinical profile compared to glafenine (9-13). They further demonstrated that the enhanced cytotoxic activity of licofelone in cells in 3D MCTSs correlated with their higher basal expression of CSCs marker ALHD1A. Because epidemiologic studies showed that the use of NSAIDs, specifically aspirin, lowered the risk of EOC in a dose- and frequency-dependent manner (14). The authors compared the activity of licofelone with another NSAID, celecoxib which is a selective COX-2 inhibitor, on MCTSs. They provided evidence that licofelone exhibits more potent cytotoxic activity than in MCTSs suggesting that dual targeting of COX/LOX have superior inhibitory effect on ovarian MCTSs than COX-2 inhibition alone. Moreover, licofelone exhibited a synergistic effect with paclitaxel on 3D spheroids. Importantly, combinatorial treatment of licofelone and paclitaxel led to a significant reduction in the expression of a number of stem-like transcripts in MCTSs and could even reverse the stem-like phenotype induced by paclitaxel treatment.

Indeed, ovarian CSCs have been implicated as a cause of chemoresistance and recurrence (15-17). Studies of matching primary and recurrent chemoresistant ovarian tumors revealed that primary tumors are enriched with CSCs and stem cell pathway mediators, especially at the completion of primary therapy (17-19).

Ovarian CSCs have been shown to exhibit distinctive metabolic phenotype as they favor oxidative phosphorylation (OXPHOS) over glucose metabolism, and shunt pyruvate to the Krebs cycle, increasing reactive oxygen species (ROS) production and inducing hypoxia-resistant metabolism (15,18,20,21). Recent studies have also shown that mitochondrial metabolism is critical for cancer stem-like cell phenotype (16,18). Targeting mitochondrial function by inhibiting complex I and III showed a reduction in OXPHOS and inhibited ovarian CSCs (21,22). In addition, licofelone has been shown to induce mitochondrial apoptosis in colon cancer cells independent of arachidonic acid inhibition (23). In this respect, Hirst and colleagues (7) showed that licofelone strongly downregulated paclitaxel-induced TCA-related genes and reduced the expression of multiple OXPHOS complexes (I, II, and IV). The effect was significantly robust in OVCAR8 spheroids that express high basal levels of stem cell markers, whereas no noticeable effect was observed in A1847 spheroids that express low basal levels of stem cell markers. The authors inferred that licofelone could be modulating the expression of stem-like markers through regulation of mitochondrial metabolism and OXPHOS.

The authors furthered their studies *in vivo* to demonstrate the efficacy of licofelone in an ovarian cancer PDX ascites model. Licofelone treatment showed minimal difference in median survival of mice as a single agent. However, in the combination therapy with paclitaxel, the median survival was significantly improved compared with vehicle alone or mono-therapy with either drug. Paclitaxel treatment significantly increased the expression
of transcripts of stemness markers CD133 and ALDH1A, whereas the combination therapy of licofelone and paclitaxel significantly reversed the expression of ALDH1A compared with paclitaxel alone. Interestingly, the percent of Ki-67-positive cells in tumor cells isolated from the ascites was significantly reduced in the paclitaxel-treated group compared with vehicle. However, in the licofelone-treated tumor cells, Ki-67-positive cells were significantly increased compared with vehicle, while the combination of paclitaxel and licofelone showed no statistical different compared with vehicle. Taken together these data further supported the observations that licofelone reverses paclitaxel-induced stemness and increases post-chemotherapy proliferative cells that can be targeted by SOC therapy.

In summary, Hirst and colleagues (7) provided a strong evidence that 3D in vitro screening is superior to traditional screening approaches for identifying new drug leads for the treatment of women with chemotherapy-refractory ovarian cancer, and perhaps more robust drug leads for other solid tumors. They showed that some molecular targeted agents with limited clinical efficacy induce stem-like phenotype after high-dose treatment.

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Footnote

Conflicts of Interest: The author has no conflicts of interest to declare.

References


