Ovarian cancer: emerging molecular-targeted therapies

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Abstract: With about 22,000 new cases estimated in 2012 in the US and 15,500 related deaths, ovarian cancer is a heterogeneous and aggressive disease. Even though most of patients are sensitive to chemotherapy treatment following surgery, recurring disease is almost always lethal, and only about 30% of the women affected will be cured. Thanks to a better understanding of the molecular mechanisms underlying ovarian cancer malignancy, new therapeutic options with molecular-targeted agents have become available. This review discusses the rationale behind molecular-targeted therapies and examines how newly identified molecular targets may enhance personalized therapies for ovarian cancer patients.

Keywords: ovarian cancer, angiogenesis, PI3K/Akt, BRCA, metabolism

Introduction to ovarian cancer and current therapies

As knowledge of the complexity and diversity of tumor cells increases, clinical trials attempt to improve the treatment of patients by using molecular-targeted agents. This demarche is based on the assumption that targeting the signaling pathways that a tumor cell depends on, especially if these are driven by the alteration of a protein, might sensitize the tumor cells to treatment without affecting the normal cells. Thus, targeted therapies are emerging for the treatment of several types of tumors characterized by specific and additive genetic aberrations. For example, in lung cancers, patients with mutant EGFRs are treated with EGFR inhibitors, such as erlotinib or gefitinib, while breast cancer patients with overexpressed HER2 are treated with agents that inhibit that pathway (eg, trastuzumab or lapatinib). Over the past decade, fundamental research has shed light on numerous signaling pathways critical for the growth and the metastasis of ovarian tumor cells. The discovery of BRCA1/2 mutations underlying hereditary ovarian cancers and the use of PARP inhibitors in the clinic prompted the development of targeted therapies for ovarian cancer patients. This review outlines the current state of the emerging molecular-targeted therapies for ovarian cancers, and focuses specifically on molecular-targeted agents that affect some of the hallmarks of cancer: angiogenesis, genomic instability, anti-apoptotic signals, and metabolism.

Ovarian cancer is the ninth most common cancer, but is the fifth most frequent cause of cancer-related deaths in women. Even though 90% of patients can be cured if they are diagnosed at an early stage (stage 1, when the cancer is limited to the ovaries), over 80% of ovarian cancer patients are diagnosed after the tumor has already metastasized (stage 2 or beyond). The most common type of ovarian cancer (which is observed in
approximately 90% of cases) is thought to originate from the epithelial cells covering the ovaries, and is known as ovarian epithelial cancer. Although ovarian cancers are all epithelial in origin, they display four distinct histologies – serous, mucinous, clear cell, and endometrioid – that correlate with distinct gene expression patterns, and with distinct sensitivity to therapies. Also, patients with clear cell histology appear to have a worse prognosis than other histologies.

At early stages (stages 1–2), current therapies include surgery, chemotherapy, and/or radiation therapy. At later stages, debulking surgery will be combined with platinum or taxane-based chemotherapy (intraperitoneal or external) and, potentially, radiation therapy. However, since 80% of patients will relapse after first-line platinum-based or taxane-based chemotherapy, the development of new therapies is needed.

A close follow-up of patients with complete clinical remission is commonly done by regularly measuring the levels of serum CA 125, an ovarian-cancer antigen. Indeed, increases in CA 125 levels (in comparison with the levels obtained at the completion of the first line treatment) allow the early detection of a relapse. Therapeutic options for patients with recurrent disease are extremely limited. For patients with platinum-sensitive disease, treatment with platinum or platinum based combinations may be considered. For other patients, enrollment into a clinical trial might be an option.

**Emerging molecular-targeted therapies**

Gene amplifications, genetic mutations, and epigenetic abnormalities may lead to aberrant activation of an oncoprotein or to the loss of function of a tumor suppressor, and, consequently, may promote tumor growth. These genetic aberrations have all been reported in ovarian cancer, thus understanding the biology underlying the development and growth of ovarian cancer may give us the keys for successful targeted therapies.

**Angiogenesis and receptor tyrosine kinases**

Angiogenesis (the formation of new blood vessels) is a critical parameter for tumor growth and survival as it provides the nutrients and the oxygen necessary to maintain tumor cell biological functions. The best-studied pathway involved in angiogenesis is the vascular endothelial growth factor (VEGF) pathway. The VEGF family consists of seven ligands (VEGF-A to -E, and placental growth factors 1 to 2) and three receptors (VEGFR1 to VEGFR3). VEGF signaling is important for normal ovarian physiology and the reproductive cycle, but ovarian cancer is able to co-opt VEGF signaling. Indeed, retrospective clinical studies and preclinical studies have shown that the VEGF family pathway is activated in ovarian tumors, and might indicate a poor prognosis or survival.

At the molecular level, VEGFR activates several signaling pathways such as the PI3K/Akt signaling cascade and the MAP kinase pathway, and therefore promotes tumor growth, survival, and metastasis. Because VEGFRs are expressed and functional in ovarian cancer cells, anti-angiogenic therapies may also have a direct anti-tumor effect. Direct inhibitors of VEGF-A using monoclonal antibodies (bevacizumab, VEGFTrap) as well as multiple small molecules inhibiting VEGFR have both been broadly developed, although most of these molecules inhibit other receptor tyrosine kinases.

For example, vandetanib inhibits VEGFR, RET, and EGFR, while sorafenib is a VEGFR, PDGFR, and c-Kit inhibitor, and pazopanib inhibits VEGFR and PDGFR.

Clinical results of two highly anticipated phase III trials using the VEGF monoclonal antibody bevacizumab (Avastin™; Genentech/Roche, South San Francisco, CA) have recently been published. Avastin slowed tumor growth, but no significant difference in overall survival was observed in one of the studies. Other clinical trials targeting VEGFR using small molecules such as pazopanib, cediranib, sorafenib, and vandetanib were performed or are ongoing. A phase II trial with pazopanib in patients with recurrent disease appears to be promising, but a phase II trial combining sorafenib with topotecan presented high toxicity and poor clinical activity, and a phase II trial found that vandetanib had no clinical activity as monotherapy for recurrent ovarian cancers.

There may be several reasons for this variability in effect. One obvious concern is the poor specificity of VEGF receptor kinase inhibitors, which suggests that while targeting the vasculature of ovarian cancer is attractive, an understanding of the broad molecular effects of each receptor tyrosine kinase inhibitor (RTKI) will be necessary to better design these therapies.

The targeting of angiogenesis through alternative pathways mediated by RTKs other than VEGFR has been well studied in both preclinical and clinical studies of ovarian cancer. EGFR, Src, and Met have overlapping functions in the activation of signaling pathways involved in angiogenesis, cell growth, survival, and metastasis.

The EGFR receptor tyrosine kinase family has four members: EGFR, ErB2/HER2, ErB3/HER3, and ErB4/HER4. Following ligand binding, EGFR dimerizes and
activates signaling pathways such as PI3K/Akt and MAPK. Overexpression of EGFR has been observed in ovarian cancer and its nuclear localization has been linked to poor prognosis. The effect of EGFR inhibitors on ovarian cancer has been clinically investigated. Pertuzumab, a monoclonal antibody against EGFR, has shown encouraging results when combined with chemotherapy. However, other EGFR inhibitors such as gefitinib or trastuzumab presented variable clinical activities, suggesting that better patient selection with the development of new prognostic biomarkers might be needed.

Expression and activation of the nonreceptor tyrosine kinase Src leads to tumor cell growth, survival, and metastasis, and is an indicator of poor prognosis in ovarian tumors. The Src kinase family has nine members: Src, Fyn, Yes, Lyn, Lck, Fgr, Blk, Hck, and Yrk. Both Src and Yes have been shown to be overexpressed and activated in late stage ovarian cancer, and are key mediators of various RTKs, such as EGFR, Met, VEGFR, or HER2. Src activation promotes angiogenesis and invasion by supporting VEGF-A expression and inhibiting the expression of anti-angiogenic factors mediated by TGFβ1. Src activation has also been linked to platinum-drug resistance. This suggests that inhibition of Src combined with paclitaxel or with an anti-angiogenic agent might have a therapeutic value by decreasing the development of resistance to these therapies. A phase II and III clinical trial using the Src inhibitor saracatinib (AZD0530) combined with paclitaxel is ongoing in platinum resistant ovarian cancer patients.

Since the first report of Met being an oncogene was published in 1984, several solid tumors have been shown to be driven by Met aberrant activation and/or expression. Papillary type one kidney tumors and ovarian cancers are two examples out of many. Aberrant activation of Met can be due to overexpression of its endogenous ligand, the hepatocyte growth factor/scatter factor (HGF/SF), as well as point mutations of the MET gene, or activation of other receptors such as EGFR, semaphorin 4D receptor, or α5-integrin that can all activate Met by heterodimerization. This indicates that Met is involved in the crosstalk of multiple signaling pathways and plays an important role in tumor growth and metastasis. Met and its ligand HGF/SF also play an important role in angiogenesis. HGF/SF was described in 1992 as a “potent angiogenic factor which stimulates endothelial cells motility and growth.” Since then, Met has been shown to regulate VEGF-A signaling. In ovarian cancer, several pre-clinical studies have identified Met as a relevant therapeutic target due in part to its role in invasion and angiogenesis, which suggests that combining Met inhibition with anti-angiogenic therapies could be beneficial for ovarian cancer patients. Since Met is also important in the development of resistance to therapies targeting EGFR, it would be interesting to examine whether dual EGFR/Met inhibition improves the effect of EGFR inhibitors in the clinic.

Genomic instability and BRCA1/BRCA2
Patients with mutations in one of the tumor-suppressor genes BRCA1 or BRCA2 are more likely to develop breast and ovarian cancers following the loss of the remaining wild-type allele (LOH). BRCA1 and BRCA2 encode for proteins that maintain the integrity of the genome by regulating the DNA damage response and repair. Although mutations in BRCA1 or BRCA2 lead to similar diseases, the proteins they code for have different functions with BRCA1 involved in both the DNA damage response and DNA repair, whereas BRCA2 is involved only in DNA repair; however, both are critical for homologous recombination (HR). When a double-strand break occurs in proliferating cells, HR will repair the DNA with high fidelity. When BRCA1 or BRCA2 are mutated and HR is compromised, the overall repair capacity of the cell is greatly reduced, and less reliable repair pathways such as nonhomologous end-joining (NHEJ) will be used, leading to increased genomic instability. It has been shown that ovarian cancer patients with BRCA mutations may be more sensitive to platinum-based chemotherapy and may have better outcomes than patients without BRCA mutations. PARP proteins are also involved in DNA repair and thus BRCA mutated cells are highly sensitive to PARP inhibition. Alterations of other members of the HR pathway such as ATM, ATR, CHK1, or CHK2 also sensitize the cells to PARP inhibitors. Since mutations of ATM and CHK2 are common in cancers with deficient BRCA and further increase genome instability, one can predict that cells with several mutations in the HR pathway will have a marked sensitivity towards PARP inhibitors. Several PARP inhibitors are currently used in the clinic for patients with BRCA mutations or methylation. In a recent randomized phase II multicenter study, the efficacy of the PARP inhibitor olaparib was compared with pegylated liposomal doxorubicin in patients with BRCA1/2 mutations and recurrent ovarian cancer, but no significant difference was reported. The existence of secondary somatic mutations able to restore BRCA functions has been proposed to explain these results.

Another way to selectively target patients with BRCA loss of function could be through the regulation of epigenetic
modifications. Posttranslational modification of histones by methylation and acetylation regulates the access of a transcription factor to the DNA, so this plays a prominent role in controlling gene expression. In tumors, it is common to observe aberrant DNA methylation that silences tumor-suppressor gene expression. In ovarian cancer the BRCA1 promoter has been shown to be hypermethylated in more than 30% of tumors. In contrast, acetylation of histones on lysine residues by histone acetyltransferases (HAT) allows the active transcription of genes. Acetyl groups are removed by histone deacetylases (HDACs), silencing gene expression. Thus, while the mechanism of action of HDAC inhibitors is not fully understood, they may allow the reactivation of silenced genes. Several promising preclinical studies in ovarian tumor cells have been performed using HDAC inhibitors either alone or in combination with other agents.

Because inhibition of HDACs has been shown to reverse epigenetic silencing, it is reasonable to speculate that combining HDAC inhibitors with paclitaxel and/or cisplatin, or with PARP inhibitors, might be of therapeutic value, especially if targeted to patients with silenced BRCA genes. A phase III clinical trial for cisplatin-resistant ovarian cancer with topotecan alone or combined with the epigenetic agents hydralazine and magnesium valproate is currently ongoing at the National Institute of Cancerologia (Mexico).

Anti-apoptosis and cell survival: the PI3K/Akt pathway

The PI3K/Akt signaling pathway plays a prominent role in the growth and survival of numerous cancers, including ovarian cancer. The three classes of the lipid kinases PI3K are made up of a regulatory (p85) and a catalytic (p110) subunit, with several isoforms for each. In cancer, inappropriately activated PI3K induces the phosphorylation of phosphatidylinositol-4,5-biphosphate (PIP$_2$), to produce phosphatidylinositol-3,4,5-trisphosphate (PIP$_3$). PI3K activation is counterbalanced by PTEN, a 3′-phosphatase that transforms PIP$_3$ into PIP$_2$. The second messenger, PIP$_3$, recruits and promotes the activation of Akt, which initiates a signaling cascade leading to anti-apoptotic signals, and tumor cell growth and survival. In ovarian cancer, genetic mutations have been found on PTEN*, PIK3CA (p110α)* (*from the Catalogue Of Somatic Mutations in Cancer PIK3R1 (p85), and AKT2. All of these mutations can lead to aberrant and constitutive activation of the PI3K/Akt signaling cascade, and to the subsequent activation of mTOR and NFKB, two targets of the Akt pathway. Because aberrant constitutive activation of the PI3K/Akt pathway promotes tumor survival and chemoresistance, small inhibitors targeting this signaling cascade may have a therapeutic value. Several clinical trials targeting the PI3K/Akt pathway are ongoing, and pan-PI3K inhibitors are currently in phase I clinical trials. For example, XL147 is in trials in combination with paclitaxel and carboplatin, and BKM120 is used as a single agent for patients with specific PIK3CA mutations. The pan-Akt inhibitor MK-2206 is in phase II trial. Small molecules targeting downstream targets of Akt such as mTOR inhibitors are also used in combination with carboplatin and taxol/paclitaxel in clinical trials. Ridaforolimus is in phase I trial, temsirolimus is in phase II trial, and the mTOR inhibitor RAD001 combined with avastin is also in phase II clinical trials. As well as this, even though MAPK mutations were suspected to induce resistance to PI3K/Akt/mTOR inhibitors, it has recently been demonstrated that a subset of patients with both PIK3CA and MAPK mutations responded to PI3K/Akt/mTor targeted therapies.

Promising preclinical data linked to the aberrant activation of the PI3K/Akt signaling cascade identify potential new therapeutic targets. For example, loss of tumor suppressors such as PTEN, DNA damage, or genetic alterations can also lead to the aberrant activation of the nuclear factor-κB (NF-κB). In cancer cells, the transcription factor NF-κB plays a complex role that promotes angiogenesis, inflammation, and metastasis. Preclinical data have shown that the NF-κB pathway is critical for ovarian cancer cells and the development of selective and direct NF-κB inhibitors may have therapeutic value for ovarian cancer patients. Moreover, humoral hypercalcemia of malignancy (HHM) is mediated by the secretion of parathyroid hormone-related peptide (PTHrP) and has been associated with gynecologic neoplasms, including ovarian cancers. Patients with HHM will present with hypercalcemia, low parathyroid hormone (PTH), and high PTHrP serum levels. At the molecular level, PTHrP binds to the PTH/PTHrP receptors and activates the PI3K/Akt/NF-κB pathway, which promotes tumor growth and metastasis. As a result, ovarian cancer patients with HHM might particularly benefit from a therapy targeting the PI3K/Akt signaling cascade.

Besides its role in promoting anti-apoptosis and cell survival, the PI3K/Akt pathway, especially mTOR, is a key node in regulating cellular metabolism. Indeed, activation of Akt increases glycolysis, decreases β-oxidation by reducing the expression of carnitine palmitoyltransferase 1A (CPT1A), upregulates the fatty acid synthase (FASN), and activates mTOR. Thus, by promoting glycolysis and anabolic reactions, the PI3K/Akt pathway promotes an anabolic metabolism shift that might favor the growth and spread of tumor cells.
Metabolism of ovarian cancer cells: from HIF1α to the mitochondria

It is now broadly accepted that metabolic reprogramming of tumor cells, either as a tumor initiator or as a consequence of tumor growth, provides a growth advantage to tumor cells. The mechanisms underlying this reprogramming are, however, not fully understood.

The hypoxia inducible factor HIF1α is critical to maintain the energy production of a cell in the metabolic shift that occurs under hypoxia. In normal tissues, oxygen is consumed by the cells to support mitochondrial function and produce energy by oxidative phosphorylation. Under hypoxia, oxidative phosphorylation is impaired because there is little or no oxygen to serve as an electron acceptor at the end of the electron transport chain, which leads to a decrease in ATP production. A metabolic shift needs to occur to compensate for this lack of energy, and this metabolic adaptation can be driven by HIF1α. In the absence of oxygen, HIF1α is stabilized and translocates into the nucleus to transcribe the genes involved in glucose metabolism such as Glut1, lactate dehydrogenase A (LDHA), or pyruvate kinase (PK) M2 (which is also a coactivator of HIF1α). By activating these genes, HIF1α promotes glycolysis, a less efficient yet reliable way to produce energy under hypoxic conditions. In tumors where HIF1α is expressed, this metabolic shift occurs even in the presence of oxygen and is known as aerobic glycolysis, or the Warburg effect. HIF1α is overexpressed in late stage ovarian cancer, but, to date, its impact on chemotherapy response or prognosis is unclear. That said, low expression of the glucose transporter Glut1, which is its downstream target, correlates with a longer disease-free survival, suggesting that HIF1α could be a therapeutic target. Since VEGF-A is also a transcriptional target of HIF1α, inhibition of HIF1α may simultaneously deprive tumor cells of their energy by inhibiting glucose metabolism and angiogenesis. Although no direct HIF1α
inhibitors are currently available in the clinic, several small molecules affecting alternative pathways have been shown to decrease HIF1α expression and activity. Topoisomerase inhibitors, HSP90 inhibitors, mTOR inhibitors and EGFR inhibitors have all shown potent anti-HIF1α activity in preclinical studies, and, with the exception of HSP90 inhibitors, are currently used at different phases in the clinic for the treatment of ovarian cancer patients. HIF1α expression may also have a prognostic or diagnostic value in those studies.

Another possible metabolic adaptation promoting rapid growth of tumors is their parasitic use of energetic resources from surrounding tissues. Recently, Nieman et al have demonstrated that adipocytes can be an energy reservoir for ovarian tumor cells. Surrounding adipocytes promote migration and invasion by transferring fatty acids to the tumor cells, which will then generate ATP via mitochondrial β-oxidation. Thus, any agents that can prevent tumor cells receiving energy from their supporting host cells could have a therapeutic effect, and this includes AMPK activators (eg, metformin) or lipid synthase inhibitors. Since Akt is known to decrease mitochondrial β-oxidation, PI3k/Akt inhibitors might prevent the parasitic usage of energetic resources by ovarian cancer cells and might have a therapeutic value. Moreover, the small molecule elesclomol (STA4783), which targets mitochondrial function and requires a functional electron transport chain to show a cytotoxic effect, is currently in phase II clinical trial for ovarian cancers. This agent may be more selective for “parasitic” tumor cells that preserve functional mitochondria, while not affecting surrounding adipocytes since these have a shifted metabolism with increased glycolysis and lipolysis.

Conclusion
Molecular characterization of ovarian cancer is already allowing for improvements in the design of targeted therapies. Over 72,000 scientific papers related to ovarian cancer have been published on PubMed, and numerous gene mutations have been identified by The Cancer Genome Atlas (http://cancergenome.nih.gov). This allows for the development of multiple therapeutic approaches with the potential to treat ovarian cancer. Several emerging targeted therapies have been highlighted in this review. They target multiple aspects of the hallmarks of a cancer cell (Figure 1) and expand the current treatment options for ovarian cancer patients.

The complexity of signaling cascades, the numerous resistance mechanisms, and the lack of specificity of certain small molecules all make it difficult to predict which therapy will be successful, or identify the appropriate patient populations. An in-depth understanding of the molecular effects that a small molecule may have toward diverse types of tumors, and how this might lead to resistance, will certainly be an advantage. The use of new targeted agents will be improved by the development of multiple biomarkers to identify which patients will benefit or be harmed by a particular treatment, and to monitor the efficacy of treatments. Understanding both the mechanisms of action and the toxicities of therapeutic agents will ultimately enhance the quality of patient care and the quality of life of ovarian cancer patients.

Other challenges that were not discussed in this review also remain. Understanding the roles played by stem cells or microRNA in the growth of ovarian tumors, improving the delivery of therapies using nanotechnologies, as well as studying the prognostic value of surgical resection of tumor necrotic tissues following therapy, or assessing the prognostic value of minimal residual disease for ovarian cancer patients, are a few examples of these challenges. A better understanding of these will improve the treatment and standard of care for patients with advanced ovarian cancer. For example, a recent prospective multicenter study showed that patients with complete cytoreduction have a better outcome than patients with residual minimal disease, a finding that will certainly have a significant impact on the care of ovarian cancer patients.

Disclosure
The author reports no conflicts of interest in this work. This review was prepared by the author in her personal capacity. Ongoing clinical trials were found using the http://www.clinicaltrials.gov and http://www.clinicaltrials.com websites, as well as Internet searches. The opinions expressed in this article are the author’s own and do not necessarily reflect the views of any institutions and/or governments.

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