Trends in Cancer

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Review

Tumor–Host Cell Interactions in Ovarian Cancer: Pathways to Therapy Failure

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Although most ovarian cancer patients are highly responsive to chemotherapy, they frequently present with recurrent metastatic lesions that result in poor overall survival, a situation that has not changed in the last 20 years. This review discusses new insights into the regulation of ovarian cancer chemoresistance with a focus on the emerging role of immune and other host cells. Here, we summarize the complex molecular pathways that regulate the interaction between tumor and host cells, discuss the limitations of current *in vitro* and *in vivo* models for translational studies, and present perspectives for the development of innovative therapies.

The Unique Microenvironment of Ovarian Cancer

High-grade serous carcinoma is the most common ovarian malignancy and is usually diagnosed at an advanced stage. Patients presenting with advanced disease have a dire prognosis with an overall 5-year survival rate of less than 40% due to the recurrence of peritoneal metastases after first-line therapy [1,2]. A feature that distinguishes ovarian cancer from other human tumors is the specific **tumor microenvironment** (see Glossary) [3,4]. Ovarian cancer is a peritoneal disease, where the dissemination of tumor cells is facilitated by the peritoneal fluid as a carrier [3,5]. This fluid, frequently occurring as large volumes of **ascites**, comprises detached tumor cells, tumor cell spheroids, and numerous types of host cells, including different kinds of innate and adaptive immune cells and activated mesothelial cells, which produce, and are targeted by a plethora of tumor-promoting soluble factors and **extracellular vesicles (EVs)**. Tumor-associated macrophages (TAMs) play a prominent role in this context as major producers of tumor protumorigenic and immunosuppressive factors [6]. Another feature characteristic of ovarian cancer is the particular relevance of the **omentum** (Box 1), a structure composed of fatty and connective tissue that covers the ventral surface of the intestines. The omentum is the preferred site of ovarian cancer metastases and represents a key player in tumor progression [1].

The standard first-line therapy for high-grade serous ovarian carcinoma is a combination of surgery and carboplatin/paclitaxel-based chemotherapy. Although most patients are initially highly responsive to this regimen, the vast majority of patients present with recurrent disease. Apart from inherent and acquired chemoresistance, a poorly understood mechanism of transient or conditional resistance is likely to be responsible for the failure of chemotherapy (Box 2). Prime resistant candidates are detached cancer cells and spheroids in the malignant ascites that express markers characteristic of stem cells [7]. However, in contrast to some other human malignancies, a clearly defined 'cancer stem cell' has not been identified in ovarian cancer and may not exist. Nevertheless, ovarian cancer cells expressing stemness markers

Trends

Cytokines, EVs, and miRNAs modulate PI_3K–AKT–mTOR, STAT3, and NFκB signaling pathways that mediate chemoresistance. Drugs for these pathways are available and at various stages of clinical development.

EV-encapsulated miRNA released by omental CAAs and CAFs blocks chemotherapy-mediated tumor apoptosis by targeting APAF-1, a central component of the apoptosome.

CAFs limit the intracellular availability of chemotherapy drugs in cancer cells by restricting the extracellular concentrations of GSH and its precursor cysteine, which is counteracted by IFNγ released by T cells.

Tumor-infiltrating T cells reactivated by PD-1 checkpoint blockade improve the cytotoxic effect of chemotherapy in animal models of ovarian cancer and are clinically tested.

Ovarian cancers have different types of resistance. The underlying mechanistic diversity requires individualized therapies that combine chemotherapy, signaling inhibition, and T cell checkpoint blockade.

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Box 1. Role of the Omentum in Ovarian Cancer Progression

The omentum is a large apronlike structure in the abdomen that serves to protect the visceral organs and is part of the peritoneal immune defense system. Its major constituent is a double layer of fatty tissue, but harbors a variety of other cell types, including mesothelial cells, fibroblasts, lymphocytes, and macrophages. Tissue-resident adipocytes and fibroblasts of the omentum are converted to carcinoma-associated adipocytes (CAAs) and CAFs by tumor-derived mediators that induce growth and a metastasis promoting microenvironment. One pathway specific in ovarian cancer is the transfer of fatty acids from CAAs to adjacent tumor cells via the fatty acid-binding protein 4 (FABP4), thereby supporting energy production for metastatic growth [67]. Other examples are the promotion of tumor cell homing and invasion into the omentum by IL-8 secreted by CAAs and CAFs [67] and the activation of the growth-promoting receptor ERBB3 by its ligand neuregulin 1 (NRG1) also released from CAAs [28].

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possess a high tumor-initiating potential, exhibit increased drug resistance, and are enriched in the ascites of patients with relapsed ovarian cancer [8]. These findings clearly support the conclusion that ascites-associated tumor cells play a pivotal role in ovarian cancer spread and resistance to therapy. There is also evidence to suggest that the properties of these cells are strongly influenced by factors of both the local and distal tumor microenvironment, the invaded host tissues, and the malignancy-associated ascites [9].

The impact of ovarian cancer cells on the immune system has been extensively studied (Box 3) and discussed in recent reviews [3,10,11]. Reciprocal interactions are less well studied, but a number of recent studies have shed new light on the question of how subverted host cells within the unique microenvironment of ovarian carcinoma impinge on cancer progression and therapy resistance (Figure 1, Key Figure). Here we discuss these new and partly unexpected biological principles, which provide potential explanations for the failure of current treatment regimens and point to the need for innovative therapeutic approaches.

EVs and miRNAs Impinge on Ovarian Cancer Resistance

The communication between tumor cells and the microenvironment depends to large extent on EVs, also referred to as exosomes. EVs are small particles that are composed of lipids, proteins, and nucleic acids, and are released by virtually all cell types and thus are present in all biological fluids, including ascites. These vesicles are either shed as microvesicles from the cell membrane, or released as exosomes when multivesicular bodies fuse with the plasma membrane. EVs can convey biological materials to surrounding cells and thereby interfere with gene expression and signaling pathways [12].

There is emerging evidence that EVs transfer miRNAs, which shape the plasticity of the cells within the microenvironment (Figure 1). Of note, EVs have a cell-independent capacity to generate mature miRNAs and contain pre-miRNAs, along with processing enzymes such as Dicer or AGO2 [13]. Given the crucial role of miRNAs in cancer, an miRNA signature associated with the survival of ovarian cancer was established based on the expression of 35 miRNAs that were shown to be linked to disease outcome [14]. A retrospective study indicated both a predictive value and prognostic relevance of this signature for patients at diagnosis. One of the miRNAs associated with good prognosis was miR-506, recently identified as a key node in a master miRNA network regulating the mesenchymal subtype in ovarian cancer [15], which is of particularly poor prognosis [16]. miR-506 targets SNAI2/SLUG, a transcriptional repressor that promotes epithelial–mesenchymal transition (EMT) and inhibits apoptosis, which involves the downregulation of genes encoding epithelial proteins including E-cadherin. Consistent with these observations, nanoparticle delivery of miR-506 suppressed EMT and reduced tumor growth in orthotopic mouse models of ovarian cancer [15]. Moreover, *in vitro* data indicate that EMT may contribute to enrichment of ovarian cancer cells with stem cell-like properties and a decreased sensitivity against paclitaxel [17]. Finally, transforming growth factor β signaling is strongly associated with a short relapse-free survival of ovarian cancer and drug resistance,

Box 2. Different Mechanisms of Chemoresistance in Ovarian Carcinomas

The vast majority of ovarian carcinomas are highly responsive to chemotherapy (Figure IA) with only a small fraction of patients showing disease progression during or shortly after chemotherapy due to a presumably genetic mechanism of inherent chemoresistance (Figure IB). Although disease control can initially be achieved, most of these patients present with recurrent tumors within 3 years (Figure IC). In a subset of patients, this relapse results from the selection of genetically altered tumor cells leading to a state of acquired chemoresistance (Figure ID), resulting from diverse genetic mechanisms as suggested by the extensive clonal heterogeneity [68]. By contrast, many of the relapsed cancers are still highly sensitive to treatment with a combination of carboplatin and paclitaxel, even after multiple cycles of relapse and chemotherapy (Figure IE). Since these cells survived and were refractory to first-line therapy, their state of chemoresistance is likely transient. This feature distinguishes ovarian carcinoma from most other human cancers, where recurrence is usually associated with genetically acquired resistance.

Transient chemoresistance may be induced, for example, by changes in the tumor microenvironment and/or stress-induced autophagy triggered, for instance, by a perturbed energy metabolism in individual cancer cells [44]. Increased autophagy has indeed been observed in dormant metastatic lesions on serous membranes in relapsed disease compared to primary ovarian cancers [69]. Tumor cell spheroids floating in the peritoneal fluid most likely play a pivotal role in causing this state of transient chemoresistance. On the one hand, these multicellular aggregates are endowed with a high tumor-initiating potential, which has been proposed to be linked to the expression of stemness-associated genes [3,70] and is increased by fluid shear stress to which cells are exposed in malignant ascites [71]. On the other hand, these cells are protected from anoikis and drug-induced programmed cell death [44], which is presumably related to their quiescence [72] and the skewing of metabolism toward anaerobic glycolysis [70]. It is therefore conceivable that a small number of tumor cell spheroids survive chemotherapy and due to their high tumorigenic potential initiate new metastatic lesions upon attachment to visceral organs. In view of the transient or conditional nature of the chemoresistant state of these cells, their interaction with the microenvironment, including host cells, EVs, soluble mediators, and the extracellular matrix, deserves particular attention.

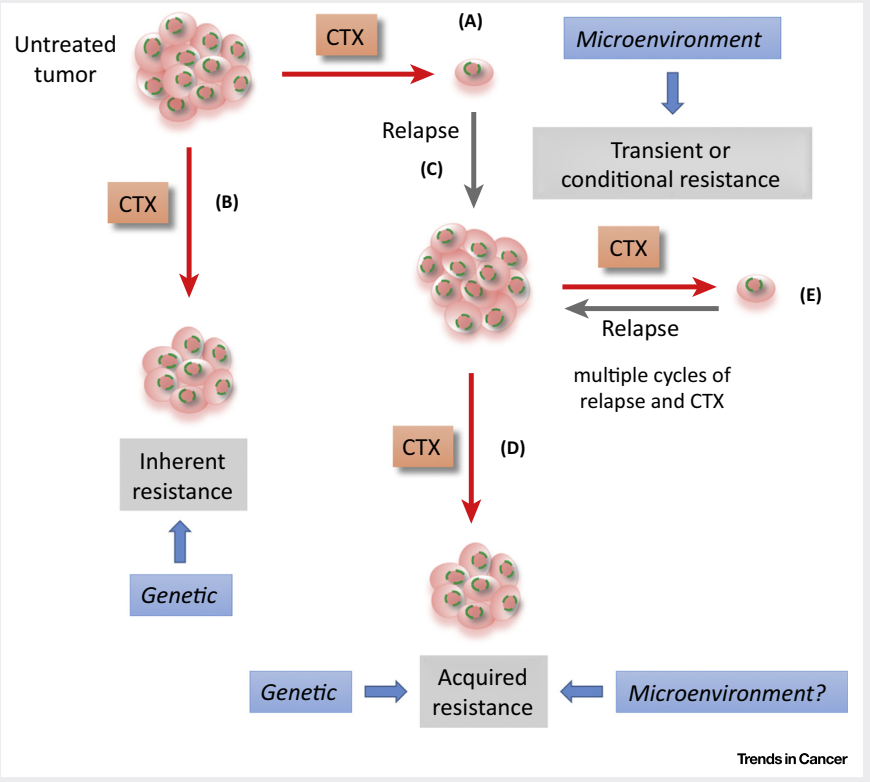

Figure I. Model Illustrating Different Mechanisms of Ovarian Carcinoma Chemoresistance. CTX, cyclophosphamide (chemotherapy).

Glossary

Ascites: in ovarian cancer, the tumor microenvironment extents into the peritoneal fluid, which at advanced stages occurs as a malignant effusion (ascites). This effusion plays a pivotal role in cancer cell dissemination throughout the peritoneal cavity due to the presence of highly tumorigenic spheroids composed of aggregated cells detached from solid tumor lesions, immune cells, and other host cells, as well as a plethora of soluble factors and EVs.

Extracellular vesicles (EVs): extracellular vesicles are shed from the plasma membrane or released from multivesicular bodies via exocytosis from almost all cell types and they play a prominent role in intercellular communication. EVs transport nucleic acids (DNA, mRNA, miRNA), lipids, and proteins between cells to regulate signaling pathways and biological functions of the recipient cells. EVs have been recognized as major components of the tumor microenvironment. They play essential roles in tumor immune escape, metastasis, angiogenesis, and tumor–stroma interactions.

Immune checkpoints: signaling pathways, including PD-1 and its ligand PD-1L, which physiologically limit immune responses, for instance, to avoid autoimmune reactions. These signaling pathways are recruited by tumor cells to circumvent immune detection and elimination, for example, via upregulation of PD-1L. Monoclonal antibodies that abrogate inhibiting signaling pathways (checkpoint inhibitors) have been shown to restore antitumor T cell activity in different human tumors resulting in intriguing clinical responses.

Immune surveillance: malignant cells are kept in check by an antitumor immune response driven by innate immune cells including NK cells and macrophages, as well as by an adaptive immune response largely dependent on antigen-specific T cells. The initial elimination phase is followed by an equilibrium phase of tumor cell proliferation and immune cell-dependent killing. Tumors develop strategies to circumvent immune detection allowing accelerated and uncontrolled tumor growth within the final escape phase.

Box 3. T Cells Are a Major Determinant of Clinical Outcome in Ovarian Cancer and Are a Promising Therapeutic Target

In a seminal study, Zhang and colleagues [73] showed that the presence of $CD3^+$ T cells is strongly associated with a favorable clinical outcome. The 5-year overall survival rate for patients with tumor infiltrating T cells was 79% and for patients without T cells was 11.9% [73]. This observation was confirmed by a meta-analysis of ten studies with 1815 patients, which also identified $CD8^+$ tumor-infiltrating lymphocytes as crucial effectors of the antitumor response [74]. By contrast, the presence of regulatory T cells (Tregs) or a low $CD8^+$/Treg ratio is associated with poor survival [75,76].

Even though the immune surveillance system can efficiently control the initiation and progression of cancers, immune evasion is a hallmark of tumor development. A major mediator of immune evasion is the PD-1 immunomodulatory receptor on T cells, which is activated upon binding by PD-L1 expressed on tumor cells and TAMs [77,78]. PD-1 checkpoint activation inhibits the proliferation, survival, and function of tumor-infiltrating cytotoxic $CD8^+$ T cells. The additional activation of the checkpoint controlled by CTLA4, another inhibitory receptor expressed on T cells, further increases the ratios of both CD4 and CD8 effector T cells to regulatory T cells (Treg) [79].

The PD-1/PD-1L pathway is believed to play a pivotal role in ovarian cancer [10]. Consistent with this notion, the PD-1 blocking antibody nivolumab showed encouraging results in a subgroup of platinum-resistant patients, including full clinical remissions [80]. Current experimental and clinical studies that evaluated immune checkpoint inhibitors against ovarian cancer and clinical issues regarding immune checkpoint inhibitors are discussed in detail in recent reviews [66,81].

Omentum: the omentum is a structure composed of fatty and connective tissue that covers the ventral surface of the intestines. It has essential functions in lipid storage, the regulation of fluid balance, and immune response in the abdominal cavity. The omentum contains large numbers of adipocytes, macrophages, and lymphocytes. Ovarian cancer cells primarily metastasize to the omentum by passive transcoelomic and active hematogenous dissemination.

Tumor microenvironment: the tumor microenvironment is the cellular and soluble environment of tumors including blood vessels, immune cells, fibroblasts, EVs, soluble signaling molecules, and the extracellular matrix. This microenvironment is generated in response to the reciprocal interactions of tumor cells and host cells and supports cancer growth and progression.

which is presumably due to the promotion of a mesenchymal phenotype by transforming growth factor β [6,18,19].

Paclitaxel resistance is also mediated through miR-433, which is released into the extracellular environment via vesicles. This contributes to cellular senescence and resistance against paclitaxel in tumor cells and probably also in bystander cells through downregulation of the tumor suppressor RB1 via its direct target cyclin-dependent kinase 6 (CDK6) [20]. A particularly intriguing molecule in the context of chemoresistance is miR-21, which is transferred from carcinoma-associated adipocytes and carcinoma-associated fibroblasts (CAFs) to ovarian cancer cells via EVs [21,22] as described in detail in the following section in the context of adipocytes.

Taken together, these findings clearly underline the therapeutic potential of approaches targeting miRNAs involved in the regulation of EMT and programmed cell death. To be able to fully exploit these therapies, it will be essential to understand the mechanisms that regulate the expression and cellular exchanges of resistance-associated miRNAs in ovarian cancer, which remain largely obscure with very few exceptions. One example is miR-193b, which is downregulated by mesothelial cell interactions, as discussed in the following section [23].

T Cell-Induced Chemosensitization

Another major determinant of clinical outcome in ovarian cancer is the patient's immune surveillance system driven by tumor-infiltrating $CD8^+$ T cells (Box 3). Recent studies identified a pivotal role for $CD8^+$ T cells in improving the efficacy of chemotherapy and have substantially extended our understanding of how T cells can exert a beneficial therapeutic effect beyond a direct antigen-specific cytotoxic attack of cancer cells (Figure 1). Xu and colleagues [24] identified miR-424(322) as a negative regulator of several mRNAs coding immune regulatory proteins, including the T cell inhibitory ligand programmed death-1 (PD-1) ligand 1 (PD-1L; CD274), in chemoresistant ovarian cancer cells. Importantly, miR-424(322) levels in tumors were associated with progression-free survival. Forced expression of miR-424(322) expression in tumor cells concomitantly reversed chemoresistance *in vitro* and *in vivo*, and blocked the PD-L1 **immune checkpoint** in T cells in mouse models, resulting in proliferation of functional cytotoxic $CD8^+$ T cells and inhibition of myeloid-derived suppressive cells and regulatory T cells. Importantly, the authors showed that $CD8^+$ T cells were indispensable for a chemosensitizing effect of miR-424(322) in mice.

Key Figure

Schematic Representation of Signaling Pathways in the Ovarian Cancer Microenvironment Impinging on the Response of Tumor Cells to Chemotherapeutic Drugs.

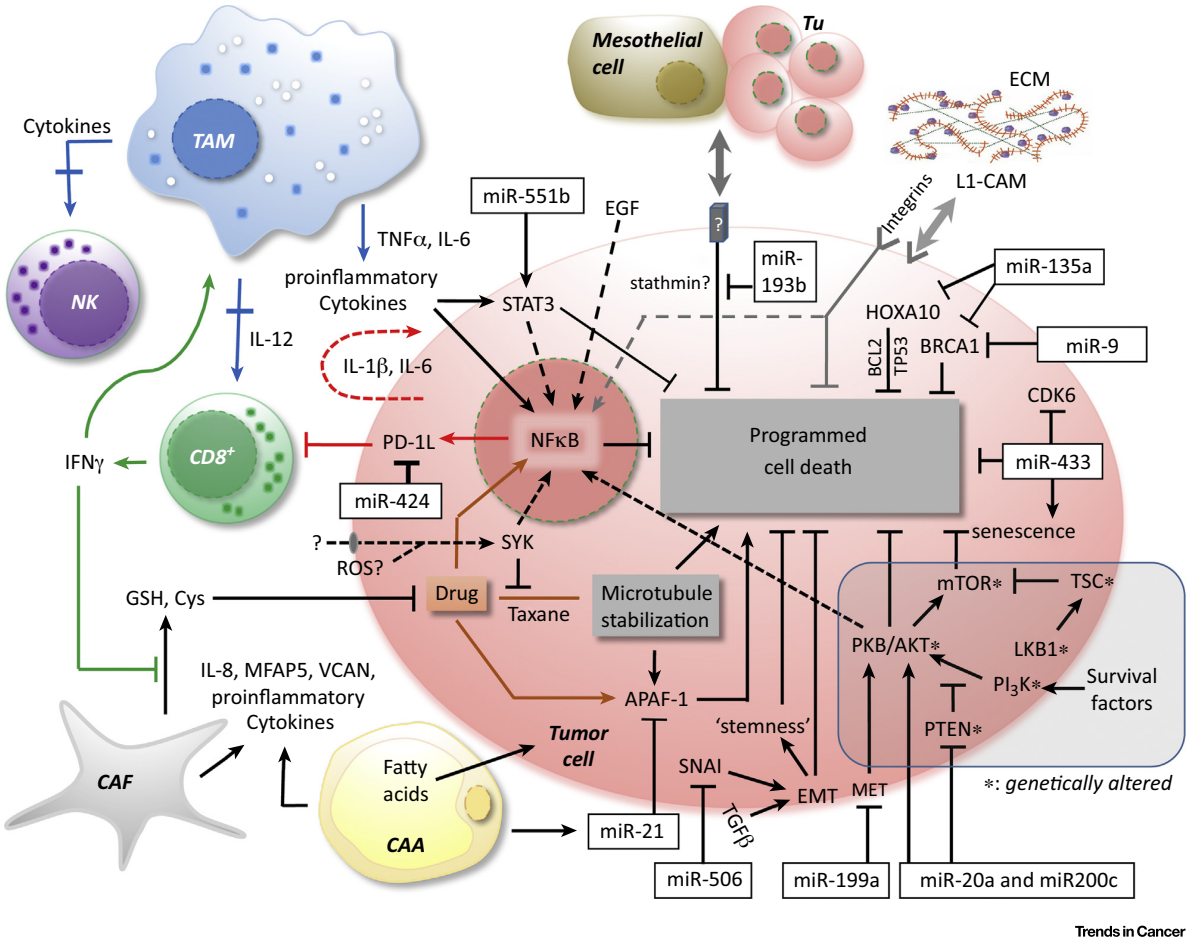

Figure 1. (A) Clinically successful first-line chemotherapy (most patients) with few surviving tumor cells. (B) Disease progression during or shortly after chemotherapy due to genetically determined inherent resistance (small fraction of patients). (C) Cancer recurrence in initially responsive patients within 3 years (most patients) with two possible consequences (D and E). (D) Multiple cycles of tumor reduction and regrowth in relapsed patients due to transient resistance presumably controlled by microenvironmental cues. (E) Acquisition of a state of stable resistance largely driven by genetic alterations.

Abbreviations: CAA, cancer-associated adipocyte; CAF, cancer-associated fibroblast; $CD8^+$, cytotoxic T cell; Cys, cysteine; ECM, extracellular matrix; EGF, epidermal growth factor; EMT, epithelial–mesenchymal transition; GSH, glutathione; IFNγ, interferon-γ; IL-x, interleukin-x; L1-CAM, L1 cell adhesion molecule; MET, mesenchymal–epithelial transition; miR, microRNA; mTOR, mammalian (or mechanistic) target of rapamycin; NFκB, nuclear factor-κB; NK, natural killer cell; PI_3K, phosphoinositide 3-kinase; PKB, protein kinase B; ROS, reactive oxygen species; STAT, signal transducers and activators of transcription; SYK, spleen tyrosine kinase; TAM, tumor-associated macrophage; TGFβ: growth transforming growth factor β; TNFα, tumor necrosis factor-α; Tu, tumor cell. Genes and proteins are named according to the HUGO gene nomenclature. References are quoted in the main text.

Consistent with these results, previous studies showed that chemotherapeutic drugs, including paclitaxel, induced the expression of PD-L1 in mouse models of ovarian cancer through activation of nuclear factor-κB (NFκB) [25], and that blockade of PD-L1/PD-1 enhanced the therapeutic effect of paclitaxel.

Although these findings clearly suggest that PD-L1-mediated T cell suppression enhances chemoresistance in ovarian cancer, the mechanistic aspects need to be addressed in further detail. Thus, while reactivated $CD8^+$ T cells may be beneficial in this scenario due to a direct cytotoxic antitumor effect, other mechanisms cannot be excluded, as suggested by the studies discussed in the following section [26].

Crosstalk of Cancer Cells, T Cells, and CAFs Regulates Chemoresistance

A novel intriguing mechanism of T cell-induced chemoresistance was recently unraveled by Wang and co-workers [26] who were able to demonstrate that $CD8^+$ T cells can counteract cancer cell resistance via nonimmune functions. These authors showed that CAFs can induce chemoresistance through the production of glutathione (GSH) and its rate-limiting precursor cysteine. GSH diminishes the accumulation of active platinum-based drugs in ovarian cancer cells by forming a drug–GSH complex that effluxes from the cell. Activated $CD8^+$ T cells interfere with this mechanism by altering the metabolism of CAFs via an interferon-γ (IFNγ)-regulated Janus kinase/signal transducers and activators of transcription 1 (JAK/STAT1) pathway to inhibit the accumulation of GSH. In addition, others have shown that IFNγ can restore the function of TAMs, regarding interleukin-12 (IL-12) secretion [27]. Thus, counteracting T cell exhaustion via inactivation of the PD-1 checkpoint can have beneficial therapeutic effects through multiple independent mechanisms.

The clinical relevance of these findings is underscored by highly significant opposite effects of stromal $CD8^+$ T cells and fibroblasts on overall survival and also by a clear association of stromal fibroblasts with response to platinum drugs [26]. The discovery of T cell-mediated abrogation of stromal-mediated chemoresistance is likely to trigger the search for additional resistance pathways involving multiple cell types of the tumor microenvironment (Figure 1).

A Unique Role for Adipocytes in Ovarian Cancer

Ovarian cancer cells preferentially home to the omentum to form metastatic lesions, either via the passive transcoelomic route or via hematogenous dissemination [28]. Different cell types of the omentum also modulate the microenvironment to promote tumor growth, metastasis, chemoresistance, and immune evasion by releasing a plethora of soluble mediators and EVs with tumor-promoting properties (Figure 1). A particularly interesting mechanism in this context is the recent discovery of a novel pathway causing resistance to paclitaxel by targeting of APAF-1 [21,22], a central component of the apoptosome and mediator of the cytochrome c-triggered autocatalytic activation of procaspase-9. This pathway comprises the shuttling of EV-encapsulated miR21 from omental carcinoma-associated adipocytes and CAFs to cancer cells, with release of miR21 targeting APAF-1 mRNA in recipient cancer cells. As APAF-1 has an essential rate-limiting function in paclitaxel-induced apoptosis [29], it is conceivable that miR21 lowers the chemosensitivity of ovarian cancer cells. These findings add a conceptually novel mechanism to the emerging picture of how host cells drive therapy resistance of ovarian cancer.

Microenvironment-Regulated Signaling Pathways Promote Chemoresistance

In ovarian cancer, the AKT, NFκB, and STAT3 pathways are instrumental in mediating chemoresistance by blocking proapoptotic mechanisms (Figure 1). The canonical phosphoinositide 3-kinase (PI_3K)–AKT–mammalian (or mechanistic) target of rapamycin (mTOR) survival pathway is deregulated in less than 70% of all ovarian cancers through genetic mechanisms including amplification or activating mutations of *AKT1*, *PIK3CA* (encoding PI_3K), or *MTOR,* and inactivating mutations of *PTEN, TSC,* or *LKB1* [30,31]. In addition, survival factors of the tumor microenvironment [6], such as insulin-like growth factors, can trigger the PI_3K signaling pathway.

A characteristic feature of ovarian cancer is the prominent role of the STAT3 pathway, which is triggered by several mediators of the malignancy-associated ascites, notably IL-6 and IL-10,

mainly derived from tumor cells and TAMs [3,5,6,32]. STAT3-inducing cytokines are mediators of tumor growth, chemoresistance, inflammation, and immunosuppression and are clearly associated with a short survival [32,33]. STAT3 is also directly upregulated by miR-551b, which contributes to increased apoptotic resistance and proliferation of ovarian cancer cells and tumor growth in mice [34].

The transcription factor NFκB, known for a long time to play a pivotal role in blocking apoptosis, is constitutively active in more than 50% of all ovarian carcinomas and is associated with poor survival [35–37]. This activation of NFκB presumably results from the presence of proinflammatory cytokines in the ovarian cancer microenvironment, including tumor necrosis factor-α and IL-6, and ligands of the epidermal growth factor family [3,5,6,32,38–40]. NFκB is also activated by platinum-based drugs, which may limit their clinical efficacy [41]. Furthermore, NFκB is activated by crosstalk with AKT and STAT3 pathways. Notably, persistently activated STAT3 has been shown to maintain NFκB constitutively active in ovarian cancer cells [42]. Protumorigenic proteins encoded by target genes of NFκB include BCLX-L, IL-6, IL-8, vascular endothelial growth factor, and PD-1L, reflecting its multifaceted role in ovarian cancer biology and therapy resistance.

Pathways triggered by cancer cell adherence and detachment, as well as by homotypic and heterotypic intercellular interactions also impinge on the regulatory circuits of programmed cell death and on the efficacy of chemotherapeutic drugs. Interaction of cancer cells with the extracellular matrix (ECM) or the adhesion molecule L1 cell adhesion molecule through integrins induced prosurvival pathways, including the activation of NFκB [43], while detachment triggers autophagy-associated survival mechanisms [44]. A recently published proteogenomic analysis of ovarian cancer cells has identified a strong association of integrin and RhoA-mediated signaling with short survival [45], consistent with the role of these pathways in invasion and apoptotic resistance [46,47].

Epigenetic regulation may also contribute to ovarian cancer chemoresistance. For example, the interaction of ovarian cancer cells with mesothelial cells induces a DNA methyltransferase-mediated repression of miR-193b expression in ovarian cancer cells [23]. As miR-193b has been reported to promote cell death following 5-fluorouracil treatment of esophageal cancer cells [48], it is possible that the repression of miR-193b by paracrine mechanisms contributes to the chemoresistance of ovarian cancer.

Finally, spleen tyrosine kinase (SYK), originally identified as a tyrosine kinase that couples immunoglobulin receptors on immune cells to intracellular signaling pathways that trigger NFκB activation and inflammatory responses, has recently been recognized as a promoter of cell survival in different cancer types [49]. Relapsed ovarian carcinomas express elevated levels of SYK and phospho-SYK, and inhibition of SYK sensitizes ovarian cancer cells to paclitaxel *in vitro* and *in vivo* [50]. The phosphorylation of tubulins and microtubule-associated proteins by SYK is presumably instrumental for induction of paclitaxel resistance. It is currently unknown how SYK and phospho-SYK are upregulated in ovarian cancer cells. SYK has been shown to be induced by reactive oxygen species (ROS) [51]. As ROS are eliminated by GSH [52], and GSH is kept at a low level in chemoresistant ovarian cancer by CAFs (see above), an involvement of ROS in the activation of SYK in resistant tumor cells is conceivable.

Experimental Models: Caveats and Perspectives

Although the findings reviewed in the preceding sections have clearly advanced our knowledge of pathways and mechanisms that promote chemoresistance of ovarian cancer, the relevance of most of these findings in clinical settings needs to be clarified. Most functional studies were performed with established cell lines or mouse models that do not faithfully reproduce human

ovarian cancer. Established tumor cell lines are especially problematic with respect to chemoresistance studies. Comprehensive gene expression analyses of multiple cell lines representing all major cancer types showed that the establishment of permanent cell lines is accompanied by the induction of antiapoptotic resistance mechanisms that do not reflect therapy-related events [53]. Furthermore, the precise origin of many ovarian cancer cell lines is unclear and/or do not represent human high-grade serous carcinoma (*TP53* mutated), which is the most common ovarian cancer. Thus, the most frequently used human line, SKOV-3 (*TP53* wt), presumably was derived from a human clear cell carcinoma cell line [54], and ID8 cells used for transplantation in syngeneic mouse models were obtained by *in vitro* transformation of murine ovarian surface epithelial cell lines [55].

Ince and colleagues [56] recently reported a technological breakthrough to culture primary ovarian carcinoma cells. Cancer cells from ascites can be grown under protocol conditions without crisis, with no apparent upper limit of population doubling and no decrease in growth rate. Importantly, these cells retain the genomic, histopathological, and molecular features of the original tumors, and the drug responses of these cell lines correlate with clinical outcomes. This experimental system is likely to solve many of the problems associated with previously established cell cultures.

Another problem to understand therapy resistance concerns genetically engineered mouse model of serous ovarian carcinoma. Most available mouse models are based on tumor genes that are not known to play a role as drivers of human high-grade ovarian carcinoma, such as *SV40-T, kras*, or *dicer* [57]. Even though these mice develop a disease resembling human carcinomatosis, the relevance of these models to understand high-grade serous ovarian carcinoma in humans is questionable. The only established mouse model that recapitulates the human disease, both genetically and clinically, is based on the simultaneous disruption of the *Tp53, Pten*, and *Brca1* or *Brca2* genes targeted to ovarian epithelial cells by a regulatable Pax8 promoter [58]. However, the use of this model is hampered by its complexity, in particular if additional genetic alterations are considered, a problem that could be solved by the recent advances of the clustered regularly interspaced short palindromic repeats (CRISPR)/CRISPR-associated protein 9 (Cas9) technology [59]. This is of the utmost importance in view of the participation of immune and other host cells in determining the efficacy of chemotherapy, as discussed earlier, an aspect that cannot be appropriately addressed in the described patient-derived xenograft mouse models [60,61].

Finally, a complex 3D organotypic model, composed of primary human fibroblasts embedded in an ECM and covered by a monolayer of omentum-derived primary human mesothelial cells, is another promising experimental system [23,57,62]. This model emulates the morphological and functional features of the *in vivo* human peritoneal microenvironment, thereby offering unique possibilities to study adhesion, proliferation, and invasion of primary tumor cells, and also to investigate the role of host cells and the microenvironment in these processes and to study the effects of experimental therapeutic interventions.

Concluding Remarks

Research in the past few years unraveled numerous novel functions of host cells in promoting the resistance of ovarian cancer to chemotherapy. This work identified a number of clinically highly relevant mechanisms, but at the same time also highlighted unresolved questions with respect to the development of novel targeted therapies (see Outstanding Questions). T cells play an essential role in this context by enhancing the efficacy of chemotherapeutic drugs, for instance, by their unexpected inhibitory effect on CAFs. However, this function is dependent on the secretion of IFNγ following T cell activation, which is largely blocked in the ovarian cancer microenvironment, but can be reactivated by inhibition of the PD-1 checkpoint. Antibodies

Outstanding Questions

Evaluating novel mechanisms and potential drug targets in clinically relevant experimental settings is an urgent challenge. These models include patient-derived primary cells, 3D and co-cultures, and mice with conditional inactivation of *Tp53, Pten,* and *Brca* genes.

It is essential to assess the advantage of combining multiple treatment modalities, including chemotherapy, inhibitors of signaling components, and checkpoint blockers.

Checkpoint blockers beyond inhibitors of PD-1 should be explored as chemosensitizing drugs.

EV-encapsulated microRNAs play a major role in intercellular communication affecting drug resistance. It is essential to elucidate the mechanisms that regulate miRNA expression as well as the biogenesis, release, and uptake of EVs to better access them as potential targets.

The complexity of the microenvironmental signaling network with respect to mediating chemoresistance needs further clarification, in particular the relevance of individual pathways and intracellular signaling mechanisms, for example, NFκB.

The cooperation of microenvironment-induced signaling and genetic alterations is poorly understood at present but may be key to eventually achieve efficacious individualized therapies.

A hallmark of ovarian carcinoma tissue and ascites is their extreme heterogeneity. Analyzing the effect of drugs on individual cells and understanding their contribution to chemoresistance will be of paramount importance to develop more efficient treatment regimens. This includes the application of omics to primary cells prior to, during, and after therapy, as well as at relapse.

Finally, the integration of data from different Omic technologies to establish global networks will promote the identification of novel resistance mechanisms and promising drug targets.

against PD-L1 also inhibited glycolytic glucose utilization and inhibit mTOR activity in tumor cells in mouse sarcoma models, pointing to an additional therapeutic benefit of therapies directed to PD-1L [63]. Based on the first clinical observations, targeting the PD-L1/PD-1 signaling axis indeed seems to offer a promising new treatment modality. It remains to be seen whether inhibitors of other immune checkpoints also have the potential to enhance the efficacy of chemotherapeutic drugs, as reported for the synergistic effect of anti-CD47 antibodies on other tumors [64,65]. Ipilimumab, a CTLA-4 antibody, is another candidate that is currently being evaluated in clinical Phase II studies [66].

miRNAs, frequently delivered to tumor cells via host cell-derived EVs, turned out as pivotal players in promoting ovarian cancer resistance. In this context, the direct targeting of the apoptosome in cancer cells by EV-encapsulated miR-21 from omental adipocytes is particularly intriguing. The identification of other miRNAs that target central resistance-promoting mechanisms, for instance PD-1L or STAT-3, attests to the relevance of these regulatory molecules. The mechanisms regulating the synthesis of these miRNAs as well as the biogenesis, release, and uptake of EVs are currently unknown, but represent interesting potential targets for pharmacological invention.

PI_3K–AKT–mTOR, STAT3, and NFκB are major intracellular signaling pathways triggered by the microenvironmental stimuli, such as soluble mediators, EVs, or cellular or ECM interactions, that mediate chemoresistance. Small molecule inhibitors are available for key components of these pathways (Box 4). As these drugs have either been approved for other clinical applications or are currently being evaluated in clinical trials, their use in chemoresistant ovarian cancer can be envisaged. The combination of chemotherapy, signaling modulating drugs, and checkpoint inhibitors may therefore provide a major breakthrough and revolutionize ovarian cancer therapy.

Chemoresistance of ovarian cancer can be inherent, acquired, or conditional. It is highly likely that these different scenarios of therapy resistance are linked to different molecular mechanisms. It will be of the utmost importance to investigate the role of defined pathways and the effect of their modulation in different experimental models reflecting relevant clinical settings.

Comprehensive Omics-based data sets are available and these will clearly be expanded by ongoing and future analyses. The bioinformatic integration of these data obtained by genomics,

Box 4. Clinical Development of Small Molecule Inhibitors of Signaling Pathways That Promote Ovarian Cancer Chemoresistance

Drugs approved for treatment of other cancers include the PI_3K-δ inhibitor idelalisib, the AKT inhibitor miltefosine [82], and the mTOR inhibitor everolimus [31]. STAT3 inhibitors are still at earlier stage of development, but the anti-IL-6 antibody tocilizumab is used for treatment of arthritis. Tocilizumab might be useful to neutralize IL-6, one of the major protumorigenic mediators in the ovarian cancer microenvironment [83,84]. The SYK inhibitor fostamatinib is currently in clinical trials for treatment of inflammatory conditions [49].

Therapeutic targeting of NFκB in ovarian cancer has been attempted with the proteasome inhibitor bortezomib [85]. While the limited data from a Phase I study were encouraging [86], results of a Phase II study were disappointing [87] and led to a discontinuation of its clinical development for ovarian cancer. However, these findings do not diminish the potential relevance of NFκB as a target in ovarian cancer, as the reasons for the clinical failure of bortezomib are not known. Several aspects need to be considered in this context. First, bortezomib is a nonselective proteasome inhibitor that also targets the immunoproteasome, which may be therapeutically undesirable [88]. More selective inhibitors, which are available now, may show a better efficacy. Second, bortezomib has off-target effects that may be counterintuitive for cancer therapy, for instance the induction of IL-8 in ovarian cancer cells [89]. IL-8 induction was prevented by IκB kinase inhibition, concomitantly with an increased therapeutic effect in mouse xenograft models. Third, advanced stage relapsed cancers may be less dependent on NFκB than tumors at earlier stages, suggesting that NFκB inhibiting drugs may have a greater impact in an adjuvant setting. Taken together, these findings show that there is an urgent need to understand the clinical failure of bortezomib and to identify novel, more selective drugs for clinical testing.

transcriptomics, miRNomics, proteomics, and lipidomics represents one of the current challenges. A major goal of this work will be the construction of global networks that connect cell autonomous and mutation-driven mechanisms with microenvironment-triggered signaling pathways. To take the extreme heterogeneity of both cancer and host cells into account, it will also be of paramount importance to perform global molecular analyses on single cells, which currently represents a technological hurdle. This requirement also applies to functional studies, which should focus on individual cancer and host cells. These studies must take the diversity in cellular responses to environmental cues and chemotherapeutic drugs into consideration to gain a deep understanding of the functional interaction of tumor and immune/host cells impinging on the efficacy of chemotherapy. It can be anticipated that this work will lead to new and unexpected insights into microenvironment-triggered signaling mechanisms promoting chemoresistance in ovarian cancer that will pave the way to more efficacious therapies.

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