Summary and perspectives

Chapter 8
Ovarian cancer remains an extremely challenging disease, in terms of unraveling its histopathological origins, as well as optimizing diagnosis and treatment. A testimony to this fact is the relatively unchanged long-term survival for ovarian cancer patients over the last 30 years (1). Importantly, the inability to improve prognosis using standard surgery and chemotherapy suggests that the ceiling has been reached for these conventional treatment modalities and new approaches are needed.

In the past decades, the re-activation of active antitumor immunity has gained prominence as a possible means of achieving curative ovarian cancer treatment. After all, ovarian cancer has proven to be immunogenic, with both cellular and humoral immune responses often present and correlating with disease outcome (2). In clinical trials on active immunotherapy in ovarian cancer, successful immune responses were induced against the targeted tumor antigen(s). However, these immunological responses have not yet translated into meaningful clinical responses (3). This discrepancy may be caused by the fact that the tumor microenvironment is typically immunosuppressive, for instance due to the presence of Treg (chapter 1 & 2) or lack of appropriate antigen presentation (chapter 3). These findings underline the extreme complexity of the immune environment in ovarian cancer.

This thesis was dedicated to gaining a better understanding of the interaction between ovarian cancer and immune cells, focusing on factors that influence the number and activity of T lymphocytes in the tumor. Ultimately, a better understanding of the factors at play here may contribute to the rational design of immunotherapeutic modalities or combinations thereof.

**Summary**

**Chapter 1** provides a general introduction on ovarian cancer and the adaptive immune response against ovarian cancer, in particular of the adaptive immune system, followed by a short outline of each chapter.

In brief, the immune system is capable of recognizing and responding to ovarian cancer cells. This translates into a survival advantage for patients with high numbers of intratumoral T lymphocytes and especially a high CTL/Treg ratio. These intratumoral lymphocytes can mount an immune response to several identified tumor antigens. At the same time, tumor specific factors may negatively impact the cytotoxic potential of effector T lymphocytes. Therefore, current tumor immunology research focuses on the identification and manipulation of such immunomodulatory factors.

In **chapter 2**, we performed a systematic review and meta-analysis of studies investigating the prognostic impact of TIL in solid tumors, aiming to establish pooled hazard ratios for survival outcomes. Studies were included in which the prognostic significance of intratumoral CD3+, CD4+, CD8+, and FoxP3+ lymphocytes, as well as ratios between these subsets, were determined in ≥100 patients. In total, 52 papers were included, 34 of which were used for pooled analysis.
In pooled analysis, CD3+ TIL had a positive effect on survival with a hazard ratio (HR) of 0.58 (95% confidence interval (CI) 0.43-0.78) for death, as did CD8+ CTL with a HR of 0.71 (95% CI 0.62-0.82). FoxP3+ Treg were not linked to overall survival, with a HR of 1.19 (95% CI 0.84-1.67). The CTL/Treg ratio produced a more impressive HR (risk of death: HR 0.48, 95% CI 0.34-0.68) but was used in relatively few studies. Smaller sample size and a shorter follow-up time were associated with an increased likelihood of finding statistically significant survival outcomes.

In conclusion, not the number of infiltrating T lymphocytes, but the ratio between CTL and Treg may be the strongest predictor of patient survival, as this ratio more accurately reflects the balance of powers within a tumor. Any future studies should have a very strict design, with large sample sizes to increase statistical power, a uniform way of analyzing survival outcomes, and an adequate follow-up period.

In chapter 3 we assessed the prognostic value of CD8+ CTL, FoxP3+ Treg, and CD45R0+ memory T lymphocytes in ovarian cancer. Immunohistochemical stainings were performed on ovarian tumor tissue and/or omental metastases from 306 stage I-IV ovarian cancer patients. In ovarian derived tumor tissue, the presence of FoxP3+ Treg was associated with high grade and advanced stage disease. High numbers of CD8+ CTL and a high CTL/Treg ratio were independent predictors for improved survival. When only advanced stage patients were included in the analysis, CD8+ CTL and FoxP3+ Treg in ovarian derived tumor tissue were both independent predictors of improved survival. The latter finding was surprising, in view of the immunosuppressive effects of Treg. Interestingly, such a contra-intuitive prognostic benefit of Treg was also reported in some other malignancies, most notably colorectal cancer and head and neck cancer (4). In our cohort, this finding may be explained by the strong positive associations between numbers of infiltrating Treg and CTL and a strong prognostic benefit of a high CTL/Treg ratio. Taken together, it seems likely that some degree of Treg infiltration accompanies an otherwise effective immune response, and the proportions of different T lymphocyte subsets determine its final outcome. Another possible explanation that was offered previously is that the role of Treg differs with tumor stage, and that their detrimental role is confined to early stage cancer (4). Also, the use of FoxP3 as a marker for Treg may slightly overestimate the Treg population, in view of some reports that FoxP3 is also expressed on a small population of CD8+ CTL.

In chapter 4, we analyzed components of the antigen processing and presentation pathway, to determine which of these are prone to downregulation in ovarian cancer and whether this impacts patient prognosis. The antigen processing and presentation pathway is instrumental in the ability of the adaptive immune system to recognize cancer cells. The final product of functioning antigen processing machinery is the presentation of a peptide on the (tumor) cell surface by the MHC class I complex, consisting of an HLA molecule with a β2m unit. In our ovarian cancer cohort, expression of a stable MHC class I complex was frequently lost, with co-expression of HLA-A/β2m observed in only 49.1% of patients and HLA-B/C/β2m in 69.8% of patients. In multivariate survival analysis, an intact HLA-B/C/β2m complex predicted improved survival.
The proteasome plays an important role in generating immunogenic peptides by cleaving proteins into smaller fragments. The proteasome exists in two main varieties, i.e. the constitutive proteasome and the immunoproteasome, as well as some intermediate forms. Stimulation with IFN-γ converts the constitutive proteasome into the immunoproteasome. The immunoproteasome is thought to be more capable of producing immunogenic peptides than the constitutive proteasome. In line with this notion, we found that expression of the immunoproteasome and MHC class I complex was associated with higher numbers of infiltrating CD8+ T lymphocytes. Furthermore, in patients with normal MHC class I expression the IFN-γ inducible immunoproteasome was more prevalent than the constitutive proteasome, and vice versa.

Expression of MB1, a component of the constitutive proteasome, was independently associated with reduced survival. This negative effect of MB1 may stem from multiple downstream pathways regulated by the constitutive proteasome. For instance, pro-survival and mitogenic signaling by the Nuclear Factor Kappa B (NF-κB) pathway is activated by proteasomal degradation of I-κB, an inhibitor that normally blocks NF-κB activation. Presence of the proteasome may thus enable such tumor promoting signaling. Indeed, a recent study in ovarian cancer suggests that NF-κB pathway activation via proteasomal I-κB degradation triggers production of pro-inflammatory chemokines, leading to a chronic pro-inflammatory tumor microenvironment. Such an environment is frequently present during tumor development, and characterized by aspecific activation of innate immunity, as opposed to a targeted adaptive immune response. This chronic inflammatory environment subsequently promotes ovarian cancer dissemination (5).

Of note, proteasome activity can clinically be modulated via proteasome inhibitor bortezomib (Velcade ®). An in vitro study showed that bortezomib treatment suppressed proliferation and increased autophagy in ovarian cancer cells in vitro (6). Moreover, bortezomib increased tumor immunogenicity in a murine ovarian cancer model, by upregulating heat shock proteins 60 and 90 and enhancing dendritic cell (DC) function (7). Despite these results, phase II trials of bortezomib in ovarian cancer patients have only shown minimal benefits (8,9).

In chapter 5 we investigated the expression of HLA-E in 270 ovarian and 150 cervical cancer samples. HLA-E is a nonclassical HLA molecule, characterized by its nonpolymorphic nature. It can only bind a very limited spectrum of peptides, most importantly the leader sequences of classical HLA class I molecules. These leader sequences are shed when the classical MHC class I molecule enters the endoplasmatic reticulum, and subsequently undergo complete processing by the antigen presentation pathway. The presentation of leader sequences by HLA-E can thus be viewed as a sensor of the integrity of the entire MHC class I pathway (10). The principal receptors for HLA-E are the inhibitory CD94/NKG2A and stimulatory CD94/NKG2C, which are mainly expressed on NK cells. An important function of NK cells is as a ‘back up’ for CTL in case of viral infection. Viruses often downregulate the MHC class I pathway in host cells, to avoid killing by CTL. NK cells can subsequently recognize and remove these cells. Thus, the presence of HLA-E on the cell surface indicates to NK cells that the MHC class I pathway is intact and no action needs to be taken.

In both tumor types, expression of HLA-E did not correlate with clinicopathological factors such
as stage, histology, and grade. In ovarian cancer, HLA-E expression was strongly associated with HLA-A, -B, and -C, consistent with the binding of HLA class I peptides by HLA-E. As mentioned above, NK cells are the principle HLA-E interacting cells. However, using antibodies against NK cell markers CD56, CD57, and Nkp46, we found that NK cells were lacking in the overwhelming majority of samples. Thus, it is unlikely that HLA-E on ovarian and cervical cancer cells interacts with NK cells. CD94/NKG2A and CD94/NKG2C can also be expressed on a subset of activated T-lymphocytes. Indeed, we found CD94/NKG2A co-expression on up to 50% of tumor infiltrating CTL (median: 12%) in a focused panel of nine fresh surgical ovarian and cervical cancer samples, whereas CD94/NKG2C expression was negligible.

Importantly, in ovarian cancer, HLA-E expression negatively affected the previously observed survival benefits of CD8+ CTL infiltration. Survival analysis revealed that the survival benefit of tumor infiltrating CTLs, as earlier identified in chapter 3, was completely neutralized in the subpopulation of patients with high HLA-E expression. In conclusion, these results suggest that HLA-E expression on ovarian and cervical cancers is a powerful immune escape mechanism, by active inhibition of intratumoral CD8+ CTL via CD94/NKG2A.

In a previous microarray study by our group, gene expression patterns were compared between ovarian cancer samples with few and many CD8+ CTLs (11). CXCL16 and its receptor CXCR6 emerged as differentially expressed, and therefore possibly involved in CTL infiltration. In chapter 6, the possible role of CXCL16/CXCR6 in tumor behavior and lymphocyte infiltration was evaluated further.

CXCL16 exists in both a transmembrane form (TM-CXCL16) and a soluble form (sCXCL16). sCXCL16 is generated by cleavage of TM-CXCL16 by the enzymes A Disintegrin And Metalloproteinase (ADAM)-10 and -17. TM-CXCL16 and CXCR6 were assessed by immunohistochemical stainings on 306 ovarian cancer tissue samples, and sCXCL16 by ELISA on serum samples of 118 of these patients. High levels of sCXCL16 were a strong predictor of decreased survival time (median survival: 57 months vs. 149 months), even after correction for age, stage, grade, histology, and residual tumor in multivariate analysis. There were no associations between CXCL16 or CXCR6 and numbers of infiltrating CD8+ CTL, FoxP3+ Treg, or CD45R0+ memory T lymphocytes.

Next, we performed in vitro experiments to confirm that ADAM-10 and -17 are involved in shedding of sCXCL16 in ovarian cancer. Incubation of an ovarian cancer cell line and primary ovarian cancer samples with ADAM-10/17 inhibitor TAPI-2 resulted in an accumulation of TM-CXCL16 on the cell surface. This suggests that ADAM-10 and/or 17 are indeed responsible for cleavage of CXCL16 in ovarian cancer. Furthermore, ADAM-17 inhibition diminished the invasive behavior of ovarian cancer cells as assessed by scratch assay. Since ADAMs are involved in the cleavage of a multitude of proteins other than CXCL16, it seems that the negative prognostic value of sCXCL16 is at least in part a reflection of ADAM activity. Indeed, since ADAM activity is difficult to assess in vivo, sCXCL16 might be a convenient pseudomarker for identifying ovarian cancer patients with highly aggressive tumors that require more intensive therapy.
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In chapter 7, we focused on activation of T lymphocytes using Gal-9, a glycan binding protein which is known for inducing apoptosis in certain T lymphocyte subsets via its receptor TIM-3. Our results demonstrate that TIM-3 is not expressed on resting peripheral blood mononuclear cells (PBMCs). Nevertheless, Gal-9 binds to resting PBMCs, indicating that (an)other receptor(s) must be present. In line with previous studies, treatment of PBMCs with Gal-9 triggered dose dependent induction of apoptosis. However, at relatively low concentrations (5-30 nM), the remaining population of viable T-lymphocytes was activated, as evidenced by upregulation of CD25 within 1 day. Moreover, this was accompanied by a strong expansion of the T-lymphocyte population, with a 4-fold increase in cell number and up to 6 cell divisions after 7 days. During this activation, a shift occurred from a naïve towards a central memory (CD62+/CCR7+/CD45R0+) and IFN-γ producing Th1 phenotype. In the presence of T-lymphocyte activating signals anti-CD3 and IL-2, Gal-9 treatment reversed the normal CD8+/CD4+ balance in favor of CD4+ T lymphocytes. Taken together, these results show that at low concentrations Gal-9 can activate T lymphocytes via an as of yet unidentified receptor. Since Gal-9 was detected in serum and ascites of ovarian cancer patients, this protein may modulate T-lymphocyte immune responses in cancer. Further, a recombinant form of Gal-9 may be suitable for use as therapeutic immunostimulatory molecule.

Perspectives

A large part of tumor immunology research, like the studies presented in this thesis, is dedicated to characterizing pro- and anti-tumor effects of immune processes. The ultimate goal of this preclinical research is to identify immunological markers for patient stratification and to identify targets for intervention, i.e. immunotherapy. However, although understanding of the immunologic microenvironment in ovarian cancer has greatly increased over the past decades, clinical applications remain to be optimized. One important hurdle that still needs to be taken in the search for effective immunotherapy is finding the Achilles heel in the immunosuppressive environment of advanced ovarian cancer.

Standardized characterization of immune infiltrates

To improve clinical success of immunotherapy, the Society for Immunotherapy of Cancer (STIC), an international collaboration of immunotherapy researchers, compiled a list of nine hurdles and accompanying ‘opportunities’ to improve translation from fundamental to clinical tumor immunology research (12). The data presented in this thesis highlight the importance of hurdle number 3: ‘complexity of cancer, tumor heterogeneity and immune escape’. To tackle this hurdle, the STIC recommends better characterization of tumors, including ‘a global standardization of the tumor microenvironment’ (12).

In colorectal cancer, standardized characterization of immune infiltrates is being taken one step further. Currently, a world wide task force is working on establishing an ‘Immunoscore’ for colorectal cancer, which is to be integrated in conventional TNM staging (13). This score was devised after it
was shown in several cohorts that the composition of the immune infiltrate in colon cancer was a better predictor for survival than TNM staging (14). The Immunoscore consists of a semiquantitative score of CD8+ and CD45R0+ T lymphocytes in the central tumor region and invasive margin, ranging from 0 (low densities of both cell types in both locations) to 4 (high densities in both locations). The task force is now working on worldwide validation of the Immunoscore in colorectal cancer, but future goals include expanding it to other cancer types (13).

In ovarian cancer, it is not yet determined whether including a similar Immunoscore in routine histopathological examination could improve the prediction of clinical outcome. However, if this turns out to be the case, implementation of such a protocol would not only add to a more precise characterization of ovarian cancer, but also assist in selecting patients most eligible for immunotherapeutic intervention. Moreover, from a research point of view, a uniform Immunoscore would deal with some of the issues mentioned in chapter 2. There, we describe how methodological heterogeneity affects outcomes of prognostic studies. A standardized way of reporting immunologic parameters will minimize these differences and improve reproducibility of biomarker studies.

The immunosuppressive environment in ovarian cancer: dendritic cells as central players?

Even though the presence of certain intratumoral lymphocyte subsets is associated with longer survival, the immune system is not capable of eradicating advanced cancer altogether. In other words, once the tumor has entered the so-called escape phase of the tumor immunogenicity concept, the immune system has lost the battle. However, knowing what exactly is responsible for the transition of equilibrium to escape could provide a window of opportunity for intervention. In recent studies, important new insights in this transition have been gained by mapping the immune infiltrate over time. Such a ‘longitudinal’ approach to anti-tumor immunity is one important aspect that is very difficult to study and has been lacking up to now. However, studies are starting to emerge in which a clear transition from a pro-immunogenic to immunosuppressive environment is described in ovarian cancer, with a prominent negative role for DCs.

DCs are professional antigen presenting cells, capable of activating naïve or memory T lymphocytes. Immature, i.e. resting, DCs reside in epithelia, which are the most common site of entry for microbes. Once they encounter a suitable antigen for presentation by MHC class I or II, DCs become activated and migrate to lymph nodes. At this point, they upregulate several costimulatory receptors on their cell surface, such as CD40, CD80, and CD86. Upon arrival in the lymph nodes, DCs can activate B lymphocytes, CD4+ and CD8+ T lymphocytes via the MHC bound antigen and co-stimulatory molecules.

The concept that defective DCs are involved in the inability of the immune system to eradicate tumors is not new (19). However, the importance of DCs in tumor progression was perhaps underestimated. Whereas the tumor immunosurveillance concept is mainly based on loss of tumor immunogenicity as causal factor in the transition from equilibrium to escape, recent studies raise the possibility that this progression depends largely on intrinsic characteristics of the immune infiltrate.

For instance, in a very elegant study the immunologic microenvironment of advanced human
ovarian cancer was recapitulated using an inducible p53-dependent mouse model (20). In this study, it was found that in the early stages of tumor development, immunocompetent DCs were able to control tumor outgrowth, consistent with the equilibrium phase of the cancer immunoeediting concept. However, in later stages a crucial shift in DC function was observed, which coincided with aggressive tumor growth. Further experiments revealed the underlying mechanism, namely that prostaglandin E2 and Transforming Growth Factor (TGF-)β secretion by tumor cells induced an immunosuppressive phenotype in DCs, characterized by decreased expression of MHC class II and the co-stimulatory molecule CD40. At the same time, increased expression of PD-L1 and increased arginase activity by DCs actively suppressed immune effector cells. Subsequent experiments in which DCs were depleted at different stages of tumor development corroborated this evidence: DC depletion accelerated tumor growth in early stage tumors, whereas it decreased tumor growth in late stage tumors (20).

Similarly, another study reported that immunosuppressive myeloid DCs play a major role in Treg recruitment, by secreting CCL22 in response to IFN-γ (21). This phenomenon was observed in late, but not in early stage ovarian cancers. Again, this points to a quite distinct change from immunostimulatory to an immunosuppressive environment which revolves around a change in DC function.

Taken together, these data demonstrate a dual effect by which tumor cells manage to take the immunologic circumstances from bad to worse: DCs are prevented from effective antigen presentation, but also induced to actively sabotage the anti-tumor immune response by suppressing effector T lymphocytes and attracting Treg.

Regaining DC functionality would therefore be a crucial step in reversing the immunosuppressive environment as whole. This could be accomplished either at a causative level (targeting the inhibitory cytokines or the production thereof by the tumor cells), or at a more symptomatic level (reprogramming the DCs). Although the causative approach may be more elegant from a physiological point of view, it may not be feasible due to the multitude of inhibitory cytokines possibly involved in DC malfunction. The study by Scarlett et al. mentioned above identifies prostaglandin E2 and TGF-β as culprits (20), but from previous research factors such as VEGF, macrophage colony stimulating factor (M-CSF), IL-6, and IL-10 are also known to negatively affect DC function (22). Targeting the DC directly may thus be more efficient. As mentioned below, encouraging results have already been reported, although the proverbial silver bullet remains to be identified.

How to reverse immunosuppression?

Many individual factors contributing to the immunosuppressive environment of advanced ovarian cancer have already been defined, often accompanied by possible remedies. For instance, as mentioned in chapter 1 and chapter 2, Treg negatively affect the function of other T lymphocytes, but they can be selectively inhibited by cyclophosphamide (15). Other inhibitory factors that have gained some notoriety over recent years include the enzyme indoleamine 2,3-dioxygenase (IDO) (16), the endothelin-B receptor (ETbR) (17), and PD-L1 (18). IDO depletes the tumor microenvironment of tryptophan, resulting in cell cycle arrest of effector T lymphocytes, and also
promotes the generation of Treg. ETbR is expressed on tumor endothelium and prevents entry of T lymphocytes into the tumor epithelium. PD-L1 directly inhibits T lymphocytes via interaction with receptor PD-1, which is expressed on activated T lymphocytes. For each of these mechanisms, specific targeting methods have been identified and tested in animal models: 1-methyl-tryptophan against IDO, and antibody mediated blockades of ETbR and PD-L1. These mechanisms are, however, not universally present in ovarian cancers. In fact, each ovarian cancer possesses a unique profile of tumor characteristics and immunomodulatory mechanisms. Thus, for immunotherapy to be successfully applied in the clinical setting, it might eventually come down to either a) designing personalized immunotherapy, based on which immunosuppressive mechanisms are at play in the individual patient or b) finding a common denominator earlier in tumor development that converts a pro-immunogenic into an immunosuppressive environment, such as the DCs mentioned previously.

Up to now, the agents with the most success in clinical trials are cytotoxic T lymphocyte associated antigen 4 (CTLA4) and PD1 blockers, albeit mostly in non-gynecologic malignancies. Importantly, since these agents target DC function, they may positively impact the DC malfunction mentioned above.

CTLA4 negatively regulates TCR signaling, by competing with the costimulatory molecule CD28 for binding of ligands CD80 and CD86. Since CTLA4 has a much higher affinity for both CD80 and CD86, CD28 is effectively outcompeted (23). Currently, the humanized anti-CTLA4 antibody ipilimumab (Yervoy®) is approved for use in advanced melanoma, increasing median overall survival by 3-4 months (23). Phase II and III trials are ongoing in a multitude of cancers, among which ovarian cancer (http://www.cancer.gov/clinicaltrials, NCT01611558).

A major difference between CTLA4 and PD1 is that PD1 regulates already activated T lymphocytes in the tumor or inflammatory environment, while CTLA4 inhibits the initial activation stages. Clinical application of PD1 blocking antibodies is not yet as far as for CTLA4. Initial trials show some promising results in melanoma, colon, renal, and lung cancer (23). Currently, there are no ongoing trials in which PD1 blockade is evaluated in ovarian cancer.

Currently, targeted delivery of costimulatory ligands to properly activate DCs is being investigated in our group, especially focusing on those belonging to the Tumor Necrosis Factor (TNF) superfamily. TNF ligands and their cognate receptors are normally expressed as transmembrane proteins. However, proteolytic cleavage and/or alternative splicing can result in the presence of soluble TNFLs (sTNFLs). sTNFLs are, unfortunately, not ideal for therapeutic use. First of all, sTNFLs have a very short serum half-life, e.g. 30 minutes for sTRAIL, leading to a lack tumor accumulation. More importantly, sTNFLs are poor activators of agonistic receptors compared to the cognate full-length transmembrane proteins.

These disadvantages can be transformed into advantages by incorporating sTNFLs in targeted immunotherapeutic approaches. In brief, an sTNFL is fused to an antibody fragment (scFv) selective for the desired target tissue, yielding a scFv:sTNFL fusion protein. While being essentially inactive when soluble, antibody fragment mediated binding converts the sTNFL to membrane-bound form that efficiently activates agonistic receptors. In addition, this approach ensures high local
TNFL concentrations in the desired location and reduces glomerular excretion. Moreover, such an approach may reduce the systemic toxicity which is otherwise likely to occur in view of the ubiquitous expression of the target molecules (24,25).

**Integrating immunotherapy with non-immunotherapeutic modalities**

In addition to local immunosuppression, the pleiotropic nature of ovarian cancer is a major hurdle to successful therapy, including non-immunotherapeutic modalities. As mentioned in chapter 1, ovarian cancer has distinct histopathologic origins and is therefore currently more viewed as a collection of separate entities instead of one illness (26). Such heterogeneity does not only exist between tumors, but also within individual tumors. For instance, it was demonstrated that a single ovarian cancer cell can give rise to six different cell subpopulations, with varying capacities for self-renewal and different tumorigenic phenotypes (27).

One consequence of these observations is that multimodal treatment is likely needed to adequately suppress or eliminate the tumor as a whole. Thus, conventional surgery and chemotherapy may be combined with immunotherapy to achieve a curative therapy. In this context, it would probably be most effective and efficient to find synergistic effects between conventional and immunotherapeutic treatment.

An example of such a synergistic effect is the induction of immunogenic cell death (ICD). Immunogenic cell death is specifically capable of inducing an immune response against antigens present in the dying cells (28). On a molecular level, ICD is characterized by the exposure of calreticulin and heat shock proteins on the plasma membrane and secretion of ATP and High Mobility Group Box 1 (HMGB1). These factors subsequently bind receptors on DC’s, resulting in phagocytosis of dying cells and the presentation of (tumor) antigens to T lymphocytes and protective immunity in syngeneic mouse models (28). Therefore, induction of ICD may provide the immunostimulatory shift searched for in ovarian cancer.

ICD can be elicited by several anti-cancer agents, such as anthracyclins, cyclophosphamide, and oxaliplatin. In ovarian cancer specifically, only a limited number of studies are available. Poly(I:C) induced ICD in ovarian cell lines and patient material (29). Similarly, anthracyclins were found to induce ICD in ovarian cancer cell line OV70 (30). Notably, with regard to anthracyclins, current Dutch standards for ovarian cancer incorporate liposomal doxorubicine as an option in case of platinum resistant disease (31). This might therefore be a lead candidate for evaluation of ICD induction.

Further research is needed to determine whether these agents can effectively induce ICD in vivo. Moreover, since the nonimmunogenic platinum-based compounds are the mainstay of ovarian cancer chemotherapy, it is not clear yet if and how ICD inducing agents could be integrated into standard therapy. In this context, using other drugs to induce ICD may be more appropriate. For instance, a recent study suggests that cardiac glycosides, e.g. digoxin, could efficiently induce ICD in tumors treated with agents other than the abovementioned ICD inducing chemotherapeutics (32). Whether these drugs can be safely administered to non-cardiac patients and if so, in which dose, will of course have to be evaluated.
Another perhaps unanticipated way to achieve immune activation is via angiogenesis inhibitors. Angiogenesis inhibitors were designed to block vascularization of tumors. However, it became evident that angiogenic factors secreted by tumor cells under hypoxic conditions, for instance Vascular Endothelial Growth Factor (VEGF)-A and CC-chemokine Ligand 28 (CCL28), recruit a number of immunosuppressive cells to the tumor (33). These include Treg, tumor associated macrophages (TAMs), myeloid-derived suppressor cells (MDSCs), neutrophils, and immature DC’s. These cells then also secrete pro-angiogenic factors, thus resulting in a synergy between pro-angiogenic and immunosuppressive effects (34).

In ovarian cancer, anti-angiogenic therapy in the form of the VEGF-A blocking antibody bevacizumab is currently the subject of many clinical trials. Up to now, bevacizumab has not yet lived up to its expectations, although small improvements in progression free survival have been observed when combined with standard chemotherapy (35). Interestingly, Treg and MDSC were shown to induce resistance to bevacizumab (34). Thus, combining anti-angiogenic therapy with immunomodulation may disrupt the pro-angiogenic and immunosuppressive tumor environment.

Concluding, there are ways to design a multimodal approach to ovarian cancer, in which immune modulation is combined with other types of therapy. Preclinical research can be used to find promising treatment combinations, with the emphasis on issues regarding dosage and timing regarding the administration of the individual compounds.

Conclusion

In conclusion, the immune system is a defining factor in the clinical course of ovarian cancer. In optima forma, it has the power to directly kill tumor cells through CD8+ CTL, thereby substantially contributing to the patients’ chance of survival. However, once an immunosuppressive environment has arisen, these effects can be largely negated. Reversing an immunosuppressive tumor microenvironment is a formidable challenge, due to the many factors at play. Finding which cell populations or tumor characteristics contribute most to this process will be key to developing successful immunotherapy strategies. In other types of cancer, substantial survival gains have already been achieved using immunotherapy. Since knowledge on ovarian tumor immunology is increasing day by day, similar steps forward might be taken in the search for curative (immunotherapeutic) treatment of ovarian cancer.

References


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