Beyond chemotherapy

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CHAPTER 1

General introduction and Outline of the thesis

GENERAL INTRODUCTION

Currently, several important questions regarding ovarian carcinogenesis and ovarian cancer therapy remain to be answered. While in the past ovarian cancer was thought to arise from the celomic epithelium that lines the surface of the ovaries, molecular profiling studies point towards extraovarian origins.[1] The major histological subtypes distinguished in ovarian cancer (*i.e.* serous, endometrioid, clear cell and mucinous adenocarcinoma) may instead originate from precursor lesions in the Fallopian tube (especially the fimbria) and endometriosis. This contributes to the molecular heterogeneity observed in ovarian cancer, and complicates viewing ovarian cancer as one disease entity in terms of clinical approach.

Ovarian cancer patients commonly present with disseminated disease. Despite current frontline treatment consisting of surgical debulking and chemotherapy, 5-year survival of these patients is still only 25-30%.[2] This can be largely attributed to the development of chemoresistance.

Platinum-based chemotherapy is still the cornerstone of systemic ovarian cancer treatment, and standard chemotherapy – consisting of carboplatin and paclitaxel given in 3-week cycles – has been unchanged since its introduction more than a decade ago. Many alternative regimens have been assessed, but failed to show superior outcome.[3] Some therapeutic improvement might be realized through alternatively dosing current standard chemotherapy, as has been shown for weekly dose-dense paclitaxel administration. However, chemotherapy seems to have reached its ceiling in ovarian cancer treatment.[4] Interest has therefore turned to the potential of incorporating molecular targeted agents that interfere with biological processes that are characteristic for the development and progression of (ovarian) cancer. Two of these hallmark phenomena are induction of angiogenesis (blood vessel formation) and evasion of apoptosis (programmed cell death).[5] Several drugs have been developed to inhibit angiogenesis or induce apoptosis.

Angiogenesis is an important feature of both ovarian physiology and ovarian carcinogenesis. These cancers are considered highly angiogenic, showing extensive vascularization and overexpression of many factors involved in angiogenesis regulation. The importance of angiogenesis in facilitating tumor growth and dissemination has been recognized ever since the early 1970s, when it was also proposed as a potential target for future cancer therapy.[6] It was the discovery of vascular endothelial growth factors (VEGFs) and their receptors (VEGFRs), which are important orchestrators of angiogenesis, that paved the way for antiangiogenic drug

development.[7] Most clinical experience today is based on studies with the VEGF-A-blocking antibody bevacizumab and VEGFR-targeted tyrosine kinase inhibitors (TKIs). These agents demonstrated clinical efficacy in a subgroup of ovarian cancer patients.[8-10] Newer insights have implied numerous other proteins in angiogenesis regulation, providing new avenues for angiogenesis inhibition. The exact place of antiangiogenic drugs in the treatment of ovarian cancer patients is currently being evaluated in a large number of clinical trials.

The tumor suppressor protein p53 mediates chemotherapy-induced cell death via an intrinsic (mitochondrial) mechanism. Inactivating mutations in the *p53 gene* occur frequently in high-grade ovarian cancers, especially in serous adenocarcinoma (up to 95%) which is the most common histological subtype.[11] Mutant p53 status contributes to chemoresistance and patients with mutant p53 cancers have a shorter progression-free and overall survival compared to those with wild-type p53.[12] Apart from intrinsic apoptosis induction, a cancer cell can be triggered to undergo apoptosis by exposure to death ligands, either in an autocrine or paracrine manner. After binding of these ligands to their receptors, which are present on the membrane of many cancer cells including ovarian cancer cells, the extrinsic apoptosis pathway is activated. Members of the tumor necrosis factor (TNF) superfamily are well-known death ligands that are being explored as anticancer drugs, amongst which are TNF-related apoptosis inducing ligand (TRAIL) and Fas ligand (FasL).[13]

The armamentarium of drugs targeting angiogenesis and apoptosis, as well as drugs directed against other hallmarks of cancer biology, is expanding rapidly. Given the heterogeneity in ovarian cancer, it is likely that any molecular targeted agent will only benefit a subgroup of ovarian cancer patients. This notion is supported by results from several clinical trials applying a wide range of molecular targeted agents, all showing – if any – benefit in a subpopulation of ovarian cancer patients. Together with toxicity and cost-effectiveness concerns, this stresses the need for adequate patient selection strategies. Discovering relevant predictive biomarkers would greatly facilitate proper patient selection. This underscores the need for uncovering crucial (cellular) determinants of response to these agents.

This thesis describes preclinical work performed to evaluate the effects of antiangiogenic and proapoptotic death receptor-targeted agents in ovarian cancer models *in vitro* and *in vivo*, and analysis of potential predictive biomarkers in human ovarian cancers.

OUTLINE OF THE THESIS

Amongst the many targeted drugs explored in ovarian cancer, angiogenesis inhibitors have been at the forefront of being integrated in the treatment of ovarian cancer patients. By far the most experience to date stems from studies with drugs blocking VEGF-driven angiogenesis, either by blocking VEGF family ligands using the VEGF-A-blocking antibody bevacizumab or the fusion decoy receptor VEGF-Trap, or by interfering with VEGFR signaling activity using TKIs. In **Chapter 2** we review the available experience from phase II/III clinical trials with these agents. Published studies as well as preliminary reports presented at the American Society of Clinical Oncology (ASCO) meetings and protocols of ongoing trials (extracted from clinicaltrials.gov) are summarized.

Both bevacizumab and VEGFR-targeted TKIs harbor the potential of suppressing VEGF-driven angiogenesis, albeit in distinct ways. Bevacizumab neutralizes VEGF-A, which is considered to be the most prominent proangiogenic ligand within the VEGF family. VEGFR-targeted TKIs have the potential of inhibiting angiogenic signaling relayed by multiple VEGFRs, which can be activated by VEGF-A as well as several other VEGF family members including VEGF-B, VEGF-C and VEGF-D. It is currently unclear how to select patients that are likely to benefit from these treatments, or how to choose between treatment with bevacizumab or a VEGFR-targeted TKI. Insight in the expression of multiple VEGF family members may guide these decisions. In **Chapter 3**, we analyzed the simultaneous protein expression of VEGF-A, VEGF-B, VEGF-C and VEGF-D in a large series of primary ovarian cancers and their omental metastases. To this end, we performed immunohistochemical staining for all four VEGF family members on tissue microarrays (TMAs) containing treatment-naïve ovarian cancer tissues from 270 patients. Staining intensities were analyzed and coupled to a comprehensive clinical database to assess correlations with clinicopathological parameters and patient survival.

Several alternative targeted agents are being explored preclinically for their potential to inhibit ovarian cancer angiogenesis. The mammalian target of rapamycin (mTOR) controls translation of several oncogenic proteins, including several factors involved in angiogenesis.[14] mTOR as well as other components of the mTOR pathway, including the central relay kinase Akt, are often activated in ovarian cancers. Inhibiting mTOR results in translational repression of VEGF-A and its transcriptional regulator hypoxia

inducible factor-1α (HIF-1α). Monitoring reductions in tumor VEGF-A expression, as an early read-out of antitumor efficacy, could potentially provide a means of non-invasive early response prediction which ultimately benefits patient selection. In **Chapter 4**, we monitored tumor VEGF-A levels *in vivo* with positron emission tomography (PET) before and during treatment with the mTOR inhibitor everolimus in a human ovarian cancer xenograft-bearing mouse model. Human ovarian cancer cells were xenografted in mice and tumor VEGF-A levels were measured before and after 2 weeks of continuous everolimus treatment using radiolabeled bevacizumab (^{89}Zr-bevacizumab). Results obtained with ^{89}Zr-bevacizumab PET imaging were correlated to *ex vivo* biodistribution data and tumor VEGF-A protein levels as measured with enzyme-linked immunosorbent assays on whole tumor lysates. Immunohistochemistry was performed to assess vascular density, tumor viability and activation of the downstream mTOR target protein S6.

Bypassing or overcoming chemoresistance is an important objective to improve ovarian cancer prognosis. Targeting tumor cells by activating proapoptotic members of the tumor necrosis factor (TNF) receptor family may provide a potential cellular pathway that can be used to circumvent chemotherapy resistance. Recombinant human TRAIL (rhTRAIL) and TRAIL receptor-targeted antibodies are in clinical trials to analyze their antitumor activity. These agents showed modest activity with limited toxicity in single-agent phase I-II trials as well as in combination studies with chemotherapy.[15] Combining classical chemotherapeutic drugs with rhTRAIL has the potential to act synergistically and overcome resistance to either of the agents alone. Gaining insight in the factors involved in rhTRAIL sensitivity may aid in predicting drug efficacy and finding rational combination therapies.[16] In **Chapter 5** the molecular determinants of rhTRAIL sensitivity and the mechanisms involved in synergy between platinum chemotherapy and rhTRAIL are investigated in an isogenic ovarian cancer cell line model, using cell survival and apoptosis assays, flow cytometry for death receptor expression, analysis of caspase expression and activation with Western blotting and PCR, as well as short interfering RNA (siRNA).

Fas is another member of the TNF receptor family which can be effectively targeted to induce apoptosis in cancer cells. However, *in vivo* systemic use of recombinant human Fas ligand (rhFasL) or anti-Fas antibodies results in marked liver toxicity and these agents are therefore not being tested clinically. A hexameric form of soluble FasL,

called MegaFasL (APO010), has been constructed to decrease liver toxicity, while conserving the capacity to activate the extrinsic apoptosis pathway through binding with Fas.[17] In **Chapter 6** we determined the apoptosis-inducing potential of APO010 in 12 human solid tumor cell lines with various resistance profiles, including platinum sensitive and resistant ovarian cancer cell lines, to assess its ability to circumvent resistance to chemotherapy and other death receptor-targeted drugs. Sensitivity to APO010 was established using cell survival and apoptosis assays, performed on cells treated with single-agent APO010 or APO010 in combination with chemotherapeutic drugs. Fas membrane expression was analyzed with flow cytometry to assess any correlation with APO010 sensitivity. To this end, expression of components of the death-inducing signaling complex (DISC), which is the upstream initiator complex in the extrinsic apoptosis pathway, was also analyzed using Western blotting and PCR analysis.

Finally, a summary of all results presented in this thesis is provided in **Chapter 7**. This is followed by a discussion on the interpretation and (clinical) implication of these findings, along with a discussion on the perspectives for future research.

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