Unravelling the role of signal transduction pathways in high-grade serous carcinogenesis

Summary

Ovarian cancer is characterized by poor prognosis. The disease often recurs despite aggressive treatment with cytoreductive surgery and chemotherapy. During the last decades, survival rates have not significantly improved, indicating the high need to unravel the molecular characteristics of ovarian cancer to constitute new therapeutic approaches. In this thesis, we aim to increase our understanding of ovarian cancer behaviour using a novel method measuring signal transduction pathway (STP) activity. Ultimately, we aim improve the implementation of targeted treatment strategies for ovarian cancer patients by stratification based on STP activity. As described in **Chapter 1**, carcinogenesis results from dysregulation of cellular activity of signalling pathways, which is frequently triggered by gene mutations. With regard to targeted treatment strategies, information on STP activity is used to provide a tailored treatment for cancer patients with specific targeted drugs. For this treatment strategy to be effective, identification of the tumour-driving STP is of most importance to enable accurate patient selection. However, current diagnostics for patient selection focus on a single molecular trait (e.g. immunohistochemical protein expression and genomic alterations) and disregard other factors, such as the tumour micro-environment. Therefore, in this thesis, we used an alternative approach to quantify functional STP activity with consideration of the tumour cell phenotype. The use of STP activity assays, enabled us to investigate six major signalling pathways, all of which were previously associated with ovarian carcinogenesis.

Identification of the tumour-driving STP requires knowledge on normal STP activity in healthy cells to determine whether a certain STP is aberrantly activated. The Fallopian tube epithelium (FTE) is recognized as the predominant cell type of origin of the most common type of ovarian cancer, the high-grade serous subtype. In **Chapter 2**, we investigated the influence of the hormonal cycle on STP activity in the fimbrial epithelium of morphologically normal Fallopian tubes. We included healthy pre- (n=17) and postmenopausal (n=8) women who had surgical interventions for benign gynaecological conditions. For the premenopausal women, hormone serum levels and histological sections of the endometrium were used to determine the hormonal phase (early follicular (n=4), late follicular (n=3), early luteal (n=5) and late luteal phase (n=5)). After laser capture microdissection, total messenger ribonucleic acid (mRNA) was extracted from the fimbrial epithelium and real-time quantitative reverse transcription-polymerase chain reaction (RT-qPCR) analysis was performed. We used STP activity assays to assess functional activity of the hormone driven pathways androgen receptor (AR) and oestrogen receptor (ER), the growth factor pathway phosphoinositide 3-kinase (PI3K) and the developmental pathways Hedgehog (HH), transforming growth factor beta (TGF-β) and canonical wingless-type MMTV integration site (Wnt). The early luteal phase demonstrated high AR and ER

pathway activity compared to the late luteal phase (*P*=0.016 and *P*=0.032, respectively) and low PI3K activity compared to the late follicular phase (*P*=0.036), while the late luteal phase showed low activity of HH and Wnt compared to the early follicular phase (both *P*=0.016). In FTE from postmenopausal women, we observed differences in AR, ER, PI3K and Wnt pathway activity in comparison to the follicular and/or luteal phase in premenopausal women. In summary, we found cyclic changes in activity of the AR, ER, PI3K, HH and Wnt pathways, indicating that STP activity in FTE is influenced by the hormonal cycle.

To increase our understanding of tumour-driving mechanisms during high-grade serous carcinogenesis, we aimed to identify early aberrations in functional STP activity in precursor lesions of HGSC in **Chapter 3**. We searched the pathology archive for tissues from patients diagnosed with serous tubal intraepithelial carcinoma (STIC) and concurrent HGSC. Then, we performed mRNA extraction and RT-qPCR analysis on STIC (n=8) and HGSC (n=7) samples to assess STP activity and compared this to STP activity in normal FTE from postmenopausal women (n=8). We found no statistically significant differences in the activity of the AR, ER, PI3K, HH, TGF-β and Wnt pathways between STIC and concurrent HGSC. However, STIC and HGSC demonstrated significantly lower ER and higher PI3K and HH pathway activity in comparison to normal FTE, suggesting these pathways as putative early drivers in the neoplastic transformation of FTE. In addition, we determined forkhead box O protein 3a (FOXO3a) expression by immunohistochemistry and found loss of FOXO3a expression in STIC and HGSC compared to normal FTE. This observation confirmed that activation of PI3K signalling by loss of FOXO is an early hallmark of high-grade serous carcinogenesis. Furthermore, HGSC were characterized by significant loss of AR and Wnt pathway activity in relation to FTE, suggesting these pathways contribute to HGSC progression.

In **Chapter 4**, we explore the activity of the previously mentioned oncogenic STPs in HGSC in relation to survival. We assessed functional STP activity in 85 primary tumour samples of patients with advanced stage HGSC and a disease-free survival below 12 months (n=52) or above 24 months (n=33). There were no significant differences in STP activity between patients with short- and long-term disease-free survival. In univariate Cox proportional hazards analysis, stratification of HGSC patients for menopausal status revealed a favourable relation between ER pathway activity and disease-free survival (hazard ratio=0.943) and overall survival (hazard ratio=0.930) in postmenopausal women (*P*=0.033 and *P*=0.041, respectively), but not in premenopausal women. We divided the postmenopausal group into four subgroups based on ER pathway activity quartiles. Survival analysis revealed that postmenopausal women in the lowest ER quartile had a shorter disease-free and overall survival (log-rank *P*=0.006 and *P*<0.001, respectively). In conclusion, in

postmenopausal women with advanced stage HGSC, low functional ER pathway activity was associated with a poorer survival outcome.

Therapy targeting the ER signalling pathway may be used as a palliative treatment option in HGSC patients. Currently, positive ER protein expression by immunohistochemistry is considered a biomarker for sensitivity to anti-oestrogen therapy. In **Chapter 5**, we conducted a systematic review of the literature on the clinical benefit of anti-oestrogen therapy in a homogenic population of ER positive metastatic or recurrent HGSC and searched for a correlation between ER protein expression and clinical response. The primary outcome was the clinical benefit rate (CBR) defined as the proportion of patients with complete or partial response or stable disease. The secondary outcome was the overall response rate (ORR) defined as the proportion of patients with complete or partial response. There were no studies with populations consisting solely of ER positive HGSC. However, we included six studies reporting on 407 evaluable patients of whom 376 were HGSC (92%) and 302 were confirmed ER positive (80%). Anti-oestrogen therapy resulted in a CBR of 27-65% and an ORR of 0-16% after approximately three months of therapy. No correlation was found between ER expression and clinical response. Therefore, ER protein expression alone is not a reliable predictor of response. This may result from the incorrect assumption that ER protein expression equals functional ER pathway activity, since in the absence of ER activating mutations, the substrate oestrogen is required to activate the receptor and initiate transcription of ER target genes. We concluded that, to apply effective ER targeted therapy, it is important to develop better predictors to identify (non-)responders.

Subsequently in **Chapter 6**, we determined the accuracy of ER protein expression as a biomarker for functional ER pathway activity. In 29 HGSC samples, immunohistochemical ER protein expression was visually scored using total percentages of stained tumour cells and ER histoscores. In addition, functional ER pathway activity was determined using mRNA measurements of ER-specific target genes. Our analysis showed that neither total percentages of ER protein expression, nor ER histoscores were significantly correlated to ER pathway activity (P=0.473 and P=0.606, respectively). Classification of HGSC into three subgroups based on ER histoscores 0-100 (n=6), 101-200 (n=15) and 201-300 (n=8) resulted in comparable mean ER pathway activity among the subgroups (P=0.356). Several HGSC in the higher ER histoscore subgroups showed low ER pathway activity, indicating that nuclear ER protein expression is not sufficient to describe transcriptional ER activation. We recommended that further studies are necessary to prove the predictive value of functional ER pathway activity regarding anti-oestrogen sensitivity in HGSC patients.

The signalling pathway involving PI3K, AKT and mammalian target of rapamycin (mTOR) proteins is considered an attractive targeted treatment option as genomic alterations in one of the components of this pathway are frequently found in ovarian cancer patients. We performed a meta-analysis of the clinical benefit of PI3K/AKT/mTOR pathway inhibitors in ovarian cancer and investigated the predictive value of current biomarkers on therapy response in **Chapter 7**. The primary and secondary outcomes were CBR and ORR, respectively. We included 233 patients from 19 studies and observed a pooled CBR of 32% (95% confidence interval (CI) 20–44%) and ORR of 3% (95% CI 0–6%) in advanced or recurrent ovarian cancer patients treated with PI3K/AKT/mTOR inhibitors. Subgroup analysis tended to favor the studies who selected patients based on current PI3K/AKT/mTOR biomarker criteria (e.g. genomic alterations or loss of PTEN protein expression), but the difference in CBR was not statistically significant from studies with unselected populations (respectively, CBR of 42% (95% CI 23–62%) and 27% (95% CI 14–42%), *P*=0.217). To better reflect true patient benefit, we excluded stable disease below six months as a beneficial outcome which resulted in a pooled CBR of 7% (95% CI 2–13%). Thus, the efficacy of monotherapy with PI3K/AKT/mTOR inhibitors in ovarian cancer patients is limited to a small subgroup and selection of patients with the use of current biomarkers did not improved the CBR significantly. We found that the overall proportion of patients with drug-related severe or life-theathing adverse events was 36%. Given the toxicity profile, we suggested that current treatment with PI3K/AKT/mTOR inhibitors should not be initiated unless in clinical trials. Furthermore, we concluded that reliable biomarkers that measure functional activity of the PI3K/AKT/mTOR pathway are needed to optimize patient selection.

Taken together, successful implementation of targeted therapy for ovarian cancer patients based on stratification by their molecular signature has been limited so far. Moreover, there remains a high unmet need for reliable diagnostics to predict targeted therapy response as protein expression and gene alterations are insufficiently reliable. Therefore, we designed a multicenter prospective, parallel-group cohort study to evaluate the clinical applicability of STP activity assays in selecting ovarian cancer patients for matched targeted therapy. As described in **Chapter 8**, the STAPOVER study aims to identify aberrantly activated STPs in recurrent ovarian cancer patients and implement *phenotype*-guided targeted therapy to improve survival and maintain quality of life. We used the normal STP activity ranges measured in postmenopausal women to define cut-off values to discriminate between normal and aberrant STP activity. Patients will be selected for treatment with existing targeted drugs with tolerable toxicity profiles to investigate whether these drugs have therapeutic value beyond their approved indications. Treatment response will be assessed by the progression-free survival ratio, in which the correlation of two consecutive lines of treatment allows the patient to serve as her

own control and compensates for heterogeneity in patient characteristics and tumour histology. Initially, the study will include ovarian cancer patients identified with a potentially tumour-promoting AR, ER, PI3K or HH signalling pathway, but the adaptive study design enables expansion of the trial with additional treatment arms including drugs targeting other pathways as well.

Finally, the main findings of this thesis are presented in a larger perspective in **Chapter 9**. In this chapter we discussed the implications of our findings for clinical practice and provided future perspectives on the targeted treatment of ovarian cancer patients.