Towards a more personalized approach in the treatment of esophageal cancer focusing on predictive factors in response to chemoradiation

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CHAPTER 8

Summary and future perspectives

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Summary

The studies presented in this thesis provide improved insight in factors determining prediction of response after neoadjuvant chemoradiotherapy (nCRT) in esophageal cancer (EC) patients as well as potential molecular targets to sensitize chemoradiation in therapy resistant cells. These findings may support a more personalized approach in the future treatment of patients with EC. In most centers in the Western world, nCRT is currently the standard therapy for patients with resectable locally advanced EC, who are medical fit enough for these invasive interventions. Although this treatment is widely appointed, unfortunately not every patient can be treated successfully. In fact, 70% will respond insufficiently of which 18% will not respond at all [1]. These rates are suggestive for a population of cancer cells that are resistant to the given nCRT in a large part of these EC patients.
It is well-known that one population of cells, the so-called cancer stem-like cells (CSCs), harbor the chemoradiation resistant traits and they should be eradicated in order to improve treatment response [2]. In **chapter 2** we give a comprehensive overview of the current literature concerning the role and importance of CSCs in EC and hand some tools for targeting these populations. In a previous work we have identified a combination of cell surface markers CD44 and CD24, CD44+/CD24-, that can be used to select for CSCs in EC [3]. As such, targeting this population in EC by modulating oncogenic pathways might lead to a reduced amount of tumor cells with CSC characteristics implicating a better response to nCRT. In regard to this, studies have shown that the Hedgehog (HH) signaling pathway is activated in EC and maintains a stem cell state [4,5]. In **chapter 3** we explored the potential of the HH pathway as a possible intervention to eradicate the cells with CSC properties. We found that the HH pathway activity is increased in patients with residual tumor disease after nCRT followed by surgery in tumor resection material, as indicated by the elevated expression of Sonic Hedgehog (SHH) and Patchted1 (Ptch1). Inhibition of the HH pathway using Vismodegib, a Ptch1 receptor inhibitor, resulted in a deselection of the CSC related CD44+/CD24- phenotype in EC cell lines. These results might reflect that inhibition of this signaling pathway could potentially improve therapy response, which warrants further exploration. Primary tumor response rates can also be improved by targeting the mTOR pathway [6]. mTOR inhibitors are often being used to reduce tumor growth [6]. However, in our hands as demonstrated in **chapter 4** mTOR inhibitors may also increase cells with the CD44+/CD24- phenotype, potentially indicating an increase in chemoradiotherapy resistant tumor cells. Conversely, mTOR activation led to a decrease in CD44+/CD24- cells, suggesting a decrease in num-

ber of resistant tumor cells. The addition of a mTOR inducer to the existing nCRT treatment regimen might enhance tumor sensibility to therapy, this however needs further exploration. A first attempt has been made in the same study to introduce a novel technique in studying drug response in cancer; a patient derived tumor organoid model. Our preliminary data on the patient derived tumor organoids indicate that the activation of the mTOR signaling pathway by MHY1485 leads to a reduction of cancer stemness hereby stressing the potential of MHY1485 in eradicating EC CSC populations. Combining this drug with conventional chemoradiation might lead to the elimination of CSCs and the bulk tumor cells.

The data in the above-mentioned chapters generated sufficient potential predictors of response (CD44, SHH, Ptch1, HIF1α) with the addition of Her2neu, based on its unfavorable effect on survival [7], to be tested as such in **chapter 5**. We tested the biological tumor markers on their predictive value of complete response using an existing prediction model consisting of pretreatment clinical parameters and PET/CT derived imaging features that already exceeded the accuracy of SUVmax in predicting complete response with corrected area under the curves (AUCs) of 0.74 and 0.54 respectively [8]. The addition of Her2neu to this model significantly improved the predictive ability with corrected AUCs of 0.81 and 0.61 respectively. Unfortunately, the addition of the remaining biological tumor markers did not significantly improve the model. Based on the value of Her2neu on complete response prediction, we advocate Her2neu to be included in the initial staging of EC patients.
nCRT and surgery are known to induce inflammatory responses. Therefore, measuring cytokines during the CROSS treatment regimen may be used as an method to predict treatment response. We found in **chapter 6** that a high platelet-activating factor (PAF) level prior to nCRT was associated with complete pathologic response and remained elevated during treatment. Interestingly, decreased concentrations of Ang-1 on the third postoperative day were associated with postoperative complications. Future research is needed to validate these predictive factors in a larger cohort of patients.
The number of positive locoregional lymph nodes is an independent prognostic factor in EC [9]. In **chapter 7**, we presented data indicating that nCRT indeed reduced the amount of micrometastases compared to surgery alone. Remarkably, even patients with pN0 staging after nCRT show micrometastases after additional immunohistochemical stainings and (potentially due to this) demonstrate a similar survival as patients that have been staged pN1. These results may also indicate that a small subset of resistant tumor cells, too small to be detected by conventional pathological and imaging

techniques, has significant impact on patients' outcome.

Future perspectives

This thesis provides promising tools how to improve treatment stratification of current therapy in EC patients. Determining Her2neu status and PAF levels should be considered to be assessed prior to therapy to give an indication of complete response. These two approaches in predicting response deserve priority as they can be relatively easy to be implemented in the clinical practice since no additional handlings are required for sampling and their potential value should therefore be explored to the fullest. Routine biopsies are part of the initial staging of the disease and routine blood samples are drawn to evaluate the overall condition of the patient in assessing nCRT eligibility.
In the long run, we are in need of new therapies as current therapy does not suffice in the majority of the EC patients [1]. Personalized medicine offers great promise as the therapy is adapted to the needs of each patient. The ideal method in achieving personalized medicine is to analyze each tumor individually on specific expression patterns based on activated oncogenic pathways and subsequently targeting these with corresponding molecular therapies. In this thesis, a start has been made in realizing this approach. Future treatment of patients with EC should include the use of the cancer genome atlas (TCGA), as mentioned in this thesis, subdividing EC patients in four subsets of identified genetic alterations. The new 8th edition staging for cancer of the esophagus are driven on data of both pathologic stage after neoadjuvant therapy (ypTNM) and pretreatment clinical stage (cTNM) typically defined by imaging and examination of needle aspiration and biopsy specimens. Imaging and pathological examination are important key elements [10]. In the future patient derived tumor organoids may also play a key role. Our patient derived tumor organoid model allows patient's own tumor cells to grow in vitro permitting endless possibilities to evaluate different therapies. Using this model, we first could potentially identify the proportion of patients which do or do not respond to conventional chemoradiation and subsequently offer potentially more effective alternative therapies. Screening the response to non-conventional therapies might reveal the 'Achilles heel' of these tumors. This is a very exciting prospect that represents the summit of personalized medicine. Future research will address whether this model could serve as a robust ex-vivo model to assess nCRT response as well as studying response to alternative drugs. Perhaps, as described in this thesis, the presence of CD44+/CD24- could be of predictive value and could be targeted by either a Hedgehog inhibitor, such as

Vismodegib, or by an mTOR inducer, such as MHY1485 in combination with nCRT. The value of these drugs in targeting CSC populations in EC needs to be confirmed either in patient material or in a larger cohort of patients.

In conclusion, this thesis provides novel approaches that may predict nCRT response to avoid unnecessary therapy in EC patients. Moreover, it provides alternative ways to chemoradiosensitize resistant cells when the current nCRT falls short. Overall, this thesis provides suggestions how to approach future EC treatment focusing on the patient as an individual rather than providing the common therapy for all.

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