Towards personalized and targeted treatment of head and neck cancer
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Chapter 9
Summary.
Despite advances in treatment strategies, the five-year survival rates after diagnosis of head and neck squamous cell carcinoma (HNSCC) remained around 50% over the last decades\(^1\),\(^2\). In addition, the available treatment options, and in particular the chemotherapeutic agent cisplatin, are often accompanied with marked toxicity. This is a large burden for the patient and the health care costs that are associated with treatment of the therapy side effects. This urges the need for ways to improve existing treatments or to develop novel therapies based on new drug targets. In this thesis, we explored RNA interference and microRNA screens to identify novel targets for HNSCC treatment. In addition, we analyzed putative HNSCC stem cell markers and investigated the relevance of this cell population in the clinic. Furthermore, we aimed to elucidate the mechanism of cisplatin resistance in order to find a biomarker that can be used to personalize treatment by predicting for which patient addition of cisplatin might be beneficial.

A plethora of data is available on the mechanism of action of cisplatin\(^3\),\(^4\), but publications on how cisplatin resistance is mediated are inconsistent and likely very dependent on the tissue origin of the tumor cells. Also, for HNSCC, the actual mechanisms of cisplatin response and cisplatin resistance are not well understood. In Chapter 2 we investigated 19 HNSCC cell lines for the expression levels of several genes that are implicated in cisplatin activity. Furthermore, we measured cisplatin accumulation and retention rates as well as cisplatin-DNA adduct levels. The only characteristic that showed a significant correlation with cisplatin sensitivity was the level of cisplatin-DNA adducts (p=0.006, Spearman's rank correlation coefficient). This suggests that the DNA adduct level resulting from cisplatin incubation is the most important determinant of cisplatin sensitivity.

In our inventory in 19 HNSCC cell lines, we focused on a small selection of genes and cellular processes that were previously implicated in cisplatin response. This approach is heavily biased by the published literature and, additionally, genes of importance might differ between tumor types. In Chapter 3 we describe how a genome-wide functional siRNA screen was used to identify all genes in the human genome that specifically determine the cisplatin response. Among the ones that proved to have major impact on cisplatin sensitivity, we found several siRNAs that target genes that are implicated in the repair of cisplatin induced DNA damage. This again emphasizes the likely importance of platinum-DNA adducts for the efficacy of cisplatin therapy, since reduced expression of genes that remove the adducts sensitizes the cell to the chemotherapeutic agent. Based on the genes found we assume that particularly the interstrand crosslinks are of importance for the response. Furthermore, we identified \textit{SHFM1}, a gene implicated in split-hand/split-foot malformation syndrome\(^5\), as an important determinant of cisplatin sensitivity, probably as a result of its role in BRCA2 stability. Both genes seem crucial for a good DNA repair response after cisplatin induced DNA damage.

The genome-wide siRNA screen also revealed a number of genes that seem essential for tumor cell viability, even without the addition of cisplatin. Such genes may serve as excellent candidates for the development of new anticancer therapies. A cluster of genes that are involved in the \textit{G2/M} phase regulation of the cell cycle was isolated and the genes were validated as potent anticancer targets in Chapter 4. One of these genes was \textit{KIF11}, which plays an important role in the assembly of the mitotic spindle\(^6\). A potent inhibitor of KIF11, ispinesib\(^7\), showed a strong antitumor effect in \textit{in vivo} experiments with HNSCC xenografts, confirming the potency of the identified hit list for identification of new drug targets.

The application of a given siRNA only decreases the expression of one gene, and it is not unlikely that other genes compensate for the loss of expression. Therefore, phenotypes might become more pronounced when inhibiting multiple genes that share certain genetic similarity at the same time\(^8\),\(^9\). MicroRNAs are excellently suited for this purpose\(^10\). In Chapter 5, we investigated tumor cell viability upon microRNA overexpression by transducing a HNSCC cell line with microRNA expressing lentiviral vectors. We compared the results with the viability of conditionally immortalized oropharyngeal keratinocytes (ciOKCs), which are considered to be semi-normal cells. We found six microRNAs that showed antiproliferative effects in the tumor
cell line but not in the ciOKCs. Identification of the spectrum of genes that are regulated by these six microRNAs might result in a list of new potential drug targets in HNSCC. Microarray gene expression profiling elucidated an important role for ATM in the proliferation of HNSCC cells. Cancer stem cells (CSCs) comprise a small subpopulation of cells within a tumor and are hypothesized to be responsible for driving tumor growth, formation of metastases and overall therapy resistance\(^{(11)}\). Genetic characterization of this population might reveal potential drug targets that specifically eliminate the CSCs. CD44 has been identified as a HNSCC stem cell marker\(^{(12)}\), but has low specificity and is not practical. **Chapter 6** describes the identification of CD98 as a more specific cancer stem cell marker for HNSCC. When CD98\(^{\text{high}}\) cells were inoculated in nude mice, tumor formation was seen in 60% of the injection sites, whereas CD98\(^{\text{low}}\) cells did not grow out to form new tumors. Expression microarray profiling showed that genes involved in cell cycle control and DNA integrity were expressed at a significantly higher level in the CSC-enriched CD98\(^{\text{high}}\) population when compared to CD98\(^{\text{low}}\) cells.

It is well known that HPV-positive HNSCCs have a more favorable prognosis than their HPV-negative counterparts\(^{(13)}\), and there might be a number of reasons for this. An explanation might be that HPV-positive tumors have a better response to the current therapies and this might be related to the number of cancer stem cells present in these tumors. In **Chapter 7** we investigated CD44 and CD98 expression in biopsies of 711 oropharyngeal squamous cell carcinomas (OPSCCs) with known HPV status. We observed a high percentage of CD44-positive cells in these tumors and again concluded that CD44 is not a very specific marker for squamous CSCs. Nonetheless, HPV-negative tumors showed a significantly higher percentage of cells expressing stem cell markers CD44 and CD98 as compared to HPV-positive tumors (p<0.001, for both markers, Pearson χ²-test). This implies that HPV-negative tumors might harbor more CSCs and this may explain a worse clinical outcome. We observed that within the group of HPV-positive tumors the ones with relatively high levels of CD98 (i.e. >50% of malignant cells stained) have a significantly worse 5-year overall and progression free survival (p=0.006 and p<0.001, respectively). These data suggest that the number of CSCs in HPV-positive tumors is lower and might determine clinical outcome. Remarkably, in HPV-negative tumors the number of CSCs seems of no clinical significance. Likely the molecular characteristics of this population might be of more relevance.

**REFERENCES**
