Targeted chemoprevention of head and neck cancer

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Chapter 8

Summary

Nederlandse Samenvatting

SUMMARY

Head and neck squamous cell carcinomas (HNSCCs) develop in the mucosal lining of the upper aerodigestive tract. HNSCC is a very heterogeneous disease, which on the one hand relates to the differences in anatomical location as well as etiology, and on the other hand to the large variety in molecular alterations. The last decades new diagnostic tools and treatment strategies have been developed, but these did neither translate in a significant decline of recurrent disease nor in an increase of the 5-years survival rates yet. To improve the clinical outcome of patients diagnosed with HNSCC, multiple efforts to develop new therapeutic strategies are required. Several years ago The Cancer Genome Atlas (TCGA) consortium published a comprehensive overview of the genomics of HNSCC. However, the detailed insights in the molecular landscape of HNSCC did not provide new therapeutic options. A major conclusion of these studies was that HNSCC is driven by tumor suppressor gene inactivation, complicating the identification of druggable targets.

Since prevention is generally more effective than cure, we focused with our research described in this thesis on the precancerous fields in the head and neck mucosal lining. These fields consist of epithelial cells containing the earliest cancer-associated genetic changes, but do not show the invasive behavior of tumor cells. Several types of these fields are known to occur, some are visible to the naked eye as lesions, some can be detected using a microscope and others can only be detected by genetic analysis. These precursor fields form an important risk factor for the development of primary HNSCC, but also for local relapses in treated patients. These precancerous fields should be diagnosed and treated, but treatment is a challenge. Targeted treatment seems most promising because of the generally low toxicity while the cells can be specifically hit at their vulnerabilities caused by the genetic changes.

As a first step described in **Chapter 2**, we generated several cellular models of precancerous fields directly from patient material. We collected biopsies of normal appearing mucosa surrounding the tumor from patients undergoing surgery to remove their primary HNSCC. The tissue collected was cultured and proliferating keratinocytes were analyzed for the presence of genetic alterations. These results confirmed the presence of genetic alterations in approximately half of the cultures. The observed changes typically occur early in carcinogenesis, such as loss of a particular region of chromosome arm 9p and mutations in *TP53*. We further noted that mutations in *NOTCH1* sometimes occur as early changes in the cultured keratinocytes. These data provided us with cellular models of precancerous fields that were used subsequently for identification and validation of druggable targets, and evaluation of promising small molecule inhibitors.

In **Chapter 3** we applied RNA-interference screens to identify therapeutic target genes of precancer cells, and compared the findings to cancer cells. By performing an array-based screen with previously identified tumor-lethal siRNAs we were able to detect genes being essential either for the precancer cells and/or for the cancer cells. This resulted in several promising candidate genes, of which *Polo-like kinase 1 (PLK1)*, was further validated *in vitro* and *in vivo*. PLK1 inhibitors were able to eradicate precancer cells as well as invasive cancer cells, but had less effect on normal primary cells. In the subsequent **Chapters 4** and **5**, we demonstrated similar results for three other hits identified with such RNA-interference screens. In these screens the HNSCC cell line panels tested were extended and included also normal primary fibroblasts to allow direct exclusion of core essential genes. These studies identified *Wee1*, *RRM1* and *RRM2* as potential target molecules and these should be considered as potential hits for targeted chemoprevention as well.

In **Chapter 6** we showed that besides the more classical gene targets, miRNA mimics are also suitable for targeting precancer cells *in vitro*. We performed a high-throughput screen with 2,048 mimics on a precancer cell line, two cancer cell lines and normal primary fibroblasts. Analysis of the data resulted in 31 miRNA mimic candidates with a precancer-specific lethal effect, interesting for further validation experiments.

Overall, this work focused on the development of targeted chemoprevention strategies for precancerous fields in HNSCC patients to prevent primary tumors and local relapses. By developing cellular models, followed by target identification and validation, several promising druggable targets and small molecule inhibitors have been identified, and are available for future clinical testing in patients with precancerous changes at high risk for HNSCC.