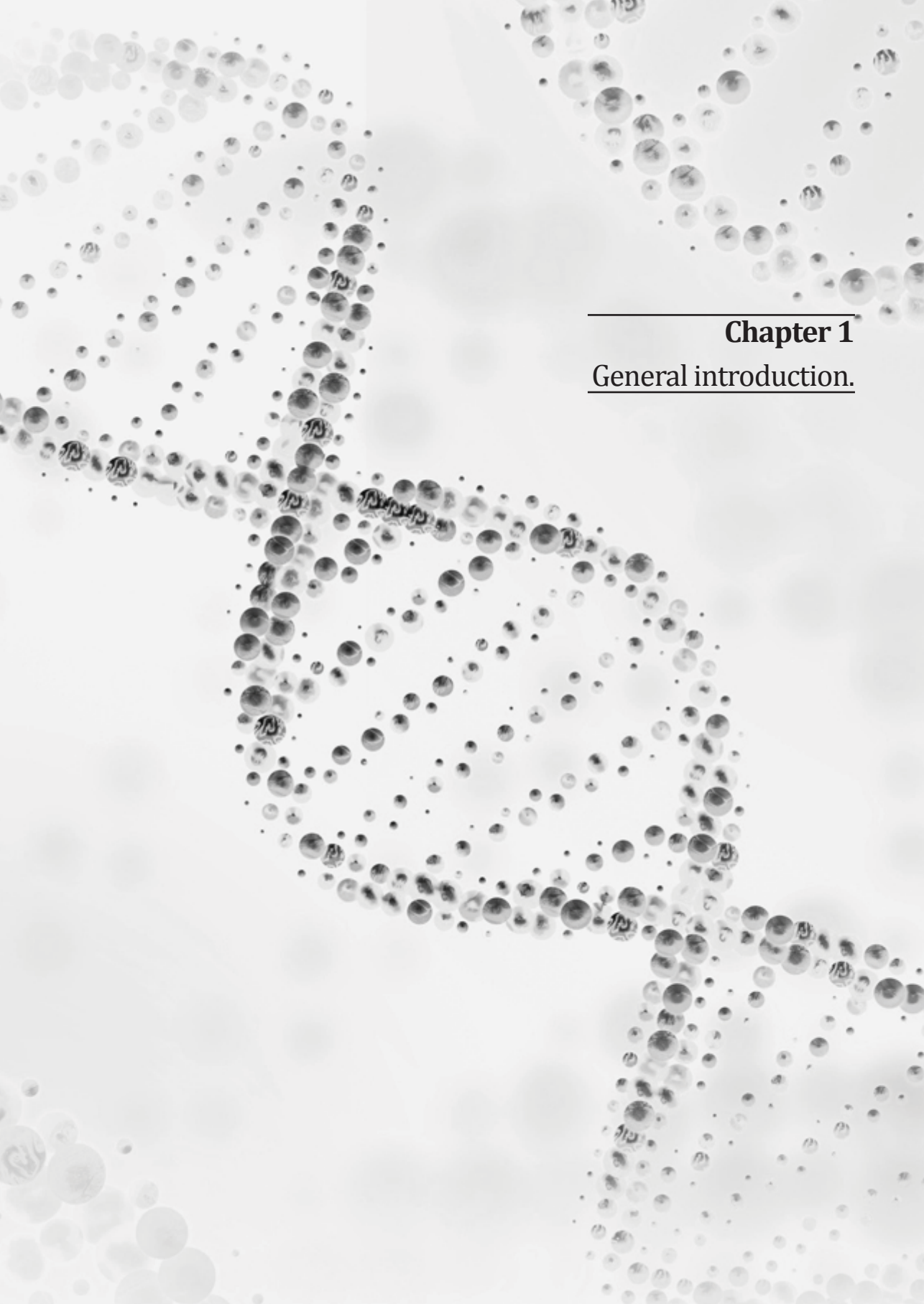

Chapter 1

General introduction.

HEAD AND NECK CANCER; EPIDEMIOLOGY, RISK FACTORS AND CARCINOGENESIS

Head and neck squamous cell carcinomas (HNSCCs) arise in the mucosal linings of the oral cavity, naso-, hypo-, and oropharynx, and larynx. HNSCCs represent over 90% of all malignant tumors in the upper aerodigestive tract. According to the latest update of the GLOBOCAN project (globocan.iarc.fr), HNSCC is the eighth most frequently diagnosed cancer type and the sixth leading cause of cancer deaths worldwide[1]. Despite advances in the treatment of HNSCC, development of locoregional recurrences, distant metastases and second primary tumors still occur frequently[2], leading to five-year survival rates after diagnosis that have remained around 50% during the last decades.

The two most important risk factors for the development of HNSCC are tobacco use and excessive alcohol consumption[3], and these seem to have a more than additive effect[4]. In addition, epidemiological studies report that a subset of HNSCCs, i.e. malignancies of the oropharynx, is caused by infection with the human papillomavirus (HPV)[5-7]. Finally, certain inherited genetic disorders, like Fanconi anemia, predispose affected individuals to the development of HNSCC[8,9].

HNSCCs usually develop in prenoplastic mucosal changes that in some cases may be visible macroscopically as white (leukoplakia) or red (erythroplakia) lesions. The large majority of these preneoplastic changes is recognized microscopically as dysplasia, and is graded as mild, moderate or severe. In 1996, Califano et al.[10] described the first progression model of HNSCC and showed that a number of genetic changes accumulate in parallel with the severity of the dysplastic changes. The initial model has been adapted, simplified, and integrated with the most critical molecular changes[2]. Early genetic changes are mutation of *TP53* and loss of chromosome arm 17p, mutation or methylation of the *CDKN2A* gene (encoding $p16^{INK4A}$) with loss of chromosome arm 9p, and amplification of chromosome 11q13 with the *CCND1* (cyclin D1) gene. Later changes encompass the gain of chromosome arm 3q (*PIK3CA* gene), loss of 10q (*PTEN* gene) and amplification of 7p (*EGFR* gene). Also frequent losses of 18q are reported (*SMAD4* gene)[2]. Next generation sequencing studies revealed frequent inactivating mutations in *TP53*, *CDKN2A*, *NOTCH1*, *FAT1*, *CASP8* and *PTEN* as well as activating mutations in *PIK3CA* and *HRAS*[11,12].

Of note, these genetic changes are typical for HPV-negative HNSCC. The subgroup of tumors caused by human papillomavirus infections show very distinct molecular characteristics, with generally less genetic changes and gene mutations.

Human papillomavirus

Human papillomaviruses (HPVs) are circular double-stranded DNA viruses that specifically infect the basal cells of epithelial mucosa. Over 120 HPV subtypes have been identified, some of which are involved in carcinogenesis[13,14]. These so-called high-risk HPV types, e.g. HPV-16 and HPV-18, can transform squamous epithelial cells through expression of the viral oncogenes E6 and E7[15-17]. Viral oncoprotein E6 binds to the tumor suppressor protein p53[18], and targets p53 for degradation. Similarly, the HPV E7 protein targets the retinoblastoma protein RB (pRB) for degradation[19], leading to the release of transcription factors that create an S-phase environment in differentiating cells to allow viral replication. Together, the HPV E6 and E7 viral oncoproteins induce and maintain an uncontrolled proliferative condition in squamous cells, which might eventually result in genetic instability and cancer.

Recent studies have shown that HPV-positive tumors represent a distinct subgroup of HNSCCs. These tumors mainly develop in the oropharyngeal region and differ markedly from HPV-negative tumors with respect to aetiology, stage, age of onset, prognosis, and molecular alterations[2]. From a clinical point of view, HPV-positive tumors are associated with a more favorable prognosis than HPV-negative HNSCCs[20-26]. As indicated above, most HPV-negative tumors harbor a mutation in *TP53*[27,28], but since the HPV E6 oncogene inhibits p53 function, mutations in *TP53* are infrequent in HPV-positive tumors[5,11,29]. In addition, it has been shown

that HPV-associated HNSCCs overexpress $p16^{INK4A}$ [28,29], while in HPV-negative tumors $p16^{INK4A}$ is usually inactivated by mutation, homozygous loss or hypermethylation. This makes $p16^{INK4A}$ expression a reliable surrogate marker for HPV-infected tumors. Expression of $p16^{INK4A}$ is usually analyzed by immunohistochemistry. HPV is usually detected by (quantitative) DNA PCR or in situ hybridization, but these assays are not always accurate particularly on archival specimen[30-32]. Therefore test algorithms have been developed such as $p16^{INK4A}$-immunostaining followed by DNA PCR on the positive cases[33]. These test algorithms may have an accuracy of 98%[34], and are much more reliable than single assays.

Fanconi anemia

Besides exposure to exogenous risk factors, there is a genetic susceptibility or predisposition for the development of head and neck cancer. The most prominent predisposition syndrome causing HNSCC is Fanconi anemia (FA). FA is a rare autosomal recessive disorder, which is clinically characterized by congenital malformations, short stature, progressive bone marrow failure, endocrine abnormalities and cancer predisposition. The FA syndrome is caused by mutations in one of at least 16 genes (FANCA/B/C/D1/D2/E/F/G/I/J/L/M/N/O/P/Q) that work in a complex as part of the FA/BRCA DNA damage response pathway[35-38]. Most FA proteins are components of the so-called FA core complex, which activates FANCD2 and FANCI by mono-ubiquitination upon DNA damage. The complex is activated by ATR when a replication fork stalls during DNA replication as a result of a DNA crosslink. The activated FA proteins subsequently localize to the site of DNA damage and recruit a wide variety of other DNA repair proteins, like BRCA2/FANCD1 and RAD51, to jointly restore the DNA by translesion polymerases and homologous recombination.

As a consequence of improper functioning of one of the FA genes, cells of FA patients show high sensitivity to DNA crosslinks. This results in defective maintenance of genome integrity and severe chromosomal instability, represented by multiple chromosomal breaks and accumulation of DNA adducts, especially after treatment with agents that damage DNA by inducing DNA crosslinks[39,40]. This chromosomal instability might explain the cancer predisposition seen in FA individuals.

Patients suffering from FA are susceptible to develop haematological malignancies, particularly acute myeloid leukemias, as well as squamous cell carcinomas, which mainly tend to arise in the head and neck and anogenital regions[8,41,42]. It was estimated that FA patients have a more than 700-fold higher risk to develop HNSCC than the normal population[43,44], and a 2000-fold higher risk of developing oesophageal cancer[45]. Moreover, these tumors present mostly without exposure to the commonly known HNSCC risk factors[8] and at a very young age (median age of 16 vs. 62 in the general population[41]). HNSCC treatment options for these patients are limited, since FA cells are hypersensitive to DNA damage inducing therapies, in particular DNA crosslinking agents, such as cisplatin and mitomycin C[36,46,47].

HNSCC TREATMENT

The treatment plan for HNSCC depends on the stage of disease. Early stage HNSCCs are mostly treated with surgery or radiotherapy. Surgical resection of tumors in the head and neck area is often challenging, since conservation of speech and swallowing is desired. For this reason, radiotherapy is sometimes preferred over surgery, and cure rates proved to be as good as those for surgery[48-50].

In the more advanced stages of disease a multimodality approach is required and surgery is frequently combined with (postoperative) radiotherapy. Furthermore, chemotherapy is increasingly part of the treatment scheme. Chemotherapy can be applied in a neoadjuvant setting, e.g. as induction chemotherapy prior to surgery. However, a large randomized phase III trial[51] recently showed that induction chemotherapy does not improve clinical outcome

and this treatment protocol is currently under debate. Chemotherapy can also be applied in an adjuvant (post-operative) setting, and effectively improves survival in many tumor types. However, the MACH-NC Collaborative Group (Meta-Analysis of Chemotherapy on Head and Neck Cancer) compared data of studies with or without chemotherapy in HNSCC patients in randomized trials[52] and found that the application of chemotherapy as an adjuvant treatment does not give a significant survival benefit in this cancer type.

In contrast, the concomitant administration of the chemotherapeutic agent cisplatin combined with locoregional radiotherapy, called chemoradiation, is associated with a significant survival benefit[53]. This approach has been adopted increasingly over the past years in an effort to improve clinical outcome and to preserve vital organs[54]. Although effective, the application of these chemoradiation protocols may have severe side effects[55-57], causing acute and long term sequelae, and hampering quality of life.

Recent research focused on the molecular characteristics of cancer cells and resulted in the development of therapies that specifically target the cancer cells. For example, the application of the epidermal growth factor receptor (EGFR) targeting drug cetuximab in the treatment of HNSCCs is justified by the fact that more than 90% of HNSCCs overexpress EGFR[58]. Targeted agents, such as cetuximab, are generally less toxic than the more classical cytotoxic chemotherapeutics, and can therefore be applied to patients unfit for chemoradiation. The addition of cetuximab to radiotherapy protocols, called bioradiotherapy, improves locoregional control[59] and the 5-years overall survival rate[60]. However, increasing evidence suggests that patients who initially show a promising response to EGFR inhibition eventually become refractory[61,62].

Therapy resistance is a major obstacle in successfully treating HNSCC. It would be ideal to be able to predict upfront whether or not a tumor will respond to chemoradiation or bioradiotherapy, since a realistic alternative (surgery with or without radiotherapy) is often at hand. The development of such more personalized treatment approaches requires the identification of biomarkers that predict response to a certain treatment.

RESISTANCE TO TREATMENT

Resistance limits the clinical success of the most effective anticancer therapies. Tumor cells may be insensitive to the therapy from the start (intrinsic resistance) or cells may develop insensitivity to the treatment (acquired resistance). Resistance to treatment, either to cisplatin or radiotherapy, may result from a variety of factors.

Insufficient DNA binding

Platinum-containing chemotherapeutic drugs, like cisplatin and carboplatin, exert their intracellular cytotoxic effect by covalently binding to the DNA. The cellular processing of platinum provided important insights into the molecular mechanisms involved in the cisplatin response (reviewed in refs. [63,64]).

Cisplatin is able to enter a cell via passive diffusion, but this process is very slow and depends on multiple factors such as sodium and potassium concentrations and pH[65]. Additionally, several transporters and gated channels have been identified that play a role in both cisplatin influx and efflux. Especially the observation that fluctuations in cellular cisplatin concentrations are paralleled by similar changes in copper, led researchers to the field of copper homeostasis. The copper transporter-1 (CTR1) was identified as an important cisplatin influx transporter[66-70]. Furthermore, copper efflux transporters ATP7A[68,71], and ATP7B[68,72-74], as well as members of the organic cation transporter (OCT) family[75], were identified to play a role in cisplatin efflux. Clinically, increased expression of these copper efflux proteins or decreased expression of influx transporters has been correlated to poor outcome in several tumor types, including HNSCC[76,77].

Once inside the cell, cisplatin undergoes aquation[63,78]. During this process, the molecule

looses the chloride groups as a result of the low intracellular chloride concentration. The hydrolyzed form of cisplatin binds to many cellular targets, but its reaction with the nuclear DNA is biologically the most relevant. The platinum atom covalently binds to the DNA and forms intrastrand, and to a lesser extent interstrand crosslinks[79]. These platinum-DNA adducts distort the structure of the DNA, leading to stalling of replication forks during the process of DNA replication. This can only be resolved by breakage of the DNA strands. Double-strand breaks are particularly hazardous to the cell and require appropriate repair. Inappropriate repair may lead to genome rearrangements and frequently to cell death. Upon detection of the cisplatin-induced DNA damage, a cell goes into growth arrest to allow repair of the damage. When damage is too extensive to repair, a cell undergoes programmed cell death, and this is desired when treating cancer patients. On the other hand, cells may survive the toxic DNA damage by either tolerating the DNA damage[80-83] or by increasing the DNA repair activities that lead to the removal of cisplatin adducts.

Increased DNA repair

Several different DNA repair pathways are evolutionary highly conserved. Intrastrand platinum-DNA adducts can for instance be repaired by nucleotide excision repair (NER)[84]. During this process, the adduct is removed by DNA incision, filling of the created gap by complementation of the undamaged DNA strand and eventually ligation. ERCC1 is one of the key factors in the NER system, and low ERCC1 expression, i.e. low NER activity, was identified as a predictive marker for successful cisplatin treatment in several types of cancer, including HNSCC[85,86]. However, interstrand crosslinks cause stalled replication forks during DNA replication, which eventually result in double-stranded DNA breaks. Two pathways are of importance in the repair of such double-stranded DNA damage, homologous recombination (HR) and nonhomologous end-joining (NHEJ). Initiation of both DNA repair pathways requires the detection of the actual DNA damage, which is the responsible task of ATM (double strand breaks) and ATR (stalled replication forks). Both proteins proved to be essential in establishing a good DNA repair response[87,88]. The main difference between the two DNA repair processes is that HR requires an undamaged template strand containing a homologous DNA sequence. This DNA strand is only available in the S- and G2 phase of the cell cycle. In contrast, during NHEJ two double-stranded DNA ends are joined, independent of the presence of complementary DNA strands and regardless of the correct repositioning of the genetic material. As a result, NHEJ may repair DNA damage in all phases of the cell cycle, but is error-prone compared to HR. Not only cell cycle phase determines which double-stranded DNA damage repair pathway is utilized, but there are indications that also the type of damage requests repair by a specific pathway. Deficiencies in FA proteins, especially BRCA1 and BRCA2, impair HR and sensitize cells to DNA crosslinking agents such as cisplatin[89,90]. On the contrary, mutations in NHEJ associated genes lead to greater radiation sensitivity[91,92].

Non-cancerous cells likely suffer less from radiation or cisplatin treatment compared to cancer cells, probably as a result of functional DNA damage pathways and correct regulation of the cell cycle. Recent studies identified a small subpopulation within a tumor that seems to have similar therapy-resistant traits.

Cancer stem cells

The processes of initiation, progression and recurrence of cancer have been extensively studied, and this led to the development of two models. The stochastic model hypothesizes that every cell within a tumor is able to maintain tumor growth and to initiate a new tumor. Individual mutations that are caused by intrinsic and environmental factors provide certain cells with a small survival benefit over the other cells, but in principle, all cells are genetically equivalent. In contrast, according to the hierarchical model, only a small subset of cells, i.e. the cancer stem cells (CSCs), is able to initiate tumor growth. CSCs are self-renewing and have the exclusive ability

to give rise to all differentiated progeny that makes up the bulk of the tumor. As a consequence it has been postulated that distant metastases are formed by CSCs that disseminated from the tumor. In addition, local recurrences are thought to be a result of residual CSCs after treatment. CSCs from multiple solid tumor types have been isolated based on differential expression of protein markers on the cell membrane. Recent studies identified such a CSC population in HNSCC which is based on the expression of the hyaluronic acid receptor CD44. It was shown that isolated CD44+ cells gave rise to new tumors *in vivo* in mouse models, whereas CD44- cells could not[93]. This study, and thereafter many others[94-98], supports the CSC concept in HNSCC, although there is still debate on the usefulness of CD44 as a HNSCC CSC marker. This is the result of the abundance and the location of CD44 expression observed in HNSCC. In mucosal epithelium the normal stem cells are assumed to reside in the basal layer. This is the place where cell division takes place, while the cells differentiate in the suprabasal layers. This ultimately leads to the terminally differentiated squames that have lost all cell organelles and form a protective layer[99]. As differentiated tumors form comparable mucosal-like structures, it is assumed that also the cells with stem cell capacities reside in these basal layers. However, it was shown in several studies that the CD44 protein is not only present on the basal cells but also on the suprabasal cells, both on normal and malignant squamous tissues[100-104], suggesting that CD44 is not the best available CSC marker.

Several lines of evidence suggest that vital processes are tightly regulated in CSCs. The CSC population is slowly cycling, providing the opportunity for accurate maintenance of DNA integrity. Also, proteins involved in DNA repair systems are adequately represented. Furthermore, CSCs express high levels of active detoxification pumps to quickly eliminate toxic compounds from the cell[105]. These characteristics led to the hypothesis that CSCs are highly resistant to the general anticancer treatments, such as radiotherapy and chemotherapy, and, consequently, that treatment failure might be explained by ineffective killing of the CSCs[106]. The identification of the HNSCC CSC population, and close examination of these cells, might lead to the development of treatments that specifically eliminate the CSCs. In theory, this will result in the complete eradication of the tumor, since CSCs are thought to maintain the tumor and to drive tumor growth. Until such therapies are available, clinicians aim to treat patients with the most effective treatment that causes the least side effects. For this reason, many studies aim to find tumor biomarkers that are able to predict upfront whether or not a tumor will be responsive to a certain treatment.

PERSONALIZED CHEMORADIATION

Cisplatin is an effective addition to radiation protocols, but the major limitation of its use is the often marked toxicity. Patients need to be hospitalized for a few days after cisplatin infusion and complications that require extra hospitalization are frequent. This is a large burden for the patient and associated health care costs. Another, less toxic agent, might at some time replace cisplatin. Cetuximab in combination with radiotherapy (bioradiation) may be a first possibility. However, bioradiation has not been compared to chemoradiation in a randomized trial. In addition cetuximab is at present very expensive, hampering clinical implementation.

Although chemoradiation is effective, several tumors do not respond well to this combination of cisplatin and radiotherapy. As there might be an alternative treatment, surgery with postoperative radiotherapy, response prediction is an important issue. Major research efforts aim to find clinical and/or biomolecular markers to predict chemoradiation response of HNSCC. The only clinical factor that shows some predictive value for chemoradiotherapy outcome of HNSCC proved to be primary tumor volume[107-114], but not all studies could confirm this[115]. Furthermore, as described above, it is well known that HPV-positive HNSCCs have a relatively favorable prognosis irrespective of treatment[20,116] and there is ongoing debate whether HPV-positive HNSCCs should not be treated by de-intensified protocols. This might turn HPV into a

prognostic factor that determines clinical management of HNSCC.
Using a candidate gene or protein approach several molecular markers have been studied to predict platinum-based chemotherapy and/or radiotherapy outcome in head and neck cancer patients. Examples of such genes are mutations in *TP53*[117-119] and expression of ERCC1[85,86,120-123], but contradictory reports have been published and none of these markers have made it to the clinic. Other groups have exploited microarray technology to determine an expression profile that predicts treatment response in head and neck cancer[115,124-131], but reported predictive profiles have not been validated.
There are several explanations for the fact that there is still no good predictor for chemoradiation response. First, most studies were performed on material derived from patients who were treated with multimodality therapy. Poor response to radiation might thus be compensated by good response to cisplatin and vice versa. Second, biomarker identification is mainly performed using sections of paraffin-embedded material or small tumor biopsies. The non-cancerous cells that are also present in these samples might blur the tumor profile, and small biopsies might not reflect all clones in a tumor. In addition, the molecular characteristics of other small subpopulations, like CSCs, will not be detected, whilst they might be highly relevant for treatment outcome (see above). Third, genetic alterations that cause protein inactivation or hyperactivation might not be detected by microarray-analysis or immunohistochemical analysis when the mutated protein is expressed at more or less normal levels. Finally, although many candidate biomarkers have been suggested in other tumor types, their predictive value might not be extrapolated to HNSCC. This emphasizes the need for in-depth examination of HNSCC CSCs and a gene-by-gene assessment of its influence on tumor response to treatment with a single therapy. This may result in the identification of genes that might serve as a biomarker for response, and, in addition, may be exploited to sensitize the tumor for drugs or radiation.

HIGH-THROUGHPUT IDENTIFICATION OF NOVEL DRUG TARGETS

New insights in biology and improvements in biotechnology have led to the development of new research tools. It has become relatively easy to interfere with complex cellular processes, even on the genetic level. Manipulating gene expression proved a powerful method to identify critical genes and elucidate pathways that play a role in cancer development and therapy resistance. This knowledge may form the base for new drug developing activities.

RNA interference

Posttranscriptional gene silencing, or RNA interference (RNAi), is based on a biological process in which small RNA molecules inhibit gene expression by targeting the mRNA for degradation. In fact, during artificial gene silencing, we use the biosynthesis routes of natural regulatory RNA molecules, the microRNAs (miRNAs). These are endogenously expressed non-coding RNAs of about 22 nucleotides long. The majority of the identified miRNAs are intergenic or oriented antisense to neighboring genes and undergo heavy processing before reaching the mature miRNA status[132]. After transcription, the primary miRNA transcript, pri-miRNA, is cropped by the Drosha-complex into a hairpin-shaped pre-miRNA. After transportation to the cytoplasm, the Dicer-complex further processes the pre-miRNA by removing the hairpin-loop, resulting in a miRNA:miRNA* duplex. Subsequently, the duplex is dissociated and the mature miRNA is incorporated in the RNA-induced silencing complex (RISC), where it interacts with its mRNA target. miRNAs exert their effect primarily through a 6-8 nucleotide long sequence, the seed region, that binds preferably to the 3' untranslated region (3'UTR) of the mRNA[133,134]. This results in mRNA degradation or translational repression. Since miRNAs do not need to be fully complementary to their target mRNA, they are able to regulate the expression of hundreds of genes simultaneously[135]. MiRNAs can be overexpressed by transient transfection of a synthetic miRNA (miRNA mimic) or by stable transduction of a miRNA encoding gene. This makes them

interesting tools to identify combinations of genes that might be lethal to cells. Moreover, the miRNAs themselves might be used as drugs although targeting to the cells of interest is still a major problem. Typical applications of miRNA (mimic) libraries are investigations of cellular processes like proliferation, migration and gene regulation[136-140]. More recently, such libraries were also employed to identify modulators of drug response[141-146].

Over the past years, the role of miRNAs has been assessed in HNSCC initiation and progression. It is now well established that deregulation of miRNA levels might result in enhanced oncogene expression. An example is the downregulation of miR-138 in HNSCC and many other tumor types[147], although its target mRNA is not yet identified. In contrast, upregulation of certain miRNAs might restrain expression of tumor suppressor genes, as was shown for miR-16 which silences the antiproliferative effects of BCL2[148] and miR-21 which inhibits apoptosis and was associated with poor survival[149].

The biomolecular pathways involved in miRNA-mediated gene regulation, can be exploited for interference of gene expression with small interfering RNA (siRNA). SiRNAs are synthetic double-stranded, 20-25 bp long RNA molecules that, like miRNAs, are processed by Dicer[150], although siRNAs are cleaved into single-stranded molecules, whereas miRNAs are dissociated[151]. When incorporated in the RNA-induced silencing complex (RISC) the single-stranded siRNA binds only to fully complementary mRNA molecules[152]. For this reason, siRNAs are assumed to influence the expression of only one gene. SiRNA:mRNA binding results in the degradation of the mRNA and a decrease in protein expression. Synthetic siRNAs can be introduced in a cell by transfection and this results in transient, specific knockdown of the desired gene.

Several other tools are available to downregulate gene expression. Short-hairpin RNAs (shRNAs) are produced from synthetic genes and can be inserted in retroviral or lentiviral vectors to transduce cells of interest. The shRNA is subsequently randomly integrated in the host cell DNA. The product resulting from transcription mimics pri-miRNA and is processed as was described for miRNAs.

Loss-of-function experiments using siRNAs have been increasingly implemented in high-throughput platforms and proved to be an important genomic research tool[153-157]. Function-specific or complete genome-wide siRNA screens are valuable, unbiased methods to identify genes that modulate drug response[158-167] and this may lead to new lead compounds[168-171].

TARGETED TREATMENT: ONCOGENE ADDICTION AND SYNTHETIC LETHALITY

Specific oncogenic pathways might be (over)activated in tumors by mutations in a key player, such as mutations in EGFR found in non-small cell lung cancer. The tumor cells may depend on these stimulatory signals and become oncogene addicted. This concept has been shown to allow targeted treatments. When a small molecule inhibitor restrains the activated oncogene, the cells may die. However, head and neck cancer seems more a disease caused by the inactivation of suppressing pathways than the activation of oncogenic pathways, hampering the design of novel treatment strategies. Though, there might be an alternative approach to target inactivated suppressing pathways by exploiting synthetic lethal interactions.

A genetic interaction in which mutations in two or more genes result in cell death, whereas alteration of only one of the genes is harmless, is defined as synthetic lethality[172]. Typically, the involved gene products take part in the same cellular process or can function as a bypass route for the other. Since tumor cells already harbor many mutations, the concept of synthetic lethality can be used to identify new drug targets that are tumor cell specific.

Several studies have reported decreased tumor cell survival by inhibition of individual genes in a specific background of mutations[173]. An important synthetic lethal interaction was recently identified. Breast cancer cells with mutated BRCA1 or BRCA2, which are involved in the repair of double-stranded DNA breaks by homologous recombination, showed to be very sensitive to inhibition of PARP, an enzyme required for single-stranded DNA repair[174,175]. Single stranded

DNA breaks that occur by natural causes or that are radiation induced, can be repaired by PARP-mediated base excision repair, but, when left unrepaired, develop into double stranded DNA breaks during replication. These are preferably repaired by homologous recombination during S/G2 phase when the homologous sister chromatid is available. This process requires BRCA1 and BRCA2. The impairment of both pathways that are important for maintenance of DNA stability, eventually results in cell death. Later it became apparent that also cells with other defects in genes involved in homologous recombination die after PARP inhibition[176]. Clinical trials are now performed to determine the safety and efficacy of PARP inhibitors in BRCA1 or BRCA2 deficient breast cancer[177-179].

Large-scale RNA interference (RNAi) screens are excellently suited to systematically identify synthetic lethal interactions in human cells. This approach can also be used to identify genes that are synthetic lethal with the oncogenic expression of a gene that is difficult to target. For example, tumor cells that depend on oncogenic KRAS expression might be eliminated by the inhibition of another, synthetic lethal, gene[180-182]. This approach might circumvent a long complicated road to KRAS drug development. Furthermore, genome-wide RNAi screens can be used to identify genes that enhance the effect of a specific anticancer drug[167,183,184]. Identification of such drug sensitizing genes might be the beginning of tackling cancer therapy resistance.

OUTLINE OF THIS THESIS

Patients suffering from advanced stage HNSCC have less than 40% chance to survive the first five years after diagnosis. Tumors may not respond well to chemoradiation, may relapse, or distant metastases may occur with major impact on outcome. Moreover, there is a lack of biomarkers that predict chemoradiation response, which hampers the personalization of therapy. In this thesis we used functional genetic screens to identify potential biomarkers for cisplatin response as well as novel therapeutic targets. In addition, we describe a novel and more suitable marker to isolate the head and neck cancer stem cell population, and studied the relevance of this marker for outcome prediction.

In spite of all the available knowledge on the pharmacodynamics and anti-tumor effect of cisplatin, the exact mechanism of platinum-based chemotherapeutic response in HNSCC is not clear. In **Chapter 2**, we aimed to identify cellular characteristics that determine cisplatin response in a large panel of HNSCC cell lines.

In an attempt to further identify genes that confer cisplatin response in HNSCC, we performed a genome-wide siRNA screen in the context of cisplatin treatment. In **Chapter 3** our findings are described on how important the role of the FA/BRCA pathway is in head and neck cancer cells in the response to cisplatin.

Besides the identification of genes that modify the effect of cisplatin, we aimed to find genes that are essential for tumor cell maintenance in general, while they are less important for the viability of normal cells. To this end we mined the data of the genome-wide siRNA screen. In **Chapter 4** the data on one cherry-picked hit is presented. Furthermore, we screened a microRNA library in order to identify microRNAs that might be lethal when overexpressed in tumor cells, while immortalized normal keratinocytes remained viable (**Chapter 5**).

Over the past years the role of cancer stem cells in the resistance to cancer treatment is increasingly emphasized (as described above). Therefore, it is important to recognize the cancer stem cells (CSCs) within a tumor and subsequently try to find ways to characterize and specifically eliminate these cells. In **Chapter 6** we identified CD98 as a novel HNSCC CSC marker, which seems more suitable and specific than CD44.

The newly identified CSC marker CD98 was used in **Chapter 7** to find an explanation for the survival benefit that patients with HPV-positive HNSCC tumors have over patients with HPV-negative tumors. HPV-positive tumors have a favorable prognosis and are very sensitive to anticancer therapy. We hypothesized that this might be the result of a lack of CSCs in these

tumors. We analyzed a large cohort of HNSCC tumors for HPV-presence and CD98 expression and correlated these parameters with clinical outcome. The results were compared to those obtained with the established HNSCC CSC marker CD44.
A general discussion of the results described in this thesis is provided in **Chapter 8**.

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