Summary

The average overall survival for head and neck cancer is around 50%, but can vary significantly between groups of patients with different characteristics. Currently only clinical characteristics are used and treatment choice (often including radiotherapy) is based on site and TNM stage, which explain only a small proportion of the variation in survival. The research presented in this thesis describes studies into the individual biological tumor properties of head and neck cancer, using messenger- and microRNA data to predict which tumors will be more radioresistant and to gain insight into the mechanisms behind this. Eventually this should lead to a better understanding of the causes for radiotherapy failure allowing an up-front adaptation of therapy to give each individual head and neck cancer patient the best chances of survival with the least amount of toxicity.

Chapter 1 gives a general introduction into head and neck cancer and reviews existing knowledge on reasons for failure of radiotherapy. The general aims and outline of this thesis are also described in this chapter.

The first question to be addressed was whether gene expression data could add useful information to known clinical factors in the prediction of outcome after (chemo-)radiotherapy for head and neck cancer. In **chapter 2** we show that gene expression can improve the prediction model and adds valuable information to known clinical factors for the prediction of local control after chemoradiotherapy for advanced head and neck cancer.

We analyzed pre-treatment gene expression data from 75 advanced head and neck cancer patients treated with primary chemoradiotherapy. In this series a published high risk signature (Chung high-risk) and a HPV expression profile (Slebos) were analyzed in a model with known clinical predictors of local control: age at diagnosis, gender, tumor site, tumor volume, T-stage and N-stage. Only tumor site (oral cavity vs. pharynx, hazard ratio 4.2 [95% CI 1.4–12.5]), Chung gene expression status (high vs. low risk profile, hazard ratio 4.4 [95% CI 1.5–13.3]) and HPV profile (negative vs. positive profile, hazard ratio 6.2 [95% CI 1.7–22.5]) significantly predicted local control after chemoradiotherapy in the multivariable model.

Chapter 3 describes the analysis of a more homogeneous series of patients, treated with single modality radiotherapy. The hypothesis was that this series would give a better insight into the cause of radioresistance, without confounding by heterogeneity or clinical factors.

Gene expression data were generated on pre-treatment biopsies of 52 T1-2 laryngeal cancer patients treated with radiotherapy. Since recurrence rates are low in this population, patients with a local recurrence were matched for T-stage, subsite, treatment, gender and age with non-recurrence patients (1:2). Gene sets for hypoxia, proliferation and intrinsic radiosensitivity did not correlate with recurrence, whereas high expression of the putative stem cell marker CD44 did (odds ratio 20.2 [95% CI 3.4-172.3]). Immunohistochemical analysis of CD44 expression on an independent validation series of 76 small laryngeal cancers confirmed CD44's predictive potential. For more insight into the function of CD44,

gene expression data of eight larynx cancer cell lines with known radiosensitivity were analyzed. In these cell lines, CD44 expression did not correlate with intrinsic radiosensitivity although it did correlate significantly with plating efficiency, consistent with a relationship with stem cell content.

In neither of the patient series in **chapter 2 and 3** published intrinsic radiosensitivity gene sets were significantly correlated with recurrence after (chemo-)radiotherapy. This was an unexpected finding, since it is known that for head and neck tumors the *ex vivo* measurement of radiosensitivity correlates with outcome after radiotherapy. It was therefore concluded that an accurate gene expression set correlating with intrinsic radiosensitivity in head and neck cancer was lacking.

Chapter 4 describes the search for an intrinsic radioresistance gene set. Having such a set would not only be helpful to predict sensitivity before start of treatment, but could also reveal biological processes that could be targeted to overcome intrinsic resistance. MicroRNA and messenger RNA expression was measured in irradiated and unirradiated samples of 32 head and neck squamous cell carcinoma (HNSCC) cell lines. Measurements on unirradiated cells correlated with resistance, whereas the response to radiotherapy seemed irrelevant for the prediction of resistance. The presence of epithelial-to-mesenchymal transition (EMT) and low expression of microRNAs involved in the inhibition of EMT were important radioresistance determinants. This finding was validated in two independent cell line pairs, in which the induction of EMT reduced radiosensitivity. For the most important microRNA (miR-203), downregulation strongly correlated with intrinsic radioresistance in cell lines and a higher recurrence rate after radiotherapy in a series of 34 laryngeal cancer patients.

In **chapter 5** we show that for hypoxia different sets of genes have been published, with almost no overlapping genes. However, almost entirely different sets of genes can come to the same conclusion. Four published gene sets were compared using expression data from 224 head and neck cancer patients from three different datasets. Although only 2% of all genes were similar in the four validated hypoxia profiles, the profiles showed a near complete correlation with each other in categorizing the 224 patients. While it was assumed by most authors that they were studying both acute and chronic hypoxia, the gene sets that were published only corresponded with an *in vitro* chronic hypoxia profile, not with the early hypoxia response profile. Additionally, this early hypoxia profile better predicted local recurrence after chemoradiotherapy.

Chapter 6 contains a general discussion of the work presented in this thesis. In this chapter possible pitfalls of the presented research are discussed. In the last part, directions for future research are explored.