Molecular-cytogenetic characterization of head and neck cancer: Identification of novel prognostic factors and gene targets for therapy [double dissertation 2]
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Cancer incidence has increased significantly over the past 50 years, with an overall lifetime risk for cancer development of approximately 45% in individuals living in the developed countries. Despite significant advances in cancer care, cancer mortality has remained virtually unchanged over the past 3 decades. Given the realization that cancer is a genetic malady, it is quite apparent that further improvement of cancer outcomes is likely to come from an improved understanding of the molecular circuitry of cancer cells.

Cancer develops when normal cells acquire specific changes in their genetic information that allow them to overcome normal growth regulatory mechanisms, invade surrounding structures and spread to distant anatomic sites. Cancer causing genetic changes invoke increased activity of genes that induce cell growth (proto-oncogenes), surrounding blood vessel ingrowth (angiogenesis), cellular dissociation from the environment and cellular migration (proto-oncogenes), and inactivation of genes that limit these processes or promote programmed cell death (tumor suppressor genes). Activation of proto-oncogenes may be acquired through gene dosage increase (as a result of chromosomal gain or genetic amplification), genetic rearrangement (translocation) or changes in single nucleotides in the blueprint of the gene (activating point mutations). On the other hand, tumor suppressor genes are inactivated through loss of genetic information (genetic deletion), inactivating mutations in the genetic code (i.e. missense/nonsense mutations) and blockage in production of proteins (i.e. promotor hypermethylation).

Normal cells may be born with mutations inherited through the germ line. More importantly, all cells acquire a significant number of somatic genetic mutations over the course of their lifetime. This as a consequence of the physiologic imbalance between inherent errors in DNA replication and exposure to mutagenic influences on the one hand, and the fidelity of intrinsic DNA repair mechanisms on the other hand. Based on the interplay of these processes, mutagenesis is a random process and the chance of inheriting oncogenic mutations is linearly related to the extent of the mutational burden. Accordingly, cancer develops at a higher rate in the setting of certain inherited mutational syndromes, increased exposure to mutagenic influences or diminished activity of DNA repair. Nonetheless, it has been under debate whether the physiologic mutation rate is sufficient to cause the large number of genetic mutations that are found in cancer cells (> 12,000 individual mutations). Studies of colorectal cancer have fueled this debate. Early in the course of their development, colorectal cancers may increase their chances of acquiring critical oncogenic mutations through inactivation of pathways maintaining genomic stability. An important form of genetic instability in colorectal cancers involves chromosomal instability (CIN). CIN may be a result of acquired defects in DNA repair, mitotic spindle formation and chromosome segregation. CIN increases genetic heterogeneity within the cellular population thereby paving the way for perpetual Darwinian selection and clonal outgrowth of cells with the fittest genomic content. This feature explains the continuous adaptation to inhibiting influences (selective pressures) that characterizes malignant behavior. Given the causative role of genetic aberrations in cancer pathogenesis, it is clear that identification of mechanisms underlying the accumulation of genetic changes and deciphering the effects of those changes is a key to understanding and modifying cancer behavior.

Head and neck cancer is a generic term that includes a variety of tumors originating within the confines of the head and neck region. The most common cancers found to originate in the region are squamous cell carcinomas of the upper aerodigestive tract and adenocarcinomas of the thyroid gland. Similarly to colorectal cancers, an array of chromosomal alterations has been described in head and neck squamous cell carcinomas (HNSCC) and thyroid cancers. However,
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mechanisms underlying the high rate of chromosomal changes in both malignancies remain obscure.

To begin to assess factors predisposing to the development and accumulation of genetic aberrations in head and neck cancer, we focused on a possible underlying role of chromosomal instability in these tumors. In order to do this, we first explored the cancer incidence and distribution in patients with an inborn defect in chromosomal stability, including Fanconi Anemia (FA), in a registry-based study. FA is caused by germline mutations in DNA repair genes and patients with FA have an increased cancer risk but the spectrum of tumors associated with this disease has remained obscure. We found a >500-fold increased incidence of squamous cell carcinoma (SCC) in FA patients relative to the general population, with a predominance of HNSCC (Chapters 1). SCC in FA patients develops in younger patients and displays an aggressive clinical course (Chapter 2). Given the low prevalence of tobacco and alcohol use, the anatomic location of SCC, and clinical and histopathological evidence suggesting antecedent viral infection, we explored a role of HPV in SCC pathogenesis in FA patients (Chapter 3). The HPV virus is an oncovirus that may contribute to CIN and malignant transformation of mucosal cells (Chapter 3). Our results demonstrate that, compared to the general population, SCCs occurring in FA patients are significantly more often characterized by an HPV-positive phenotype. FA associated (HN)SCC were also significantly less common to feature p53 mutations, reminiscent of the established ability of the HPV-oncovirus to inactivate the tumorsuppressive function of the p53 protein (Chapter 3). As a possible contributing factor for the high rate of HPV-induced squamous cell carcinogenesis among the FA population, our data suggest a high rate of Arg72 polymorphisms among the FA population, a genetic polymorphism that was previously associated with a higher sensitivity for HPV E6 oncoprotein-induced degradation of p53 (Chapter 3). Our findings strongly suggest that chromosomal instability is a key underlying mechanism of (HN)SCC development in humans and provide further evidence for a contributing role of HPV virus in (HN)SCC development. Beyond these biological implications, the identification of Fanconi Anemia as a novel risk factor for (HN)SCC development is associated with significant clinical implications. These include the need for careful otolaryngological and gynaecological follow-up of FA patients, suspicion of Fanconi Anemia in young patients presenting with SCC and opportunities for cancer prevention through HPV vaccination of FA patients.

To explore a contribution of chromosomal instability in the pathogenesis of sporadic HNSCC, we studied the dynamics of chromosomal alterations in HNSCC, focusing on effects of tobacco exposure and defects in mitotic machinery on induction of CIN (Chapters 4 and 5). Our data confirm the presence of CIN, but contrary to previous reports, no association between CIN and tobacco exposure was observed in HNSCC. We did find defects in mitotic fidelity, specifically spindle assembly checkpoint integrity, which may be a contributor to CIN in HNSCC, offering new venues for targeting. As radiation has also been proposed to induce CIN in normal cells, we assessed for chromosomal aberrations in thyroid neoplasms occurring in patients exposed to fallout from during the 1986 Chernobyl nuclear power plant accident (Chapter 6). However, no evidence for CIN could be demonstrated in these tumors, suggesting alternate mechanisms are involved in the pathogenesis of radiation associated thyroid cancers.

In addition to delineation of mechanisms underlying accumulation of genetic changes, a key goal of this effort was the identification of genetic changes critical for cancer development and progression. Given their close association with disease biology, such factors may be predictors of tumor behavior and attractive therapeutic targets. The value of this paradigm has been convince-
ingly demonstrated with the example of chronic myeloid leukemia (CML), which can be cured by targeting its key genetic alteration (BCR-ABL). Given the random nature of cellular mutagenesis, the majority of genetic mutations in cancer cells involve genes non-essential for cancer pathogenesis or occur in non-coding genomic regions. Accordingly, the challenge for modern genetic analysis is the identification of critical cancerous mutations among the large pool of genetic noise.

Dramatic progress in the field of (cancer) genetics, including the completion of the Human Genome Sequencing Project in 2002 and development of high-throughput genetic screening, has enhanced our ability to decipher the cancer genome. In order to identify biologically relevant genetic alterations, we applied genome-wide screening tools including comparative genomic hybridization, spectral karyotyping and cDNA microarray analysis to the genetic analysis of HNSCC and thyroid cancer and correlated identified genomic profiles with clinical behavior (Chapters 7 to 17). These analyses revealed a myriad of known and novel genetic aberrations including gains, amplifications, translocations, deletions and gene expression changes. In HNSCC, it was possible to directly correlate genetic profiles with cancer outcome. These efforts revealed several genetic alterations associated with adverse clinical outcome (Chapters 10 to 13). Molecular analysis of these changes may help improve prognostic stratification and associated treatment selection of HNSCC. We included a poignant example of the promising features of modern genome-wide screening tools in the improvement of clinical decision making, as described in Chapter 14 of this thesis. Using high-throughput gene expression profiling, we were able to convincingly differentiate metastatic HNSCC to the lung from primary lung SCC, a differentiation with significant clinical implications.

The identification of molecular prognosticators of well-differentiated thyroid cancer is limited by the long natural history of the disease. In order to circumvent this issue, we compared genetic profiles in the context of pathological progression from well-differentiated, to poorly differentiated and undifferentiated (anaplastic) thyroid cancers. These efforts revealed an array of novel genetic changes that may be associated with aggressive behavior and tumor progression (Chapter 15). We validated the prognostic significance for one of these alterations (as described in the next section). In addition, focused analysis of subsets of well-differentiated thyroid neoplasias including classical papillary thyroid carcinomas, follicular variant papillary thyroid carcinoma, follicular thyroid carcinoma and follicular thyroid adenoma suggests that the pathogenesis of some papillary thyroid carcinomas (the follicular variant papillary thyroid carcinomas) is more closely related to that of follicular thyroid tumors (carcinomas/adenomas) than to the classical papillary carcinomas (Chapters 16 and 17). If confirmed, these findings may have major implications for the clinical classification of benign and malignant thyroid tumors.

Several of the genetic aberrations identified by genomic screening analyses were found to be of particular interest based on association with disease biology, including 3q26 amplification in HNSCC and 1q21 amplification in thyroid cancer. We applied traditional positional cloning methods to identify putative oncogene(s) driving selection for 3q26 amplification in HNSCC (Chapters 18 to 21). These efforts revealed amplification and associated overexpression of the known oncogene PIK3CA, previously described in ovarian and cervical cancers (Chapter 20). Moreover, we identified amplification and overexpression of a previously unknown gene within the region that was designated squamous cell carcinoma-related oncogene (SCCRO) (Chapter 21). In corroboration with our CGH data showing 3q amplification associated with dismal HNSCC outcome, overexpression of both PIK3CA and SCCRO was associated with adverse clinical outcome
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of HNSCC (Chapter 22). Since the mere amplification and overexpression of a gene does not unequivocally implicate a gene in cancer pathogenesis, we proceeded with functional characterization of the identified gene targets. We showed that PIK3CA amplification renders HNSCC resistant to p53-mediated apoptosis that is independent of PTEN (Chapter 20). These data suggest a major role of PIK3CA amplification in HNSCC pathogenesis and reveal important clues for segregation of cell death and cell survival signals. Functional analysis of SCCRO demonstrates it is critical for both transformation and maintenance of the malignant phenotype in cell lines derived from HNSCC carrying 3q26 amplification. Further experiments underline the oncogenic activity of SCCRO by showing it to be a key mediator of the hedgehog signaling pathway, which is commonly dysregulated in human tumors (Chapters 21-24). These data identify two novel molecular targets that may be key factors for HNSCC pathogenesis and reveal important clues for novel targeted treatment approaches.

Although successful, the positional cloning strategy applied in the identification of PIK3CA and SCCRO was rather laborious and time consuming. Accordingly, we aimed to identify candidate oncogenes of 1q21 amplification in thyroid cancer by combining the results of CGH with gene expression profiling. This approach showed that MUC1 was consistently upregulated in thyroid cancers with gain/amplification of chromosomal region 1q21 (Chapter 25). Independent prognostic significance of MUC1 upregulation was demonstrated and validated in thyroid cancers from two independent patient cohorts. These data suggest that MUC1 up-regulation plays an important role in the pathogenesis of aggressive thyroid cancers and may aid the accuracy of prognostic stratification of the disease. We are currently validating the role of MUC1 in a prospective study and determining mechanisms of action in aggressive thyroid cancers.

In conclusion, the current era of cancer research is abounding with discovery, consequent to the completion of the human genome project and development of novel high-throughput analytic approaches. Along with the discovery of novel aberrations, many new prognostic markers and therapeutic targets have been identified. These changes have opened new venues for the treatment of solid tumors. As an example, the association of EGFR mutation with responsiveness to anti-EGFR directed therapy in lung cancer has certainly raised expectations. However, several barriers still remain. The genomic complexity and perpetual genetic evolution in cancer cells has made inclusive characterization of the cancer genome difficult. Accordingly, the effects of novel discoveries have not affected clinical care or patient outcome as much as anticipated. The work presented in this thesis is a starting point for continuing investigation. We have identified new models, genetic aberrations, and developed the reagents to continue to address the questions raised. It is our hope that we contribute to the understanding of cancer biology and ultimately the care of patients afflicted with this deadly disease.