Molecular analysis of the HPJ-JT syndrome and sporadic parathyroid carcinogenesis

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Chapter 11

Summary

In this thesis tumourigenesis and tools for improved diagnosis of parathyroid tumours were studied with a special focus on parathyroid carcinomas.

In ***chapter 2*** we described a large, previously unreported Dutch kindred in which 13 affected members presented with either parathyroid adenoma or carcinoma; in 5 affected individuals, cystic kidney disease was found. Additionally, pancreatic adenocarcinoma, renal cortical adenoma, papillary renal cell carcinoma, testicular mixed germ cell tumour with major seminoma component, and Hürthle cell thyroid adenoma were also identified. We determined that the disease in this family was linked to the presumed *HRPT2* locus on chr 1q and we were able to localize the region of the gene to 14cM. We concluded that HPT-JT is a clinically heterogeneous syndrome and that the HRPT2 gene might play a role in development of several tumours.
The family described in chapter 2 was of great importance for the discovery of the *HRPT2* gene as described in ***chapter 3***. By combining data from 26 families suffering from the HPT-JT syndrome, the *HRPT2* region was narrowed down to 12 cM. Using a positional candidate cloning approach in 14/26 cases from fourteen families, heterozygous germline mutations in c1orf28 (*HRPT2*) were found. Inactivating somatic mutations in this gene were also found in 3/48 parathyroid (cystic) adenomas supporting the probable tumour suppressor effect of the gene. The gene is evolutionary conserved and consists of 17 exons encoding a protein of 531 amino acids and was named parafibromin. Parathyroid tumours show higher malignancy frequencies in HPT-JT syndrome than in other familial parathyroid tumour syndromes such as MEN1. Here we suggest that mutations in *HRPT2* may be responsible for this difference. To test this hypothesis we analyzed, as described in ***chapter 4***, 60 different (benign and malignant, mostly sporadic) parathyroid tumours. *HRPT2* somatic mutations were detected in all (4/4) sporadic parathyroid carcinoma cases and 5/5 HPT-JT tumours and 2/3 FIHP tumours. No mutations were detected in any of the other (benign) tumours. ‘‘Two-hits’’ (double mutations or one mutation and loss of heterozygosity at 1q24-32) affecting *HRPT2* were found in 2/4 sporadic carcinomas, 2/5 HPT-JT-related and 2/3 FIHP related tumours.
These data supported the role of *HRPT2* as a causative gene in the development of parathyroid malignancy both in familial and sporadic tumours and it provided evidence of a role for *HRPT2* as a tumour suppressor gene. We hypothesized that *HRPT2* mutation is an early event that may lead to parathyroid malignancy and therefore suggested that mutations of *HRPT2* are markers for malignant potential of parathyroid tumours, both familial and sporadic.
In ***chapter 5*** we hypothesized, based on this high prevalence of *HRPT2* gene mutations and biallelic inactivation in parathyroid carcinoma, that loss of parafibromin, the protein product of the *HRPT2* gene, could distinguish carcinoma from benign tissue. To study this, a novel antiparafibromin monoclonal antibody was generated and immunostaining on both benign and malignant parathyroid tumours was tested. We reported that the loss of parafibromin nuclear immunoreactivity had a high sensitivity and specificity in diagnosing carcinoma. Parafibromin thus seems a promising molecular marker in the diagnoses of parathyroid carcinoma.
In ***chapter 6*** the morphological, immunohistochemical and molecular characteristics of 26 primary parathyroid carcinomas and seven metastases were studied. Down-regulation of the calcium sensing receptor (CASR) was demonstrated in 31% of carcinomas, and this was significantly correlated with a high Ki-67 proliferation index. Chromosome 1q and chromosome 11q LOH were found in 12 of 22 (55%) and 11 of 22 (50%) carcinomas tested, respectively. Combined 1q and 11q LOH was seen in 8 of 22 (36%) carcinomas, in contrast to the low percentage of LOH reported in both regions in adenomas. We concluded that both loss of CASR protein expression in

combination with an increased proliferation rate and the combined 1q/11q loss could be used as supportive criteria in the diagnosis of parathyroid carcinoma.
Furthermore, the high percentage of LOH at 1q found in our set of sporadic parathyroid carcinomas seemed to confirm the tumour suppressor function of *HRPT2* also described in chapter 3 and the importance of the gene in the development of malignant parathyroid tumours. The high percentage of LOH of 11q also suggested involvement of the *MEN1* gene in the tumourigenesis of a part of parathyroid carcinomas. This hypothesis was tested in ***chapter 7*** where we evaluated the role of *MEN1* and *HRPT2* mutations in sporadic parathyroid tumours fulfilling histological criteria for malignancy. Formalin fixed, paraffin embedded (FFPE) parathyroid carcinoma tissue from 28 Dutch cases was studied. *HRPT2* (27/28 cases) and *MEN1* (23/28 cases) were analyzed by direct sequencing. Somatic MEN1 mutations were found in 3/23 (13%) sporadic parathyroid carcinoma cases, six *HRPT2* mutations were found in 4/27 cases (15%). These results again confirmed the role of *HRPT2* in sporadic parathyroid cancer formation, but also showed parathyroid carcinomas with *MEN1* mutations possibly suggesting that an adenoma with a *MEN1* mutation can progress into a carcinoma when untreated.
In ***chapter 8*** we undertook expression profiling of 53 hereditary and sporadic parathyroid tumours. A class discovery approach identified three distinct groups, mainly consisting of respectively (1) adenomas, (2) *HRPT2* mutated tumours, and (3) hyperplasia. The most robust cluster identified in this study consisted of sporadic parathyroid carcinomas, tumours from HPT-JT patients (both benign and malignant), and tumours from two FIHP patients. Eleven of 12 of these cases were shown to carry *HRPT2* mutations. We concluded that parathyroid tumours with *HRPT2* mutations follow pathways distinct from that of other tumours. Based on the expression data, we confirmed the differential expression of Histone H1, amyloid βA4 precursor protein, Cyclin D1 and E-cadherin using IHC.
The objective of the study described in ***chapter 9*** was to develop a Multiplex Ligation-dependent Probe Amplification (MLPA) based genomic assay for the rapid diagnosis of parathyroid carcinomas using a combination of known chromosomal amplifications and deletions. In this study we again confirmed that parathyroid tumours with *HRPT2* mutations follow pathways distinct from that of other benign and malignant tumours. We suggested that genes on chromosome 1p, 3q but especially chromosome 13 play a role in *HRPT2* driven tumourigenesis.