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

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Chapter 10

Concluding Remarks

In this thesis, parathyroid tumourigenesis was studied focusing on the underlying defects and the diagnosis.

HRPT2 gene

During the last 20 years, new insights in the pathogenesis, diagnosis and management of parathyroid tumours became apparent. An important milestone was the recent discovery of the *HRPT2* gene. The *HRPT2* gene is ubiquitously expressed, evolutionary conserved and consists of 17 exons encoding a protein of 531 amino acids, referred to as parafibromin.
Germ-line mutations in this gene are responsible for the *HPT-JT* syndrome. Furthermore, sporadic parathyroid carcinomas often show (somatic) mutations in the *HRPT2* gene (this thesis, chapter 4 and chapter 7). The percentage of identified *HRPT2* mutations in sporadic parathyroid carcinomas varies in different publications, partly due to different inclusion criteria. In 70% of carcinomas with local recurrence or metastasis *HRPT2* mutations have been observed.[3;6;9;11;16] In a Dutch cohort of parathyroid carcinomas selected primarily on histological grounds (i.e. with vasoinvasion and capsule invasion), the prevalence of *HRPT2* mutations was only 15%, although mutation analysis was performed in archival paraffin embedded tissue.
Somatic *HRPT2* mutations were also reported in HPT-JT associated tumours other than parathyroid. Somatic *HRPT2* mutations were found in two renal carcinomas, one clear cell carcinoma and one Wilms tumour.[32] Also, somatic mutations were identified in benign ossifying fibromas of the jaw.[26] Interestingly, these tumours showed retained expression of parafibromin. As IHC is not a quantitative analysis it could be possible that haploinsufficiency might play a role in tumour formation, which also might explain the benign behaviour in contrast to the aggressive behaviour of parathyroid tumours with total loss of expression of parafibromin due to double mutations in *HRPT2* or to the combination of one mutation and loss of the wildtype allele. Frequent allelic imbalance (LOH) of the *HRPT2* locus was detected in different subtypes of sporadic renal tumours and LOH analyzed by microsatellite markers and arrayCGH of the *HRPT2* locus is associated with an adverse clinical outcome. [18,24] A role of the *HRPT2* was also suggested in tumour types other than typically found in the HPT-JT spectrum, as illustrated in chapter 2 where tumours of the thyroid, testis and pancreas were found in a large HPT-JT family. Also uterine tumours are found to be associated with *HRPT2*.[3] Selvarajan et al showed altered immunohistochemical parafibromin staining in breast carcinomas.[28] In the future the development of knock-out mouse models for HPT-JT could help to gain more insight in the role of *HRPT2* in the development of all these tumours

HPT-JT syndrome

Patients with germ-line *HRPT2* mutations show a wide variation of clinical features. Such individuals can develop tumours in different organs or tissues, mostly in the parathyroids, kidneys, or jaws. Additionally, tumours in the thyroid, testes, pancreas (this thesis) and uterus [3] are described. HPT-JT has an autosomal dominant mode of inheritance, with incomplete penetrance as reported in the large Dutch family described in this thesis (chapter 2). The incomplete penetrance might also explain the relatively high percentage of germline mutations found in apparently sporadic parathyroid carcinomas (this thesis, chapters 7 and 4).[29] Some individuals with germ-line *HRPT2* mutations develop only parathyroid gland tumours. The latter is illustrated by the finding that about 5% of the patients suffering from familial isolated hyperparathyroidism (FIHP) carry *HRPT2* mutations.[6;22] Despite the reported rarity of

HRPT2 mutations in FIHP, FIHP patients with aggressive tumours are likely to carry *HRPT2* mutations and are therefore serious candidates for *HRPT2* germ-line testing. [14]

Parafibromin

Parafibromin is evolutionary conserved and binds to RNA polymerase II as part of a PAF1 transcriptional regulatory complex. PAF is comprised of five subunits that include PD2/hPaf1, parafibromin, hLeo1, hCtr9 and hSki8. The mechanism by which loss of parafibromin function can lead to neoplastic transformation is poorly understood. It has been suggested that parafibromin is involved in transcriptional regulation, histone modification, cell proliferation (including cell cycle progression[7;12], apoptosis[19] and wnt signalling.[23,2;27;30-32]
We suggested by both gene and protein expression that Histone 1 Family 2 (HIST1H1C), amyloid beta precursor protein (APP), and E-cadherin (CDH1) might play a role in HRPT2 driven tumourigenesis.
APP overexpression both at the mRNA and protein level[17] and abnormal cleavage is associated with the neuropathological abnormalities of Alzheimer's disease. It was recently shown that a soluble cleavage product of APP has a growth promoting effect in thyroid, skin, pancreas, colon and oral squamous cells by activating MAP kinase, epithelial growth factor[10;25], serine protease inhibitors[21], PKC and Ras pathways. [15]
Although a role for APP in EGF mediated growth of parathyroid cells similar to that of the mechanism in thyroid cells[25] can be expected, the direct interaction between parafibromin and APP has to be elucidated. Konishi et al[16] concluded that HIST1H1C has a role in transmitting apoptotic signals, while Lin et al[19] suggested that proapoptotic activity of endogenous parafibromin is also likely to be important in its role as a tumour suppressor.
E-cadherin is a cell adhesion molecule that interacts with the wnt signalling pathway. A role for parafibromin in Wnt signalling is also reported[23], in which parafibromin is thought to activate the Wnt/Wg target gene transcription by directly associating with beta catenin. Cyclin D1 (*CCND1*) was initially cloned and recognized as an oncogene in the development of the parathyroid tumours[1]. We demonstrated both on gene expression as well as on protein level overexpression of CCND1 in parathyroid carcinomas. Two recent publications showed evidence that parafibromin downregulation causes indeed an increase in CCND1 protein levels. [30;32]

Diagnosis of parathyroid carcinoma

Diagnosis based on histology alone is sometimes difficult because unequivocal diagnostic findings can be absent in individual cases and histological features of malignant and benign parathyroid tumours overlap. As a result of this histopathologic uncertainty, the best possible diagnosis can be unsatisfying referring to entities like "equivocal carcinoma" or "atypical adenoma". Recently in the WHO atlas[8] it is favoured to use the term atypical adenoma.
As the majority of parathyroid carcinomas with aggressive behaviour carry *HRPT2* mutations, somatic DNA sequence analysis of this gene in tumours is a valid approach for the diagnosis of both HPT-JT and sporadic parathyroid carcinoma. Despite the presence of mutation ''hot-spots'' in exons 1, 2, and 7 of HRPT2 where approximately 80% of all mutations occur[4;9;11], the time and resources for molecular analysis of *HRPT2* are beyond the means of most surgical pathology laboratories. We and others[5;9;13] showed the absence or reduced staining of parafibromin in sporadic and HPT-JT carcinomas. Conversely, two recent studies[5;13] have shown that negative parafibromin immunostaining is almost invariably associated with *HRPT2* mutations and confirm that loss of parafibromin staining strongly predicts parathyroid malignancy. A point to remember however is that HPT-JT adenomas might also show

reduced staining possibly indicating their potential to progress into carcinomas.[9;13] Also, additional information is needed regarding the reproducibility and the use of parafibromin in atypical adenomas/equivocal carcinomas in order to predict possible clinical behaviour.[20] Despite this, parafibromin testing seems to be a promising molecular marker for the diagnosis of parathyroid carcinoma. However, an exceptionally positive staining for parafibromin could still be compatible with *HRPT2* mutation in the case of missense mutations, for example. In addition, we have shown that molecules such as APP, E-cadherin, CASR might play a role in *HRPT2* driven tumourigenesis. Immunohistochemical analysis of APP, E-cadherin and CASR (i.e. strong cytoplasmic staining of APP, irregular membranous staining or deposits/ droplets in the cell of E-cadherin and absence of clear membranous staining of CASR) might give circumstantial evidence to support the diagnosis of malignancy. There is no role for *MEN1* mutation testing in parathyroid tumours suspected for malignancy since parathyroid adenomas often show somatic mutations of *MEN1* together with loss of the wild-type allele.

Future perspective

There are still several aspects of parathyroid disease requiring further investigation: Can biomarkers be identified that can be used for molecular imaging of (abnormal) parathyroid glands? Such biomarkers might be highly expressed membrane bound molecules specific for parathyroid tissue. Although parathyroid carcinoma is a rare disease, in individual cases the disease can take a dramatic course. For such cases, the identification of specific parathyroid tumourigenesis pathways that can be targeted by designer molecules might be crucial. A third issue that should be addressed concerns the switch from secondary to tertiary hyperparathyroidism. What are the molecular switches that lead to such autonomous behaviour of an individual parathyroid gland? Only such insights might lead to the finding of novel therapies.

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