Karolinska Institutet

Institutionen för Onkologi-Patologi

Genetic and Epigenetic Mechanisms in Primary Hyperparathyroidism

AKADEMISK AVHANDLING

som för avläggande av medicine doktorsexamen vid Karolinska Institutet offentligen försvaras i Radiumhemmets föreläsningssal, plan 01, Karolinska Universitetssjukhuset, Solna

Fredagen den 11 januari 2013, kl 09:00

av

Luqman Sulaiman

M.D.

Huvudhandledare:

Jamileh Hashemi, PhD
Inst. för Onkologi-Patologi
Karolinska Institutet, Stockholm, Sweden

Bihandledare:

Professor **Catharina Larsson**,
Inst. för Onkologi-Patologi
Karolinska Institutet, Stockholm, Sweden

Associate professor **Inga-Lena Nilsson**,
Inst. för molekylär medicin och kirurgi
Karolinska Institutet, Stockholm, Sweden

Christofer Juhlin, PhD
Inst. för Onkologi-Patologi
Karolinska Institutet, Stockholm, Sweden

Fakultetsopponent:

Professor **Per Hellman**
Inst. för kirurgiska vetenskaper, Endokrinkirurgi
Uppsala universitet, Uppsala, Sweden

Betygsnämnd:

Professor **Peter Zaphiropoulos**
inst. för biovetenskaper och näringslära
Karolinska Institutet, Stockholm, Sweden

Docent **Ove Törring**
Inst. för klinisk forskning och utbildning
Karolinska Institutet, Stockholm, Sweden

Docent **Lars Feuk**
Inst. för immunologi, genetik och patologi
Uppsala universitet, Uppsala, Sweden

Stockholm 2013

ABSTRACT

Primary hyperparathyroidism (PHPT) is a common endocrine disorder characterized by abnormally excessive secretion of parathyroid hormone (PTH) and elevated serum calcium. PHPT patients can develop a wide range of complications affecting many body organs such as the skeleton, kidneys and heart. In the majority of patients, PHPT is due to a solitary adenoma and less frequently due to multiglandular disease. Very rarely PHPT is caused by a parathyroid carcinoma.

This thesis aimed at a better understanding of the genetic as well as epigenetic mechanisms involved in this disease in order to improve future patients management.

In **study I** we have investigated large parathyroid adenomas (≥ 4 grams) and detected frequent *MEN1*, but rare *HRPT2*/*CDC73* mutations and low MIB1 proliferation index. The majority of the tumors had loss of parafibromin expression and positive APC expression. Furthermore, gain of chromosome 5 was the most unique and frequent copy number alteration detected in this group, while very rarely detected in unselected adenomas. We concluded that a subset of large parathyroid adenomas have distinct genetic profile and pronounced clinical features reflected by significantly higher serum calcium.

In **study II** we examined the role of constitutional *APC* mutations in parathyroid tumors from two patients with *APC* mutation-associated familial colorectal cancers. Pathological revision confirmed the benign nature of both tumors. None of them had somatic mutations or DNA copy number alterations of the *APC* gene and both tumors displayed strong APC and parafibromin expression with low MIB index. Although the *APC* 1A promoter was hypermethylated, promoter *APC* 1B was unmethylated and this was consistent with normal *APC* mRNA expression. Our results supported the benign nature of the parathyroid tumors and excluded a possible association between constitutional *APC* mutations and parathyroid tumorigenesis.

In **study III** we defined the molecular cytogenetic profile of *CDC73/HRPT2*-mutated parathyroid tumors. All the carcinomas displayed frequent DNA copy number losses on chromosome 1p and 13 while the adenomas did not display any significant alterations. All the carcinomas were diploid at the *CDC73* gene locus, but three adenomas had loss at this locus. The *CDC73* promoter was unmethylated in all the tumors. The carcinomas displayed more loss of heterozygosity (LOH) events than the adenomas, and two carcinomas had LOH at the *CDC73* locus. These results suggest that *CDC73*-mutated parathyroid adenomas exhibit a partly unique cytogenetic profile in addition to that of carcinomas and unselected adenomas.

In **study IV** analyses of promoter methylation status in a panel of benign and malignant parathyroid tumors revealed frequent hypermethylation of *APC* 1A, *β-catenin* and *RASSF1A* promoters in the adenomas which correlated with reduced mRNA expression. Parathyroid carcinomas were hypermethylated for *APC* 1A and exclusively for *SFRP1*. No changes in global methylation could be detected and tumor groups with known *MEN1* or *CDC73* mutations did not display different methylation profile. We concluded that aberrant promoter methylation of *APC* 1A, *β-catenin*, *RASSF1A* and *SFRP1* can play a role in parathyroid tumorigenesis and hypermethylation of *SFRP1* can act as a potential epigenetic marker for parathyroid carcinomas.

ISBN 978-91-7549-009-0