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Alterations of DNA methylation in parathyroid tumors

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ABSTRACT

Parathyroid tumors are common endocrine neoplasias associated with primary hyperparathyroidism, a metabolic disorder characterized by parathormone hypersecretion. Parathyroid neoplasia are frequently benign adenomas or multiple glands hyperplasia, while malignancies are rare. The epigenetic scenario in parathyroid tumors has just begun to be decoded: DNA methylation, histones and chromatin modifiers expression have been investigated so far. The main findings suggest that DNA methylation and chromatin remodeling are active and deregulated in parathyroid tumors, cooperating with genetic alterations to drive the tumor phenotype: the tumor suppressors menin and parafibromin, involved in parathyroid tumorigenesis, interact with chromatin modifiers, defining distinct epigenetic derangements. Many epigenetic alterations identified in parathyroid tumors are common to those in human cancers; moreover, some aspects of the epigenetic profile resemble epigenetic features of embryonic stem cells. Epigenetic profile may contribute to define the heterogeneity of parathyroid tumors and to provide targets for new therapeutic approaches.

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Abbreviations: MEN1, multiple endocrine neoplasia type 1; HPT-JT, hyperparathyroidism- jaw tumors; CpG, cytosine phosphate guanine; 5hmc, 5-hydroxymethylcytosine; TET1, tet (ten-eleven-translocation) methylcytosine dioxygenase 1; PTH, parathormone; CASR, calcium-sensing receptor; VDR, vitamin D receptor; HIC1, hypermethylated in cancer 1; EZH2, enhancer of zeste homolog 2.

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1. Introduction

Parathyroid tumors are frequently occurring endocrine neoplasias, often associated with clinical features of primary hyperparathyroidism (PHPT). Parathyroid tumors are mainly benign parathyroid cell proliferations, presenting as single or multiple adenomas and diffuse hyperplasia, while parathyroid cancers are rare. Parathyroid adenomas are heterogeneous ranging from small, poorly active, in term of parathormone (PTH) release, lesions to large, clinically aggressive displaying histological features similar to malignancy. These latter neoplasms are identified as atypical adenomas, whose risk of recurrence and/or metastasis is yet unclear but certainly very small (Guarnieri et al., 2012). Parathyroid tumors differ from classical carcinogenesis as they do not show progression from benign to malignant lesions and precancerous lesions have not been identified.

The genetic background of parathyroid tumors is mainly characterized by loss of two oncosuppressor genes: loss of menin, the protein encoded by *MEN1* gene, and loss of parafibromin, encoded by *CDC73/HRPT2* gene, occur in parathyroid lesions of the multiple endocrine neoplasia type 1 (MEN1) syndrome and Hyperparathyroidism-Jaw Tumor (HPT-JT) syndrome, respectively. *MEN1* gene mutations also occur in about one third of sporadic parathyroid adenomas, while somatic mutations of *CDC73* gene are found in up to 70% of sporadic parathyroid cancers (Shattuck et al., 2003).

The heterogeneity of the parathyroid tumors biological and clinical presentation may be defined by epigenetic deregulation. Epigenetic modifications at both the DNA and chromatin levels are involved in embryogenesis and cell fate reprogramming, modulating the two-way relationship between transcription factor binding and chromatin structure (Nashun et al., 2015) and are present in all human cancers, where cooperate with genetic alterations to drive the cancer phenotype (Baylin and Jones, 2016). Reversibility of these changes makes them targets for therapeutic manipulations, and a number of small molecules targeting chromatin-based mechanisms are currently in clinical trials (Jones et al., 2016). Epigenetic gene regulation is achieved through the collaboration of multiple regulatory pathways involving sequence-specific DNA-binding transcription factors, ATP-dependent nucleosome remodeling, long non-coding RNAs ad DNA methylation.

Here, the DNA methylation and chromatin modifications described in parathyroid tumors are reviewed (Fig. 1). MicroRNAs have also been investigated in parathyroid tumors and data have been recently reviewed (Vaira et al., 2016), while data about other epigenetic regulation are still lacking.

2. Global DNA methylation in parathyroid tumors

The aberrant methylation of cytosine phosphate guanine (CpG) islands in genes promoter has been widely investigated in cancer and found to be mainly correlated with a loss of gene expression as an alternative mechanism to gene deletion or mutation for the loss of tumor suppressor gene function. As a consequence, the epigenetic silencing can modulate several cellular processes, such as cell division, metastasis, cell survival and angiogenesis, all of which are responsible for promoting tumor development (Baylin and Chen, 2005). Moreover, promoter epigenetic silencing has been frequently correlated with prognosis in many solid tumors and suggested as a possible improvement of the clinical course prediction beyond standard staging (Sandoval et al., 2013; Muscarella et al., 2011). In benign and malignant parathyroid tumors, despite the clear usefulness to perform large scale DNA methylation analysis, few studies were published to date describing the global aberrant methylation at CpG genes promoter islands.

Global promoter methylation by LINE-1 in parathyroid tumors did not differ from that detected in normal glands (Juhlin et al., 2010; Sulaiman et al., 2013) (Table 1), differing from cancers, which often display global DNA hypomethylation compared to normal tissue.

Different CpG sites located near the promoter regions of more than 14 000 predicted genes were screened by Starker and Collaborators using an interesting model cohort composed of normal, benign and malignant parathyroid tissues (3 normal parathyroid tissues, 14 adenomas and 7 carcinomas). A gradient of CpG hypermethylation level from normal tissues to adenomas and carcinomas was identified in a subset of genes involved into key pathways linked to parathyroid tumors. Specific genes such as *RIZ1*, *APC*, *RASSF1A*, *CDKN2A/p16* and *CDKN2B/p15*, *RB1*, *WT1*, *GATA4*, *PYCARD*, *SFRP1*, *SFRP2* and *SFRP4* were found hypermethylated, in line with the hypothesis that the cell cycle and transcription along with the WNT pathway would represent biological processes deranged in the onset of the parathyroid neoplasm (Starker et al., 2011).

The consistent involvement of DNA methylation of CpG islands in parathyroid tumorigenesis was more recently confirmed by measuring the 5-hydroxymethylcytosine (5hmC) and the expression level of its catalyzing conversion enzyme tet (ten-eleven-translocation) methylcytosine dioxygenase 1 (TET1) (Table 1). Both enzymes, TET1 and TET2, were previously reported as deregulated in many cancers and represent an alternative marker to study the epigenetic modifications (Rawłuszko-Wieczorek et al., 2015; Tahiliani et al., 2009). For many cancers, aberrant genome-wide patterns of cytosine DNA methylation in CpG dinucleotides distinguish tumor from normal tissue and contribute to disease progression by altering the transcriptome. In a systematic analysis of 17 parathyroid carcinomas compared with 43 benign adenomas, 5hmC was specifically associated with malignancy (100% of carcinomas, 0% of adenomas), while loss of TET1 expression, detected by RTqPCR and immunohistochemistry, was variable and heterogeneous. Therefore, the involvement of TET2 and other alternative mechanisms were hypothesized in the modulation of 5hmC (Barazeghi et al., 2016).

A number of open questions remain about the opportunity to perform additional large-scale methylation analysis in parathyroid tumors by using new commercially available and denser Methyl Arrays. Consequent validation of high-throughput data related to the new identified CpG islands are demanded by using a more specific technical approach as pyrosequencing and/or bisulphite DNA sequencing to better identify the CpGs of the islands specifically related the gene expression modulation. Open questions are mainly related to the difficulties to access the rare parathyroid carcinoma samples and, moreover, to collect suitable normal parathyroid samples for the comparative studies. It should be noted that due to ethical considerations, samples of normal parathyroid glands from normocalcemic patients are only occasionally obtained by inadvertent removal during thyroid surgery, an event that is inversely related to the experience of the operating surgeon. Normal parathyroid tissue obtained from hypercalcemic patients during parathyroidectomy for parathyroid tumors might not be epigenetically “normal” since they are influenced by hypercalcemia.

Moreover, the role of other CpGs located inside the genes remains almost uninvestigated in parathyroid tumors as well as in many other solid tumors. Intragenic DNA methylome are widely

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