



Oxyphilic carcinoma of the thyroid gland

Karmen STANKOV
Giovanni ROMEO

DIPARTIMENTO DI MEDICINA INTERNA, CARDIOANGIOLOGIA ED
EPATOLOGIA, UNITA' OPERATIVA DI GENETICA MEDICA,
POLICLINICO S. ORSOLA - MALPIGHI, BOLOGNA, ITALY

Oxyphilic tumors of the thyroid gland are rare tumors characterized by the presence of Hürthle cells - mitochondrion-rich, eosinophilic epithelial cells. Hürthle cell carcinomas (HCC) of the thyroid behave in a more aggressive fashion as compared to other well-differentiated thyroid cancers. Many recent studies have been focused on the further elucidation of pathogenesis and the role of mitochondrial hyperplasia in carcinogenesis of these neoplasms. The importance of combining morphological and genetic approaches in the study of HCC has been emphasized by the difficulties encountered in establishing adequate differential diagnostic criteria between benign and malignant forms, as well as by the resistance of HCC to radio and chemotherapy. It has been well documented that the Hürthle cells are characterized by profound aberrations in the nuclear and mitochondrial genome and by alterations in oncogenes, tumor suppressor genes and other key genes involved in energy metabolism, proliferation and apoptosis.

KEY WORDS: Adenocarcinoma; Thyroid Neoplasms; Adenoma, Oxyphilic; Chromosomes; Genetics; Mitochondria; Apoptosis

INTRODUCTION

The definition and natural history of Hürthle cell carcinoma (HCC) is not well understood. This is largely attributable to the rarity of disease since Hürthle cell tumors represent less than 5% of all thyroid malignancies (1,2).

Hürthle cells (Askanazy cells, oxyphilic cells) are thyroglobulin-producing, mitochondria-rich, thyroid epithelial cells. They are found in a variety of thyroid conditions, including Hashimoto thyroiditis, Graves's disease, nodular goiter, and thyroid neoplasms. Oxyphilic features have also been described in parathyroid, pancreatic, hepatic, renal, pituitary and salivary gland tissue (3).

The term *Hürthle cell tumor*, which is widely used in the pathology literature, designates oncocytic neoplasms in the thyroid gland. Oncocyte (from the Greek word *onkoustai*, to swell) is the generally accepted term for those cells exhibiting the characteristic phenotype, featuring a finely granular eosinophilic cytoplasm in histology sections and an increase in the number of mitochondria ultrastructurally. This increase is responsible for the swollen

appearance of oncocytic cells.

PATHOLOGICAL AND CLINICAL SPECIFICITIES OF HÜRTHLE CELL CARCINOMAS

Oncocytic neoplasms in the thyroid gland represent a distinct subtype within the group of follicular tumors, according to the World Health Organization Committee for the Histological Typing of Thyroid Tumors (4). In 1951, the American Cancer Society, recommending the classification of all thyroid tumors with oncocytic features as malignant, discarded the terms *Hürthle cell tumors* and *Hürthle cell adenoma*, and applied the term *Hürthle cell carcinoma* in order to emphasize their malignant nature. Since then, there has been a disagreement on its malignancy potential and, therefore, its prognosis and treatment. Many authors (1-3,5) confirmed the opinion that morphologic differentiation between benign and malignant Hürthle cell tumors is possible and that biological behavior correlates well with pathologic features. According to a more recent classification (7), Hürthle cell tumors are defined as being composed of at least 75% Hürthle cells and are classified as benign (Hürthle cell adenomas [HCA]) or malignant (Hürthle cell carcinomas [HCC]) (4). These two entities are distinguished based on the identification of capsular or vascular invasion, or on the presence of metastatic disease (5-7). Predicting the clinical behavior of HCC and therefore selecting appropriate treatment is controversial. Reports in the literature vary with regard to the clinical behavior of the HCC. Some authors

Address correspondence to:

Dr. Karmen Stankov, Dipartimento di Medicina Interna, Cardioangiologia ed Epatologia, Unita' Operativa di Genetica Medica, Policlinico S. Orsola - Malpighi, Pad. 11, via Massarenti 9, 40138 Bologna, Italy, E-mail: stankov@med.unibo.it

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believe that HCC behave in the clinically indolent fashion, similar to other well-differentiated thyroid cancers, while others believe them to be very aggressive (8,9). It is important to recognize that the overall mortality rate of oncocytic carcinoma appears to be considerably higher than that of papillary or follicular carcinoma. It is not known whether the increased mortality is related to the intrinsic biological and proliferative properties of oncocytic carcinomas or to decreased competence in iodine 131 uptake (3).

The behavior of apparently benign Hürthle cell tumors is equally confusing. Several authors have reported tumors initially diagnosed as adenomas that later recurred or metastasized. Unfortunately, the inability to accurately predict the clinical behavior of Hürthle cell tumors has led some authors to advocate aggressive surgical treatment for all Hürthle cell tumors (3,11).

Recent critical histopathological appraisal of HCC demonstrates that morphology is a powerful predictor of the clinical outcome (11). Many clinical and pathological criteria were applied to predict the clinical behavior of HCC. Nuclear atypia, the number of mitoses, cellular pleomorphism, necrosis, and percentage of Hürthle cells, have been shown to be of little significance (9). In the study of Stojadinovic et al. (1), patients were stratified according to degree of invasion into low- and high-risk groups. Significant predictors of outcome of HCCs are the extent of invasion, size greater than 4 cm, extra thyroidal extension, and initial nodal or distant metastasis. These criteria support an individualized treatment approach based on extent of invasion.

Fine needle aspiration (FNA) cytology is the most accurate diagnostic test in the evaluation of thyroid nodules. However, FNA cannot distinguish benign from malignant follicular or Hürthle cell neoplasms. Furthermore, intraoperative frozen section evaluation rarely yields additional useful information for the differential diagnosis of these neoplasms. The diagnosis of carcinoma requires the histological documentation on permanent section of tumor invasion, either into blood vessels or beyond the tumor capsule. In many cases, tumor invasion is a focal finding and apparent only upon careful analysis of multiple histological sections. Complicating this clinical dilemma, optimal surgical management of adenomas vs. carcinomas differs significantly; adenomas can be treated with lobectomy, whereas patients with carcinoma may benefit from total thyroidectomy. Since a definitive diagnosis can rarely be made, either pre- or intraoperatively, patients with adenomas may undergo surgery that is more extensive than necessary, and conversely, patients with carcinomas may receive less than adequate surgery (11).

Lobectomy alone is adequate therapy for low-risk patients (1). Total thyroidectomy is recommended for the high-risk patients to allow follow-up and detection of recurrent disease. Adjuvant therapy options are limited, but it would seem prudent to consider external beam radiotherapy for locally advanced cancers with

or without nodal metastases. Because most HCCs do not concentrate radioiodine, ablative doses of radioactive iodine have not been effective in salvage treatment of patients with locoregional or distant disease recurrence. Current chemotherapy for advanced disease is poor, but it may be considered for the high-risk patients in the setting of a controlled clinical trial. Octreotide has been ineffective for the treatment of metastatic HCC. The significant risk of relapse with high-risk HCC (73%) and the lack of effective adjuvant local or systemic therapies provide an adequate basis for an aggressive initial surgical approach to widely invasive carcinomas (1).

Until the identification of the Hürthle cell variant of papillary thyroid carcinoma (HCPTC), there was no adequate explanation as to why some Hürthle cell tumors developed lymph node metastases, particularly in tumors that were morphologically classified as Hürthle cell adenomas (8). Until recently, the diagnosis of HCPTC was based on the presence of papillary architecture and not on the characteristic nuclear features of this entity. The recognition of the diagnostic nuclear features of papillary carcinoma is confounded by the fact that they are often obscured by the nuclear hyperchromasia that accompanies Hürthle cell metaplasia. Because RET/PTC gene rearrangements are specific to PTCs, the diagnosis of Hürthle cell PTC (HCPTC) has recently been expanded to include those tumors that lack papillary architecture but harbor a RET/PTC gene rearrangement (5,8).

GENETIC PREDISPOSITION TO NONMEDULLARY THYROID CANCER

The identification of genes that place individuals at high risk of breast, ovarian, and colorectal cancer has greatly advanced our understanding of cancer predisposition over the past decade (12, 13). All cancers are genetic in origin because they arise from mutations in a single somatic cell, but the genetic changes in sporadic cancers are confined to a particular tissue. In inherited cancers, a predisposing mutation is present in all somatic cells and in the germ line, which enables the transmission of risk to the next generation. Cancer genetics offers a model of how information on the genetics of inherited cancers could affect identification of individuals at increased genetic risk (14,15).

Families with recurrence of nonmedullary thyroid cancer (NMTC) have been repeatedly reported in the literature (16-20) and the proportion of these familial nonmedullary thyroid carcinomas (FNMTC [MIM 188550]) is estimated to be 3%-7% of all thyroid tumors. Thyroid cancers range in their biological behavior from a most indolent growth pattern (occult thyroid cancer) to the most lethal cancer (anaplastic thyroid cancer). Currently, the definition of familial NMTC is based on having two first degree relatives affected by thyroid cancer without other familial syndromes. Familial NMTC is a clinical entity characterized by a phenotype

more aggressive than that of its sporadic counterpart and it almost exclusively includes patients with papillary or Hürthle cell cancers (21). Most published pedigrees are compatible with inheritance of one autosomal dominant gene with reduced penetrance, but polygenic inheritance cannot be excluded. The Utah Population Database resource was used to systematically study familial clustering of 28 distinct cancer site definitions among first-degree relatives of cancer probands. The highest familial risk (8.60) was found for thyroid tumors, a finding which is in favor of an important role of genetic predisposition in the etiology of NMTC (22). Therefore, the International Consortium for Study of Genetic Susceptibility to NMTC has been established in 1996. This Consortium comprises 48 centers in 13 different countries, mainly in Europe, and includes Australia, USA, Canada, Israel, Morocco and Argentina. With the collaboration of clinicians and pathologists, and with the support of patient families, 720 blood samples and clinical information from 261 families were collected. DNA samples have been prepared from NMTC pedigrees and 305 lymphoblastoid cell lines have already been established. Tumor tissue and its normal counterparts from 150 samples of sporadic cases of NMTC have also been obtained from the patients of the members of the Consortium. This is, to our knowledge, the largest existing collection of samples from NMTC families and provides the most important basis for studies aiming at the identification of the genes of the major responsibility for predisposition to NMTC. The genes for familial papillary thyroid carcinoma are yet to be identified, whereas that for Hürthle cell cancer TCO (thyroid tumors with cell oxyphilia) has been previously mapped to chromosome 19p13.2 (MIM accession number 603386) (23, 24). An extensive genome wide scan in the study of McKay et al. (25) of a large Tasmanian pedigree with a follicular variant of papillary thyroid carcinoma (fvPTC) revealed a common haplotype on chromosomal region 2q21 (MIM 606240) in 7 of the 8 patients with fvPTC.

MITOCHONDRIAL GENOME

Mitochondrial abnormalities have been described in many thyroid tissues (26). It is known that rapidly growing cancer cells have an increased glycolytic rate (27). Tumor cells have been associated with changes in mitochondrial size, number, distribution, morphology, membrane lipid composition, membrane potential, loss of electron transport components, deficiencies in energy-related functions, and impaired protein synthesis (28).

Mammalian cells possess two different and interdependent genomes, which comprise a dual genetic system. The diploid (2N) nucleus of a human cell contains approximately 6 billion base pairs, whereas mitochondria contain a 16,569 base pair genome repeated in 100-1000 copies per cell. Mitochondrial genomes (mt-genomes) are short circular molecules that, with

the exception of viruses, represent the most economically packed forms of DNA in the whole biosphere. Mitochondria are semi-autonomously functioning organelles, which contain a resident genome, and replicate, translate and transcribe their own DNA. Mitochondria are responsible for generating approximately 90% of cellular adenosine triphosphate (ATP) through the process of oxidative phosphorylation. Mitochondrial DNA (mtDNA) comprise 0.1-1.0% of the total DNA in most mammalian cells. Each organelle contains 2 to 10 copies of mtDNA molecules, and each human cell contains more than 100 copies of mtDNA. Mutated mtDNA molecules and wild-type mtDNA molecules can coexist in the same cell, tissue or organ in a state called heteroplasmy (29). The supercoiled, double-stranded circular molecule, which represents human mtDNA, contains 37 genes coding for the 13 polypeptides of the mitochondrial respiratory chain, 22 tRNAs and 2 rRNAs necessary for synthesis of the polypeptides. The electron respiratory chain lies within the inner mitochondrial membrane and produces ATP by oxidative phosphorylation. The oxidative phosphorylation system consists of five multi-subunit enzymatic complexes, which assemble the gene products of approximately 74 nuclear genes and the 13 mitochondrial genes (29).

The majority of proteins in mitochondria are encoded by the nuclear genome, and intergenomic communication is necessary for mitochondrial synthesis and function. The oxidative phosphorylation (OXPHOS) activity occurs within the mitochondrial environment; these organelles are to a large extent the "power plant" of the cell (30). Under normal physiological conditions, a small fraction of the oxygen consumed by mitochondria is converted to superoxide anions, H_2O_2 and other reactive oxygen species (ROS). Mitochondria are the major intracellular source and primary target of ROS, which are generated under normal conditions as by-products of aerobic metabolism in animal and human cells. It has been established that defects in the respiratory chain lead to enhanced production of ROS and free radicals in mitochondria. In addition, H_2O_2 has been proposed to be involved in signal transduction pathways. Recently H_2O_2 has been proposed to be involved in the communication between mitochondria and the nucleus. The mtDNA copy number has been suggested to be increased by a feedback mechanism that compensates for defects in mitochondria harboring mutated mtDNA and a defective respiratory system (31).

The mitochondrial genome is more vulnerable to oxidative damage and undergoes a mutation rate ten times higher than in the nuclear genome (31). The factors known to be needed for mammalian mtDNA maintenance are all encoded by nuclear genes and transported into the mitochondria. The DNA processing enzyme activities are dependent on several factors, including deoxynucleotide triphosphates (dNTP) concentrations within the mitochondria, the availability of ATP, and several metal cofactors (32,

33). Tumor formation is often associated with mtDNA mutations and alterations in mitochondrial genomic function. Mitochondrial aberrations have been identified in cancer of the bladder, breast, colon, thyroid gland, head and neck, kidney, liver, lung, stomach and in the hematologic malignancies. Altered expression and mutations in mtDNA-encoded complexes I, III-V, as well as mutations in the hypervariable regions of mtDNA comprise some of the mitochondrial genomic aberrations found in cancer tissue. Findings of mtDNA mutations in tumor cells are consistent with reports that tumor cells are subjected to constitutive oxidative stress (28,30).

Mitochondrial DNA is thought to be more susceptible than nuclear DNA to mutagen-induced damage for several reasons: mtDNA polymerase γ replicates the DNA with poor fidelity, mtDNA is a molecule without histones to which chemical carcinogens can easily bind, and mtDNA is particularly susceptible to the high concentration of ROS in mitochondria (34,35). ROS are generated in mitochondria of normal mammalian cells as a by-product of normal respiration and in other subcellular localizations as a function of biochemical reactions using oxygen. At high levels, ROS are toxic to the cell, but at low levels, ROS have physiological functions, including the activation and modulation of signal transduction pathways, the modulation of activities of redox-sensitive transcription factors, and regulation of mitochondrial enzyme activities (34-36). It has been demonstrated, both *in vivo* and *in vitro* that ROS are involved in all stages of tumorigenesis: initiation, promotion and progression (37,38).

CYTOGENETICS OF HÜRTHLE CELL TUMORS

Great attention has been paid in recent years to combining morphological and genetic characteristics in oxyphilic tumors and to the further elucidation of pathogenesis of these neoplasms. DNA content profiles after flow cytometry are commonly abnormal. Thyroid oncocyctic neoplasms, including histologically benign tumors, are often aneuploid or polyploid (39, 40), but the demonstration of aberrant DNA content does not help in differentiating adenomas from carcinomas (40). A comparative genomic hybridization study detected numerical aberrations in Hürthle cell tumors in a higher percentage (81%) than that reported by conventional cytogenetic analysis (39). This study showed the presence of multiple chromosomal gains with polysomy of chromosomes 5 and 7 plus extra copies of other chromosomes, usually chromosomes 12, 17, 19 and 20. However the most prominent finding has been an association between loss of chromosome 2 and aggressive forms of thyroid cancer characterized histologically by a poorly differentiated phenotype. It may be speculated that those oncocyctic neoplasms with loss of chromosomal DNA and/or chromosome 2 monosomy are at greater risk of progressing and acquiring a clinically aggressive behavior. As suggested

by the results of Tallini et al. (39), showing frequent numerical aberrations in the oncocyctic tumors, it was further speculated that the aberrant accumulation of mitochondria in these neoplasms might represent an obstacle to the correct segregation of chromosomes during mitosis and predispose the tumors to selective chromosomal gains and losses (3).

Both Hürthle cell carcinomas and adenomas frequently show chromosome copy number changes. A recent study (41) using interphase fluorescence in situ hybridization (FISH) analysis has shown chromosome 7 to be one of the most common chromosome gains in HCC and HCA. Frequent abnormalities included a variety of gains either of chromosomes 7 and 12, or 5 and 7, or gains of all three chromosomes, suggesting a variety of pathways to Hürthle cell tumorigenesis. Chromosome losses were identified only in the tumors of patients who died of the disease. Chromosome 22 was the most common loss identified and may have a prognostic value in HCC of the thyroid (41).

The study using the In Situ End-Labeling (ISEL) technique (42) showed a very high occurrence of nuclear DNA fragmentation in Hürthle cell tumors with a parallel absence of immunoreactivity for activated caspases. Peritumoral thyroid and oxyphilic nonmalignant lesions as well as nonoxyphilic benign and malignant tumors, showed a focal pattern of DNA fragmentation in a lower percentage of cases. This peculiar genomic DNA fragmentation pattern in Hürthle cell tumors may be the consequence of or even the key to a rigid response of oxyphilic tumors to ischemic stimuli, leading to a necrotic rather than an apoptotic response to stress conditions (42).

Somatic mtDNA mutations have also been described in Hürthle cell tumors (28,38,43). The compensatory increase of abnormal mitochondria that occurs in Hürthle cells is greatly facilitated by the increased replication rate of mitochondria with deletions and/or mutations over that of normal mitochondria. A high percentage (nearly 100%) of Hürthle cell tumors display the mitochondrial common deletion and/or somatic mitochondrial point mutations, probably because of the high susceptibility of mtDNA to damage by ROS and mutagens (28). Another study performed by these authors shows that every Hürthle cell tumor displays a relatively high percentage (up to 16%) of mtDNA common deletions, while sequence variants of the ATPase 6 gene are significantly more prevalent in patients with Hürthle cell tumors (38). Homoplasmic mtDNA mutations may reflect a replicative advantage for the cells containing certain mtDNA mutations and/or tumorigenic properties of mtDNA mutations. The hypothesis proposed by another study (43) suggests that missense mutations found in Hürthle cell mtDNA represent damage from oxidative stress, which induces further alterations in OXPHOS and increased production of ROS. In other words, cause and effect cannot be separated at present (43). An interesting additional

finding is that no mechanism related with nuclear mismatch repair genes has been found to be active in human mtDNA repair to date (28), although the repair of strand breaks and alkali-sensitive sites has been demonstrated in rodent and human mtDNA (32).

In the studies on Hürthle cell lesions of thyroid (43) and Warthin's tumors (44), it has been shown that mtDNA deletions are present in the normal epithelial cells of thyroid and parotid gland, respectively, prior to oxyphilic change and tumor development. The 4977-base pair common deletion of mtDNA has been documented also in Hürthle cells of Hashimoto's thyroiditis, as well as in oncocytic lesions of the parathyroid and kidney, and in thyroid tumors without Hürthle cell features. This supports the conclusion that in some cases mtDNA deletions precede tumor development (38).

MOLECULAR CHARACTERISTICS OF HÜRTHLE CELL TUMORS

Increases in mitochondrial content and mtDNA synthesis have been observed to occur in the absence of nuclear DNA replication. The alteration of mitochondrial mass and mtDNA in the cell might be uncoupled from the cell-cycle-controlled biosynthesis of chromosomal DNA. Human lung fibroblasts treated with cell-cycle-arresting drugs show similar amplitude of increase of the mitochondrial mass as those treated with H₂O₂. These results may imply that while overall cell division is arrested, mitochondrial biogenesis is unaffected and mitochondria continue to proliferate, indicating that mild oxidative stress may induce an increase in mitochondria and mtDNA via the pathway that bypasses cell-cycle control (45). These authors have reported that mtDNA copy number is increased in the lung tissues of elderly human subjects. In addition, mtDNA mutation, oxidative DNA damage and lipid peroxidation are increased during aging in human cells (35). In light of these results, it is important to emphasize that oxyphilic cells with an accumulation of mitochondria occur in parenchymas and in tumors with low proliferative index and reduced turnover, i.e. in stable cells with a very long intermitotic interval, in contrast to the cells of the digestive and respiratory tracts and of their tumors, which divide too quickly or die/desquamate too soon to allow the accumulation of abnormal mitochondria, which is the hallmark of oxyphilic cells (28).

This observation is in accordance with the recent results of Volante et al. (46), showing that Hürthle cell tumors have a low proliferative index, as detected by Ki-67 immunostaining. In the same tumor samples they have found a surprisingly high mean expression of E2F-1 (46), the member of the transcription factor family involved in developmental, tumorigenic and apoptotic processes (47). E2F-1 is not overexpressed in nonneoplastic cells having oxyphilic changes, such as nodular goiter or Hashimoto thyroiditis, indicating that E2F-1 overexpression seems to be the result of a particular metabolic status of these

malignant cells, rather than related to the morphological oxyphilic changes. Since mitochondria are necessary for apoptosis initiation, their frequent alterations in oxyphilic tumors at both mtDNA and enzymatic levels (43,48,49) could explain why the possible death signal triggered by E2F-1 could give rise to apoptotic processes rather than to true apoptosis in these tumors.

E2F-1 has been shown to function as both an oncogene and a tumor suppressor in a tissue specific manner. In *in vivo* studies, the loss of E2F-1 reduces the frequency of pituitary and thyroid tumors, and greatly lengthens the lifespan of Rb-1(+/-); E2F-1(-/-) mice, demonstrating that E2F-1 is an important downstream target of pRB during tumorigenesis (50).

Apoptosis is a highly complex biochemical process that may differ according to the cell or tissue model and the pro-apoptotic signal-transducing pathway that is activated (51). In normal human fibroblasts, Esteve et al. (52) showed that during the apoptotic process, the mitochondria from apoptotic tissues show oxidative damage of mtDNA and lower membrane potential than controls. Most of the current data are compatible with the notion that the inner mitochondrial membrane transmembrane potential ($\Delta\psi_m$) decrease constitutes an irreversible event of the apoptotic process regulated by members of the Bcl2/Bax family. An inhibitor of apoptosis, Bcl-2, exerts its action by reducing the production of ROS in neurons (51).

The overexpression of cyclin D1 may contribute to cell proliferation and to subsequent mutations of the tumor suppressor gene p53, by facilitating the entrance of quiescent cells into and their passage through the cell cycle. Cyclin D1 forms a multimer complex with cyclin-dependent kinase (CDK), which facilitates the transition through the restriction point of the cell cycle by inactivation of the retinoblastoma tumor-suppressor protein (pRb) as a result of its hyperphosphorylation by CDK. Hyperphosphorylation of pRb leads to the release of E2F transcription factors and other proteins from their complexes with pRb, which activates genes coding for positive regulators of cell proliferation. The degree of cyclin D1 expression was significantly higher in the columnar tall cell variant of papillary carcinoma, compared to both conventional papillary thyroid carcinoma and minimally invasive follicular carcinomas (53-55). In the study of Erickson et al. (56) it has been shown that diffuse cyclin D1 protein expression is more common in Hürthle cell carcinomas as compared with HCA.

The series of Hürthle cell neoplasms examined in the study of Anwar et al. (57), lacked expression of Rb-1 in both benign and malignant lesions, including the absence of Rb-1 nuclear immunoreactivity in Hürthle cell metaplasia in the case of Hashimoto's thyroiditis. This staining pattern for Rb-1 in Hürthle neoplasms suggests that mechanisms other than Rb alteration are involved in this category of thyroid neoplasia, similar to many other biological and molecular aspects that distinguish this sub-

set of thyroid neoplasms.

The study of Hoos and Stojadinovic et al. (58) demonstrates that tissue microarray-based profiling allows identification of molecular markers that are associated with patient prognosis. Stratification of Hürthle cell neoplasms based on capsular and/or vascular invasion revealed a good correlation between molecular phenotype and clinical outcome, showing that the Ki-67(+), Bcl-2(-) phenotype was significantly associated with the diagnosis of widely invasive carcinoma as compared to normal tissue or other diagnoses. The finding of Bcl-2 down-regulation is in accordance with the results of Müller-Höcker et al. (59) and Máximo et al. (28). Mitochondria are central regulators of apoptosis, which is initiated via the opening of mitochondrial permeability transition pore (mtPTP). The mtPTP can be stimulated to open by the uptake of excessive Ca^{2+} , increased oxidative stress, decreased mitochondrial transmembrane electrochemical gradient ($\Delta\psi$), ADP, ATP and adenine nucleotide translocator (ANT) ligands, such as atractyloside (60,61). Optimal functioning of ATP synthesis is required for the commitment of cells to traverse the cell cycle. In human leukemia (HL-60) cells, a small decrease in ATP levels induce accumulation of cells in G_1 phase, while larger decreases induce accumulation in G_2 phase (62).

Many studies have shown that the expression of cell cycle-regulatory proteins is altered in HCC in comparison with normal thyroid tissue (63,64). The overexpression of murine double minute-2 homolog (mdm-2) is a common mechanism of p53 inactivation in human cancers, since mdm-2 forms a tight complex with p53 gene and can inhibit p53-mediated transactivation. In the cells overexpressing mdm-2, it also shuttles the p53 protein into degradative pathways, thus contributing to the proliferative potential of these cells (65). The induction of the mdm-2 and p21 *in vivo* can be attributed to synchronous up-regulation of mdm-2 and p21 expression. In contrast with the results of Müller-Höcker et al. (63), the more recent study (58) showed that the nuclear accumulation of p53 was present in a small number of patients with HCA and HCC, suggesting inactivation of the p53 protein, most probably by mdm-2, which was overexpressed. Since the p53 protein half-life is short and basal expression levels are low in normal cells, immunohistochemistry cannot detect the high wild-type p53 levels reported previously (63). Therefore, in cancer cells, most of the p53 mutations lead to the product that has lost its cell-cycle regulatory function, and this product accumulates in the nuclei and can be demonstrated by immunostaining (58).

The defects in the apoptosis-inducing pathways can eventually lead to the expansion of a population of neoplastic cells resistant to chemotherapy and irradiation, as is the case of HCC. Other anti-apoptotic Bcl-2 family members also seem to be involved in the resistance of tumors to apoptosis. For example, Bcl-xl can

confer resistance to multiple apoptosis-inducing pathways. Together with the down-regulation of p53 and the overexpression of the growth-promoting oncogene E2F1, it seems likely that the necessary balance between pro- and antiapoptotic triggering factors is deeply disturbed in Hürthle cell carcinoma. This is emphasized by defects in ATP production and mutations and sequence variants of genes in the mitochondrial genome, including all of the 13 OXPHOS system genes (38,66,67). A great majority (81.5%) of mtDNA somatic point mutations in the study of Máximo et al. (38) were transitions, suggesting that they result in most instances from the action of reactive oxygen species on the mtDNA (68). The aforementioned missense mutations were detected in coding regions of complex I and in complex V genes, which involve the ATPase 6 gene. Hürthle cell tumors display a relatively high percentage of the mtDNA common deletions (CD), especially in tumors with D-loop mutations (38).

A partial deficiency of cytochrome-c oxidase (COX) (complex IV of mitochondrial respiratory chain [MRC]) has been found in oncocytic nodules of hyperplastic or adenomatous parathyroid glands and Hürthle cell lesions (48,43). Defects inducing a decrease of electron transfer in the respiratory chain are bound to enhance ROS production. Thus, COX defects may have similar effects as the addition of the respiratory inhibitors. Relations between ROS production and mtDNA damage have been mostly associated with complex I. Complex I is statistically much more likely to be affected by random mutations of mtDNA or by the "common" 4977-base pair deletion (69).

The mechanism of participation of the H^+ -ATP synthase in apoptosis could be mediated via ROS. In this regard, and because of the coupling between mitochondrial respiration and oxidative phosphorylation, the down-regulation of the H^+ -ATP synthase in cancer cells would limit the flux of electrons down the respiratory chain, and therefore, the generation of the superoxide radical, a promoter of DNA damage and likely the signal for induction of the mitochondrial cell-death pathway (70).

It would be of great interest to determine the expression levels and functional activity of H^+ -ATP synthase in oxyphilic tumors, especially in view of the results of Savagner et al. (67). This study showed no evidence for defects in the mitochondrial respiratory chain complexes I to IV, with significantly lower levels of ATP in six HCA and one HCC fresh tumor sample. These authors suggested the possibility of a coupling defect, supported by overexpression of uncoupling protein 2 (UCP2), in which case mitochondrial proliferation could be an adaptive response to primary nuclear abnormality. Another hypothesis is that primary proliferation of mitochondria leads to increased production of ROS, which can be counteracted by overexpression of UCP2 (67, 38).

The overall activity of oxidative phosphorylation in the cell is the result of both the bioenergetic competence of the organelles and

of the cellular mitochondrial content. The content of mitochondria in the cells is regulated both during development and by cell type-specific programs. It has been shown that in human liver cancer, a parallel down-regulation of the bioenergetic (β -F1-ATPase) and structural (Hsp60 and mtDNA) components of mitochondria occurs, strongly suggesting that liver carcinogenesis is accompanied by the repression of the program of mitochondrial biogenesis that is responsible for the proliferation of mitochondria in the hepatocyte. In contrast, in kidney and colon carcinomas, the specific down-regulation of the expression of the bioenergetic marker of oxidative phosphorylation (H^+ -ATP synthase) suggests that oncogenesis in these tissues only affects the mechanisms that control the program of differentiation of mitochondria, which is linked to the control of the translation of oxidative phosphorylation mRNAs (70).

In a study on the rat fibroblastic cell line (67), cells were cultured in the presence of different concentrations of Antimycin A (AA), a mitochondrial respiratory chain blocker acting on complex III. In morphological analysis, using the ISEL assay, apoptosis represented the most common cell death process in these cells treated with 100 and 200 μ M of AA, resulting in a decrease of \sim 34% and \sim 30%, of ATP levels, respectively, as compared with untreated control. Within the very narrow range of AA concentrations, between 225 and 250 μ M, most of the dying cells underwent a particular mode of cell demise, which was characterized by events common to both the apoptotic and necrotic processes. The typical implosion process of apoptosis was consequently followed by the rupture of plasma membranes and extensive cell lysis, suggesting the outcome of necrosis. The ATP levels at this step of respiratory inhibition were not yet below the threshold of less than 20% (71), determining shift toward necrosis. In such a view, aponecrosis may be a response to an insult with a magnitude high enough to partially destroy the ATP stores of the cells, suggesting that ATP is an important downstream regulator, representing a switch in the decision between apoptosis and necrosis (72).

The RET proto-oncogene (MIM [164761]) which maps to the chromosomal region 10q11.2 is not normally expressed in follicular epithelium and is rearranged in papillary thyroid carcinoma (PTC). These somatic chromosomal mutations (inversions or translocations) always involve intron 11 of RET and lead to the juxtaposition of its intracellular tyrosine kinase (TK) domain to the 5' portion of different donor genes constitutively expressed in the thyroid. By fusing with the intracellular domain of RET, these genes contribute a novel amino-terminal portion which makes RET activation ligand-independent. Somatic rearrangements of the RET proto-oncogene are present in up to 66% of the sporadic PTCs and at a low frequency in familial PTC. Several chimeric RET/PTC oncogenes, which differ in their RET fusion partners

have been identified (73,74).

The results of the studies already published (5,75,76) show that a subset of Hürthle cell adenoma and carcinoma exhibit nuclear features of PTC that are attributable to specific gene rearrangements, resulting in expression of RET/PTC oncogene. The absence of RET/PTC activation in all the samples of hyperplastic nodules with oncocytic metaplasia analyzed, suggests that RET/PTC activation may be considered as a secondary event in Hürthle cell adenomas or carcinomas, i.e. subsequent to the occurrence of genetic alterations determining oncocytic metaplasia.

CONCLUSION

Despite all the controversies regarding the diagnostic criteria between HCA and HCC, the etiology of the excess of mitochondria in Hürthle cells, as well as the degree and the causes of a deficit in energy production, it has been clearly shown in many published studies that Hürthle cells are characterized by profound aberrations in the nuclear and mitochondrial genome and by alterations in oncogenes, tumor suppressor genes and other key genes involved in energy metabolism, proliferative and cell senescence processes. Considering the aggressiveness of HCC, it would be of great importance to establish precise diagnostic criteria based on molecular patterns and biochemical assays. Analysis of familial clustering of disease as well as positional cloning efforts will further contribute to the identification of genes predisposing to Hürthle cell carcinoma.

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