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REVIEW ARTICLE

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Hürthle cell tumours of the thyroid. A review with emphasis on mitochondrial abnormalities with clinical relevance

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Abstract The molecular and enzymatic abnormalities of mitochondria in oxyphilic / oncocytic / Hürthle cells and their respective tumours are reviewed in a series of sections: "Mitochondria, ageing, neurodegenerative diseases and cancer", "Mitochondrial abnormalities in Hürthle cells and Hürthle cells tumours", "Mitochondrion-related alterations in tumours with and without Hürthle cell/oncocytic features", "True and secondary oxyphilia", and "Bcl-2 expression, apoptosis and necrosis in Hürthle cell tumours and tumour-like lesions". The clinicopathological and pathogenetic meaning of the data on record on these topics are discussed, with an emphasis on thyroid pathology.

Key words Thyroid carcinoma · Hürthle cell tumours · Oxyphilic tumours · Mitochondria · Mitochondrial genes

Introduction

It is difficult to find a subject in thyroid pathology that has created more controversies through the years than that of so-called Hürthle cell tumours. Curiously, the development of molecular pathology and the application of molecular techniques to the study of these tumours have not (yet?) contributed to clarification of the issues at stake, which may be individualised, admittedly in an oversimplified way, as follows:

a) Is there any similarity between the mitochondrial abnormalities seen in mitochondrial degenerative diseases and those found in Hürthle cells? What are the most frequent nuclear and mitochondrial DNA alterations that can be found in Hürthle cells and Hürthle cell tumours, and how do such alterations interfere with cell and tissue metabolic activities?
b) Is it possible to distinguish between "true" Hürthle cell tumours of the thyroid and thyroid tumours with "secondary" oxyphilia (e.g. papillary oncocytic carcinomas)? In other words, are there different aetiopathogenic mechanisms behind the accumulation of mitochondria in different types of Hürthle cell lesions?
c) And, finally, is it possible to find a relationship between the accumulation of abnormal mitochondria in the cells of Hürthle cell tumours and the biopathological features of such tumours, taking into account that Hürthle cell tumours appear to be equally aggressive as their non-Hürthle cell counterparts, or even more so?

The existence of very good reports on the morphological and clinicopathological characteristics of Hürthle cell tumours [3, 32, 46, 59] led us to focus the present review upon the molecular and enzymatic abnormalities of mitochondria in Hürthle cells and Hürthle cell tumours, with an emphasis on those with actual or potential clinical relevance. We will address the issues highlighted above in five sections devoted to "Mitochondria, ageing, neurodegenerative diseases and cancer", "Mitochondrial abnormalities in Hürthle cells and Hürthle cells tumours", "Mitochondrion-related alterations in tumours with and without Hürthle cell/oncocytic features", "True and secondary oxyphilia" and, finally, "*Bcl*-2 expression, apoptosis and necrosis in Hürthle cell tumours and tumour-like lesions". However, before addressing those issues, we will briefly review some of the basic aspects of "Nomenclature", "Light and electron microscopy", "Histological diagnosis", "Malignancy and prognosis", and "Cytogenetics, oncogenes and oncosuppressor genes" in Hürthle cell tumours and tumour-like lesions.

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Nomenclature

Hürthle cell tumours are thyroid tumours composed exclusively or predominantly of Hürthle cells, a name considered to be a synonym for oxyphilic, eosinophilic, Askanazi or oncocytic cells [18, 26, 27, 50]. For the sake of simplicity we will use the designation Hürthle cells when referring to the thyroid and the designations oxyphilic and oncocytic cells, and oncocytoma, when referring to other organs (namely salivary gland, kidney and parathyroid).

In the thyroid, Hürthle cells are not restricted to follicular cells and follicular cell-derived tumours. Indeed, some medullary thyroid carcinomas are composed of cells that are morphologically indistinguishable from Hürthle cells derived from the follicular cells [14]. The present review deals only with the latter.

The occurrence of Hürthle cell transformation in medullary thyroid carcinoma fits in with its occurrence in other neuroendocrine tumours throughout the body. This finding, together with the prominence of Hürthle cells (or oxyphilic cells in general) in endocrine organs, salivary glands, kidney and other parenchymatous organs (and in their respective tumours), in contrast to the rarity of oxyphilic cells in the tumours of the digestive and respiratory tract, suggests that this alteration occurs in parenchymas and in tumours with low proliferative index and reduced turnover, i.e. in stable cells with a very long intermitotic interval (e.g. if the cells of the digestive and respiratory tract and of their tumours divide too quickly or die/desquamate too soon to allow the accumulation of abnormal mitochondria that is the hallmark of Hürthle /oxyphilic cells).

Light and electron microscopy

Hürthle cell tumours are usually composed of pleomorphic or polygonal large cells with abundant, granular and acidophilic cytoplasm and large nuclei with prominent nucleoli. The only exception in terms of the large cell size – cells are usually smaller in carcinomas than in adenomas – is found in the so-called poorly differentiated Hürthle cell carcinomas [46], which are composed of rather uniform, small cells. Electron microscopy and immunohistochemistry with antimitochondrial antibodies have repeatedly confirmed that the cytoplasmic granularity of Hürthle cells is due to the presence of a large number of mitochondria [44, 50, 57]. The amount and the morphological characteristics of the mitochondria vary greatly from case to case [44]. The same holds true for the histochemical features namely those linked to mitochondrial enzymatic activities [15, 40, 43, 45, 64].

The aforementioned findings support the assumption that the appearance of Hürthle cells involves a whole spectrum of morphological and histochemical alterations, i.e. it does not seem to be a black-and-white phenomenon. We will return to this point when addressing the difference between "true" Hürthle cell tumours and thyroid tumours with "secondary" oxyphilia. For the moment it should be stressed that one Hürthle cells occurring on a background of Hashimoto's thyroiditis or nodular goitre cannot be distinguished either morphologically or histochemically from adenomatous or carcinomatous Hürthle cells [42].

It also does not seem possible to separate, Hürthle cells of the thyroid from the oxyphilic cells found in other organs on the basis of their morphology. The same is true of the aneuploid DNA content, which appears to be common to benign and malignant Hürthle cell and to oxyphilic cell tumours in the different settings [5, 48, 60].

Histological diagnosis

The diagnosis of Hürthle cell tumours of the thyroid is one of the most debated issues in thyroid pathology. Since there are excellent reviews on record [30, 32, 50, 59] we will only briefly address a few points.

1. We wish to stress the usefulness of searching for thyroglobulin and calcitonin by immunohistochemical methods whenever a specifically Hürthle cell tumour is encountered in a fine-needle aspirate, since Hürthle cell transformation can also occur in medullary carcinomas of the thyroid.
2. It should be emphasised that an infarct type of necrosis, often large, is frequently observed in Hürthle cell tumours, either without apparent cause or as a consequence of a fine needle aspiration procedure (see below the discussion on the putative reasons for this propensity of Hürthle cell tumours to ischaemic necrosis).
3. We acknowledge the need to look for vascular or capsular invasion by tumour cells, as in any other encapsulated follicular tumour of the thyroid, in order to make the diagnosis of malignancy in a Hürthle cell tumour.
4. We stress that the existence of many uni- and multinodular lesions of the thyroid composed of Hürthle cells, with or without Hashimoto's thyroiditis in the background, as in common nodular goitres, raises the problem of the differential diagnosis between hyperplastic and neoplastic nodular lesions. This problem remains unsolved for the time being despite the demonstration of the clonal nature of many of the nodules of multinodular goitres.
5. We acknowledge the existence of a Hürthle cell variant of papillary carcinoma. At variance with many authors, we concur with Hedinger et al. [30] in the individualisation of this variant of papillary carcinoma whenever neoplastic cells with cytoplasmic Hürthle cell features have nuclei with the typical nuclear features of papillary carcinoma [3, 56, 57].

On the other hand, we agree with Carcangiu et al. [11] and Rosai et al. [50] that Hürthle cell tumours displaying

hyperchromatic nuclei and prominent nucleoli, i.e. lacking the typical features of papillary carcinoma nuclei, should be classed in the group of Hürthle cell tumours (Hürthle cell adenomas or Hürthle cell follicular carcinomas) regardless of their papillary or follicular growth pattern.

Malignancy and prognosis

Since several exhaustive reviews have already been published on this subject [11, 16, 39, 46, 59, 72], we will just pinpoint the three most important issues from a clinical standpoint.

1. The first issue has a historical flavour. In the 1950s the American Cancer Society was recommending the classification of all thyroid tumours with Hürthle cell features as malignant. Subsequent studies have clearly demonstrated that – as for any other encapsulated follicular neoplasm – the conventional criteria of vascular and capsular invasion should be applied to the prediction of malignant behaviour in Hürthle cell tumours [11, 59].
2. The second issue concerns the incidence of malignancy in Hürthle cell tumours. Despite some numerical variations from series to series, it is almost unanimously acknowledged that the percentage of Hürthle cell tumours in which capsular and/or vascular invasion is found, making the diagnosis of Hürthle cell follicular carcinoma possible, is higher than the percentage of non-Hürthle-cell follicular tumours displaying signs of malignancy [11, 16, 39, 46, 72].
3. The third issue concerns prognosis. As a first point it is worthwhile stressing that the prognostic factors associated with Hürthle cell carcinomas do not differ from those found to carry meaningful information in non-Hürthle cell carcinomas [11, 16, 39, 46, 72]. It remains controversial, however, whether or not the category of Hürthle cell follicular carcinoma per se carries a worse prognosis. Some authors claim that Hürthle cell follicular carcinomas spread to the perithyroid soft tissues and give rise to distant and regional lymph node metastases more often than do conventional follicular carcinomas [72]. It is also worthwhile stressing that the overall mortality rate appears to be higher among patients with Hürthle cell carcinoma [11] than among patients with papillary [10] or follicular carcinoma [34]. This higher mortality may be related to the poor responsiveness of Hürthle cell carcinomas to radioiodine therapy as a consequence of the decreased efficiency of Hürthle cells in iodine uptake and hormone synthesis [9, 65].

Since most series on record, if not all, lack a proper multivariate analysis of the numerous clinicopathological factors that can contribute to the prognosis, we are not sure whether Hürthle cell carcinomas, regardless of whether they are papillary or follicular, are indeed more malignant than their non-Hürthle-cell counterparts. Our scepticism gained strong support from the data recently reported by Evans and Vassilopoulou-Selin [16], showing that when the cases are stratified by the extent of thyroid and extrathyroid invasion there are no statistically significant differences in the rate of local recurrence, rate of nodal or distant metastasis, or patient's survival between Hürthle cell carcinoma and conventional follicular carcinoma.

The higher prevalence of malignancy in Hürthle cell tumours and the putative higher aggressiveness of Hürthle cell carcinomas and oncocytic meningiomas [49] contrast with the trends towards benignity or low malignancy of most oxyphilic tumours (oncocytomas) in other organs [50].

Cytogenetics, oncogenes and oncosuppressor genes

The cytogenetic and molecular cytogenetic alterations, as well as the alterations in oncogenes and oncosuppressor genes, observed in Hürthle cell tumours and oncocytomas tend to be organ specific, i.e. to resemble those seen in their non-mitochondrion-rich counterparts rather than those in oxyphilic tumours of other organs [13, 59, 61, 66].

Exceptions to the aforementioned "rule" include the detection of loss of heterozygosity for 10q in Hürthle cell tumours and renal oncocytomas [60], and the report of a hyperdiploid karyotype with trisomy 7, which is frequently seen in Hürthle cell tumours, in a salivary gland oncocytoma [36].

Although it is difficult or even impossible to achieve a critical evaluation of the huge amount of data on record, it seems that the Hürthle cell tumours do not differ substantially from their non-Hürthle-cell counterparts regarding the prevalence of aneuploidy, trisomy of chromosomes 5, 7 and 12, and mutations of oncogenes and/or oncosuppressor genes (see below). We still do not know whether monosomy of chromosome 2 may indeed be a marker of a subset of Hürthle cell tumours as suggested by Tallini et al. [61]. Nor do we know whether the prevalence of *ret* and *trk* rearrangements is similar in the Hürthle cell variant of papillary carcinoma and in common papillary carcinomas.

The same holds true for *ras* activation. In fact, it is known that the *ras* oncogene is frequently involved both in Hürthle cell tumours and in benign and malignant conventional follicular tumours (for thorough reviews see [51, 61]), but we still do not know whether it is possible to establish a difference between Hürthle cell and non-Hürthle-cell tumours. There is, however, enough evidence to state that H-, K-, and N-*ras* mutations do not appear to be particularly prevalent in Hürthle cell carcinomas [61].

In most series [41, 46], as in our own (unpublished results) there is an apparent association between overexpression of p53 and a subset of Hürthle cell carcinomas. Papotti et al. [46] proposed the notion that such overexpression allowed the identification, together with a solid/trabecular growth pattern, small cell size and absence

of immunoreactivity for bcl-2, of a group of clinically aggressive, poorly differentiated Hürthle cell carcinomas. It remains to be seen whether these Hürthle cell carcinomas differ significantly from conventional poorly differentiated carcinomas of the thyroid. The mechanism underlying p53 overexpression in these settings also remains unclarified [41, 46].

There is reduced immunoexpression of E-cadherin, together with a trend to a diffuse cytoplasmic pattern, both in benign and malignant Hürthle cell tumours and in papillary, poorly differentiated and undifferentiated thyroid carcinomas [21, 55]. The loss of E-cadherin expression is associated with aberrant CpG island methylation [21]. As with *ras* and *p53* alterations, there is no apparent reason to link any E-cadherin alteration specifically with the Hürthle cell phenotype. With the exception of *bcl*-2 (see below) the same holds true for other oncogenes, oncosuppressor genes, growth factors and growth factors receptors, although isolated studies have indicated overexpression of *N-myc*, TGF-α, TGF-β, IGF-1 and somatostatin receptor in Hürthle cell carcinomas [23, 51].

A last point that should be mentioned is the need to be very cautious in the interpretation of immunohistochemical results obtained in Hürthle cell tumours, because of their tendency to give rise to false-positive immunostaining with many antibodies. This problem may be solved, partially at least, by blocking the endogenous biotin [7].

Mitochondria, ageing, neurodegenerative diseases and cancer

It is known that rapidly growing cancer cells have an increased glycolytic rate [71]. Tumour 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. Though it is likely that alterations in the expression/function of proteins involved in the energy metabolism play an important part in tumour biology, specific changes in genes coding for mitochondrial proteins have not yet been firmly established.

The mitochondrial DNA (mtDNA) is small, with a molecular weight of 10^7, and incorporates a sequence of 16,569 bp. The genome encodes 13 essential components of the apparatus of cellular energy production and is absolutely vital for life. MtDNAs also incorporate two classes of structural RNAs and 22 mitochondrial transfer RNAs. These transfer RNAs assist in the expression of the mtDNA within the mitochondria [67].

The high copy number of mtDNA and the cytoplasmic location of the mitochondria contribute to a unique genetic system hallmarked by five principles (for a thorough review see [68]). One of these principles is related to the fact that mitochondria undergo replicative segregation at cell division; since there are typically 1–10 mtDNAs per organelle [70], a mixture of mutant and normal mtDNAs (heteroplasmy) is produced when a mutation occurs; over time, the proportion of mtDNAs can drift to 100% mutant or normal mtDNAs (homoplasmy) [47, 68, 69]. The fifth unique aspect of mtDNA genetics is its high mutation rate, about 10–20 times that of the nuclear DNA (nDNA) [68].

Although it is beyond the scope of the present review to discuss the type(s) of DNA repair mechanisms that may be operative at the mtDNA level, there appears to be an inverse relationship between nuclear mismatch repair deficiency (MIN or RER phenotype) and the occurrence of mtDNA mutations and/or deletions [38, 62].

Since the original report by Luft et al. [35], mitochondrial disorders have usually been regarded as neuromuscular diseases. However, over the past 10 years, a number of mtDNA mutations that cause human disease in a number of nonneuromuscular organs have been identified [68].

Quantification of the common 4.977 kb mtDNA deletion (common deletion) has shown that mtDNA deletions accumulate markedly in the basal ganglia and various cortical regions in human brain after age 75. The cause of the somatic mtDNA mutations is likely to be oxidative damage, which increases with age in the mtDNA of several tissues and pathologic conditions (for a thorough review see [12]).

These observations led to the hypothesis that somatic mtDNA mutations accumulate in postmitotic tissues with age as a result of mitochondrial reactive oxygen species (ROS) damage. This mechanism (oxidative damage plus time) may explain the presence of numerous Hürthle cells in Hashimoto's thyroiditis in adults and old patients, in contrast to their absence in Hashimoto's (autoimmune) thyroiditis of young patients [9]. The same holds true for the occurrence of isolated or clustered Hürthle cells or oncocytic cells in several endocrine and, less often, exocrine glands of elderly subjects.

Somatic mtDNA mutations have also been described in various tumours and tumour cell lines (see below). These mutations include intragenic deletions [31], missense and chain-termination point mutations [47], and alterations of homopolymeric sequences that result in frameshift mutations [24]. In principle, these mutations could contribute to neoplastic transformation by modifying the cellular energy capacities, increasing mitochondrial oxidative stress and/or modulating apoptosis [47, 71], but it must be admitted that it is somewhat paradoxical to associate the neoplastic development with abnormalities in the organelles that are mainly responsible for cellular energy-related functions. The situation is even more complicated if one tries to clarify the putative pathogenic role played by the numerous and abnormal mitochondria that stuff the cells of Hürthle cell/oncocytic tumours.

Mitochondrial abnormalities in Hürthle cells and Hürthle cells tumours

Numerous key components of the oxidative phosphorylation (OXPHOS) machinery have been identified histologically or immunohistochemically in Hürthle cell le-

sions of the thyroid and oncocytic lesions of the kidney, parathyroid and salivary gland [15, 40, 45, 64], but only a partial deficiency of cytochrome-*c* oxidase [complex IV of mitochondrial respiratory chain (MRC)] has been found in oncocytic nodules of hyperplastic or adenomatous parathyroid glands and Hürthle cell lesions [40]. Oncocytes have traditionally been regarded as cells with active metabolism and high levels of oxidative enzymes [64]. However, this does not appear to correlate with adequate cellular performance and, at least in the thyroid gland, Hürthle cells exhibit decreased efficiency [65]. These findings suggest a defect in the energy production machinery of the cell and indicate that the increased mitochondrial content may be compensatory, presumably owing to the involvement of the nuclear genes controlling mitochondrial number through a sort of a feedback mechanism [2].

Using the differential display method in the study of a series of thyroid tumours, we found overexpression of the subunit Core-I of complex III of the MRC in two Hürthle cell follicular carcinomas (personal unpublished results). A similar overexpression is, however, shared by follicular carcinomas and microfollicular adenomas without Hürthle cell features, thus showing that such overexpression cannot be linked with Hürthle cell transformation per se (personal unpublished results).

Although the type of cellular derangements causing the abnormal accumulation of mitochondria in Hürthle cell and oncocytic neoplasms is obscure, these tumours have features in common with mitochondrial encephalomyopathies, including some of the ultrastructural alterations of the mitochondria. In both types of disease, mitochondria have often accumulated in large aggregates and display a variety of morphological alterations, including abnormal cristae and inclusions [44]. Cases of infantile oncocytic (histiocytoid) cardiomyopathy are also characterised by abnormal accumulation of mitochondria in the cardiac myocytes. One of the patients with infantile oncocytic cardiomyopathy featured a set of systemic pathological abnormalities with oncocytic changes in the endocrine glands [53].

Mitochondrion-related alterations in tumours with and without Hürthle cell/oncocytic features

In cancer, some MCR gene alterations have been described both in genes encoded by nDNA and in genes encoded by mtDNA. One nDNA-encoded protein of MCR which, it has been proposed, plays a major part in the regulation of MCR is the adenine nucleotide translocator [33]. Increased expression of this gene was found in renal carcinomas and renal and salivary oncocytomas [17]. Elevated expression of several mitochondrial and nuclear genes has been described in some precancerous tissues and transformed cell lines [20, 63]. Some other alterations in both nuclear and mitochondrial genes of MRC were reported: amplification of mtDNA in acute myeloid leukaemia [4], up-regulation of mitochondrial proteins in melanoma cells [54], and increased expression of genes encoding mitochondrial proteins both in papillary thyroid carcinomas and in some other thyroid tumours [29].

Mutations (deletions) in mtDNA have also been described in gastric cancer, colorectal cancer, renal cell carcinoma, Hürthle cell tumours, and oncocytic tumours of the kidney and parathyroids [6, 31, 37, 38, 40, 43, 47, 60, 62]. Defects of cytochrome-*c* oxidase and the common deletion of mtDNA occur frequently in Hürthle cells of Hashimoto's thyroiditis and Hürthle cell tumours [37, 43].

We have looked for the presence of the mitochondrial common deletion and mitochondrial somatic point mutations in Hürthle cells tumours. A high percentage (nearly 100%) of Hürthle cell tumours display the mitochondrial common deletion and/or somatic mitochondrial point mutations (Table 1). In lesions with moderate Hürthle cell changes, i.e., in which the cytoplasm is only relatively more abundant and granular than that of the normal thyrocytes, we have also found the mitochondrial common deletion [37].

"True" and "secondary" oxyphilia

Knowledge of the mechanism(s) that lead(s) to increased mitochondrial number causing Hürthle cell changes of the thyroid follicular cells is small. In Hashimoto's thyroiditis, it can be assumed that such an increase is secondary to the chronic autoimmune response, possibly resulting from oxidative damage of the follicular cell [43]. It has been shown that over-replication of mitochondria can be the result of a compensatory effort secondary to aberrantly functioning mitochondria [2]. The same mechanism is proposed by Katoh et al. [32] as an explanation for the oxyphilia in papillary carcinomas and other thyroid lesions (e.g. nodular goitre) associated with thyroiditis; this group includes a subset of papillary carcinomas with dense stromal lymphoplasmacytic infiltrate, and large, pink granular neoplastic cells [3]. It has been advanced that in this setting the immune response and cytokine(s) production may also be associated with growth stimulation [58].

In the absence of any known environmental factor affecting tumour cells, it has been proposed by Katoh et al. [32] that the oxyphilia in Hürthle cell tumours not associated with thyroiditis must be due to somatic mutation(s) in one (or several) gene(s) directly or indirectly affecting mitochondrial number. The target gene(s) could be members of the mitochondrial genome or nuclear gene(s) controlling the mitochondrial number. Based upon these assumptions, Katoh et al. [32] suggested that the oxyphilia of thyroid tumours may be either "primary", due to somatic mutation(s), or "secondary", due to alterations induced by oxidative damage or other environmental aggressions.

The distinction between "true" and "secondary" oxyphilia [32] is very interesting from a conceptual stand-

Table 1 Summary of the data on mtDNA alterations in tumours with and without Hürthle cell/oncocytic features

Type and/or site of mutations	Conventional tumours		Hürthle cell/oncocytic tumours	
	Present	Absent	Present	Absent
Common deletion	Liver [19], stomach[a] [38]	Stomach [6], colon and rectum [24, 25, 47]	Thyroid [37, 43, 64], kidney [60]	
Other deletions	Stomach: 50 bp (D-loop) [6], Kidney: 264 bp (ND1) [31] Colon and rectum: 15 bp (ND1) [25]	Colon & rectum [47]	Thyroid: large deletions[a]	
Coding region (point mutations)	Colon and rectum: 12S rRNA; 16S rRNA; ND1, ND4L; ND5; CYT b; COXI; COXII; COXIII [24, 25, 47] Stomach: ND1; ND5; $tRNA^{Leu}$; $tRNA^{Ser}$; COXI[a]		Thyroid: ND1; ND5; COXI[a]	
D-loop (point mutations)	Stomach [1, 62], colon and rectum [1, 24]	Colon and rectum [47]	Thyroid[a]	

[a]Máximo et al., unpublished results

point, providing "true" oxyphilia is not identified with genetic mechanism(s) and "secondary" oxyphilia with environmental factors. In fact, apart from mutations on nuclear genes controlling mitochondrial number that may lead, by definition, to "true" Hürthle cell tumours – this possibility has been confirmed by the finding of consistent alteration at chromosome 19p13.2 in familial Hürthle cell tumours [8, 28] – the role of somatic mitochondrial mutations that can be caused by environmental factors within the setting of "secondary" oxyphilia must be considered. Polyak et al. [47] have shown that the majority of mutations of mtDNA in human colorectal tumours were transitions at purines, which is consistent with ROS generation.

The compensatory increase of abnormal mitochondria that occurs in Hürthle cells is greatly facilitated by the higher division rate of mitochondria with deletions and/or mutations over that of normal mitochondria [47, 67]. This higher rate of replication of mutated or deleted mitochondria also explains the trend towards homoplasmy of the long-lasting pathological processes: both in neurodegenerative mitochondrial disorders and in Hürthle cell/oncocytic tumours there is a tendency towards the progressive accumulation of mitochondria with mutated and/or deleted mtDNA. Tumour homoplasmy requires two further steps: first, a process of mitochondrial selection leading to the replacement of all mitochondrial genomes in the neoplastic cells with a mutant form; and, secondly, the clonal growth of such neoplastic cell [47]. Polyak et al. [47] demonstrated the occurrence of homoplasmic mutations in the mtDNA of human tumour cells, suggesting that this reflects either a selective growth advantage provided by the aberrant mitochondria or the role of concurrent nuclear gene mutation(s).

In other words, if "true" and "secondary" oxyphilia are considered as steps of a spectrum, rather then well-defined categories, it seems logical to predict that the progression from the former to the latter will be accompanied by a progressive shift from heteroplasmy to homoplasmy.

The issue of the pathogenic relationship between carcinogenesis and mitochondrial abnormalities, or vice versa, is beyond the scope of the present review. It is nevertheless worthwhile stressing that the timing of the occurrence of the somatic mutations of mtDNA (or the environmental aggression) in relation to the initiation of the neoplastic process may lead to "true" or "secondary" Hürthle cell tumours. If the factor that leads to the increased amount of mitochondria occurs in an already initiated neoplastic cell, the resulting tumour will most probably display the architectural, biochemical, and clinicopathological features of the corresponding conventional tumour (papillary, follicular or medullary) and will progressively acquire cytoplasmic oxyphilia ("secondary" oxyphilia). If the mitochondrial hit occurs in a normal cell that will later develop into a neoplasm through a series of mutations, the final product will be a "primary" Hürthle cell tumour.

The aforementioned hypothesis is supported by the results we have obtained in the analysis of a series of thyroid tumours and adjacent normal thyroid parenchyma. In this study we observed the presence of mitochondrial abnormalities in the granular, acidophilic cells (not typical Hürthle cells) occurring in the vicinity of Hürthle cell tumours, thus suggesting that the factor that led to the Hürthle cell changes was already present in the "normal" thyroid tissue [37].

Bcl-2 expression, apoptosis and necrosis in Hürthle cell tumours and tumour-like lesions

Bcl-2 is localised in the outer mitochondrial membrane and has a role in the normal nucleo-mitochondrial communication circuits; it may therefore be involved in tumour cell transformation [42]. It has been shown that *bcl*-2 expression is down-regulated or even absent in Hürthle cell lesions (Hashimoto's thyroiditis) and Hürthle cell tumours [42]. We have also found a marked reduction of *bcl*-2 expression in Hürthle cell adenomas and carcinomas, as well as in almost every mitochondrion-rich tumour or tumour-like lesions (e.g. tall cell variant of papillary carcinoma and nodular goitre) (personal unpublished observations). It is tempting to suggest that mitochondria in Hürthle cells have abnormalities that can lead to the incapacity for *bcl*-2 to be localised in the mitochondrial outer membrane [42]. Mitochondrial abnormalities at the membrane level would then be responsible for such reduced or null expression of bcl-2 in Hürthle cell tumours [42].

Mitochondria also provide a major switch for the initiation of apoptosis. This switch is thought to involve the opening of a nonspecific mitochondrial inner membrane channel, the mitochondrial permeability transition pore (mtPTP). The mitochondrial inner membrane space contains a number of cell-death-promoting factors, including cytochrome-*c*, apoptosis-inducing factor (AIF), and latent forms of specialised proteases called caspases. Opening of the mtPTP and the accompanying death of the cell can be initiated by the mitochondrion's excessive uptake of Ca^{2+}, increased exposure to ROS, or decline in energetic capacity. Thus, a marked reduction in mitochondrial energy production and a chronic increase in oxidative stress may activate the mtPTP and initiate apoptosis [22].

The situation of Hürthle cells and Hürthle cell tumours is therefore complex with regard to the apoptotic process: Hürthle cells lack *bcl*-2, a well-known anti-apoptotic factor and, on the other hand, the abnormalities of their mitochondria (e.g. with regard to cytochrome-*c* oxidase) may indeed contribute to block apoptosis [52].

We still ignore if there is any relationship between the aforementioned alterations of the apoptotic mechanisms and the neoplastic development of Hürthle cell tumours [47, 52]. There is, however, evidence enough to suggest that the apoptosis blockade in Hürthle cell tumours may play an important biological role at three levels.

1. By allowing the progressive accumulation of abnormal mitochondria in long living neoplastic cells, they lead to the progressive shift from heteroplasmy to homoplasmy.
2. The increased survival of the neoplastic cells of Hürthle cell tumours could contribute to the allegedly worse prognosis of such tumours (see above). Alternatively, it is conceivable that the putative worse prognosis of Hürthle cell tumours may reflect their more advanced stage in comparison with that of conventional thyroid tumours. (The accumulation of mitochondria could be an indirect sign of long-lasting lesions occurring in older patients and responding poorly to radioiodine therapy.)
3. Finally, it is tempting to advance that the deficiency of the apoptotic response in Hürthle cell tumours may provide an explanation for the high prevalence of ischaemic necrosis in these tumours, either spontaneously or after fine needle aspiration procedures. The blockade of apoptosis in Hürthle cell tumours may cause the rigidity of the response to ischaemic stimulus, abruptly leading to large foci of necrosis whenever a certain threshold of aggression is attained. Further and more detailed studies are obviously necessary to evaluate the contribution of additional factors for this tendency to ischaemic necrosis of Hürthle cells tumours, namely the intrinsic vulnerability of Hürthle cells to ischaemia and a putative reduced degree of angiogenesis in Hürthle cell tumours relative to their non-Hürthle cell counterparts.

References

1. Alonso A, Martin P, Albarran C, Aquilera B, Garcia O, Guzman A, Oliva H, Sancho M (1997) Detection of somatic mutations in the mitochondrial DNA control region of colorectal and gastric tumors by heteroduplex and single-strand conformation analysis. Electrophoresis 18:682–685
2. Attardi G, Yoneda M, Chomyn A (1995) Complementation and segregation behavior of disease-causing mitochondrial DNA mutations in cellular model systems. Biochim Biophys Acta 1271:241–248
3. Berho M, Suster S (1997) The oncocytic variant of papillary carcinoma of the thyroid: a clinicopathologic study of 15 cases. Hum Pathol 28:47–53
4. Boultwood J, Fidler C, Mills KI, Frodsham PM, Kusec R, Gaiger A, Gale RE, Linch DC, Littlewood TJ, Moss PA (1996) Amplification of mitochondrial DNA in acute myeloid leukaemia. Br J Haematol 95:426–431
5. Bronner MP, Clevenger CV, Edmonds PR, Lowell DM, McFarland MM, LiVolsi VA (1988) Flow cytometric analysis of DNA content in Hürthle cell adenomas and carcinomas of the thyroid. Am J Clin Pathol 89:764–769
6. Burgart LJ, Zheng J, Shu Q, Strickler JG, Shibata D (1995) Somatic mitochondrial mutation in gastric cancer. Am J Pathol 147:1105–1111
7. Bussolati G, Gugliotta P, Volante M, Pace M, Papotti M (1997) Retrieved endogenous biotin: a novel marker and a potential pitfall in diagnostic immunohistochemistry. Histopathology 31:400–407
8. Canzian F, Amati P, Harach HR, Kraimps JL, Lesueur F, Barbier J, Levillain P, Romeo G, Bonneau D (1998) A gene predisposing to familial thyroid tumors with cell oxyphilia maps to chromosome 19p13.2. Am J Hum Genet 63:1743–1748

9. Carcangiu ML, DeLellis RA (1992) Thyroid gland. In: Damjanov I, Linder J (eds) Anderson's Pathology, 20th edn. Mosby, St. Louis, pp 1943–1979
10. Carcangiu ML, Zampi G, Pupi A, Castagnoli A, Rosai J (1985) Papillary carcinoma of the thyroid. A clinicopathologic study of 241 cases treated at the University of Florence, Italy. Cancer 55:805–828
11. Carcangiu ML, Bianchi S, Savino D, Voynick IM, Rosai J (1991) Follicular Hürthle cell tumors of the thyroid gland. Cancer 68:1944–1953
12. Corral-Debrinski M, Horton T, Lott MT, Shoffner JM, Beal MF, Wallace DC (1992) Mitochondrial DNA deletions in human brain: regional variability and increase with advanced age. Nat Genet 2:324–329
13. Damiani S, Pasquinelli G, Eusebi V (1999) GANT-like gastrointestinal pacemaker cell tumours with oncocytic features. Virchows Arch 435:143–150
14. Dominguez-Malagon H, Delgado-Chavez R, Torres-Najera M, Gould E, Albores-Saavedra J (1989) Oxyphil and squamous variants of medullary thyroid carcinoma. Cancer 63:1183–1188
15. Ebner D, Rodel G, Pavenstaedt I, Haferkamp O (1991) Functional and molecular analysis of mitochondria in thyroid oncocytoma. Virchows Arch [B] 60:139–144
16. Evans HL, Vassilopoulou-Sellin R (1998) Follicular and Hürthle cell carcinomas of the thyroid: a comparative study. Am J Surg Pathol 22:1512–1520
17. Faure-Vigny H, Heddi A, Giraud S, Chautard D, Stepien G (1996) Expression of oxidative phosphorylation genes in renal tumors and tumoral cell lines. Mol Carcinog 16:165–172
18. Flint A, Lloyd RV (1990) Hürthle-cell neoplasm of the thyroid gland. Pathol Annu 25:37–52
19. Fukushima S, Honda K, Awane M, Yamamoto E, Takeda R, Kaneko I, Tanaka A, Morimoto T, Tanaka K, Yamaoka Y (1995) The frequency of 4977 base pair deletion of mitochondrial DNA in various types of liver disease and in normal liver. Hepatology 21:1547–1551
20. Glaichenhaus N, Leopold P, Cuzin F (1986) Increased levels of mitochondrial gene expression in rat fibroblast cells immortalized or transformed by viral and cellular oncogenes. EMBO J 5:1261–1265
21. Graff JR, Greenberg VE, Herman JG, Westra WH, Boghaert ER, Ain KB, Saji M, Zeiger MA, Zimmer SG, Baylin SB (1998) Distinct patterns of E-cadherin CpG island methylation in papillary, follicular, Hürthle's cell, and poorly differentiated human thyroid carcinoma. Cancer Res 58:2063–2066
22. Green DR, Reed JC (1998) Mitochondria and apoptosis. Science 281:1309–1312
23. Gulec SA, Serafini AN, Sridhar KS, Peker KR, Gupta A, Goodwin WJ, Sfakianakis GN, Moffat FL (1998) Somatostatin receptor expression in Hürthle cell cancer of the thyroid. J Nucl Med 39:243–245
24. Habano W, Nakamura S, Sugai T (1998) Microsatellite instability in the mitochondrial DNA of colorectal carcinomas: evidence for mismatch repair systems in mitochondrial genome. Oncogene 17:1931–1937
25. Habano W, Sugai T, Yoshida T, Nakamura S (1999) Mitochondrial gene mutation, but not large-scale deletion, is a feature of colorectal carcinomas with mitochondrial microsatellite instability. Int J Cancer 83:625–629
26. Hamperl H (1962) Benign and malignant oncocytoma. Cancer 15:1019–1027
27. Hamperl H (1962) Oncocyten und Oncocytome. Virchows Arch 335:283–452
28. Harach HR, Lesueur F, Amati P, Brown A, Canzian F, Kraimps JL, Levillain P, Menet E, Romeo G, Bonneau D (1999) Histology of familial thyroid tumours linked to a gene mapping to chromosome 19p13.2. J Pathol 189:387–393
29. Haugen DR, Fluge O, Varhaug JE, Lillehaug JR (1999) Increased expression of genes encoding mitochondrial proteins in papillary thyroid carcinomas. J Endocrinol Invest 22:7
30. Hedinger CE, Williams ED, Sobin LH (1988) Histological typing of thyroid tumors. In: Hedinger CE (ed) International histological classification of tumours, vol 11. Springer, Berlin Heidelberg New York
31. Horton TM, Petros JA, Heddi A, Shoffner J, Kaufman AE, Graham SD, Gramlich T, Wallace DC (1996) Novel mitochondrial DNA deletion found in a renal cell carcinoma. Genes Chromosom Cancer 15:95–101
32. Katoh R, Harach HR, Williams ED (1998) Solitary, multiple, and familial oxyphil tumours of the thyroid gland. J Pathol 186:292–299
33. Kholodenko BN (1984) Control of mitochondrial oxidative phosphorylation. J Theor Biol 107:179–188
34. Lang W, Choritz H, Hundeshagen H (1986) Risk factors in follicular thyroid carcinomas: a retrospective follow-up study covering a 14-year period with emphasis on morphologic findings. Am J Surg Pathol 10:246–255
35. Luft R, Ikkos D, Palmieri F (1962) Severe hypermetabolism of non thyroid origin with a defect in the maintenance of mitochondrial respiratory control: a correlated clinical, biochemical and morphological study. J Clin Invest 41:1776–1804
36. Mark J, Dahlenfors R, Havel G, Bockmann P (1991) Benign parotid oncocytoma with the chromosomal abnormality trisomy 7. Anticancer Res 11:1735–1737
37. Máximo V, Soares P, Rocha AS, Sobrinho-Simões M (1998) The common deletion of mitochondrial DNA is found in goitres and thyroid tumors with and without oxyphil cell change. Ultrastruct Pathol 22:271–273
38. Máximo V, Soares P, Seruca R, Sobrinho-Simões M (1999) Comments on: Mutations in mitochondrial control region DNA in gastric tumours of Japanese patients. Eur J Cancer 35:1407–1408
39. McDonald MP, Sanders LE, Silverman ML, Chan HS, Buyske J (1996) Hürthle cell carcinoma of the thyroid gland: prognostic factors and results of surgical treatment. Surgery 120: 1000–1005
40. Müller-Höcker J (1992) Random cytochrome-*c*-oxidase deficiency of oxyphil cell nodules in the parathyroid gland. A mitochondrial cytopathy related to cell ageing? Pathol Res Pract 188:701–706
41. Müller-Höcker J (1999) Immunoreactivity of p53, Ki-67, and Bcl-2 in oncocytic adenomas and carcinomas of the thyroid gland. Hum Pathol 30:926–933
42. Müller-Höcker J (2000) Expression of BCL2, Bax and Fas in oxyphil (Askanazy) cells of Hashimoto thyroiditis. Virchows Arch (in press)
43. Müller-Höcker J, Jacob U, Seibel P (1998) Hashimoto thyroiditis is associated with defects of cytochrome-c oxidase in oxyphil Askanazy cells and with the common deletion (4,977) of mitochondrial DNA. Ultrastruct Pathol 22:91–100
44. Nesland JM, Sobrinho-Simões M, Holm R, Sambade MC, Johannessen JV (1985) Hürthle-cell lesions of the thyroid: a combined study using transmission electron microscopy, scanning electron microscopy, and immunocytochemistry. Ultrastruct Pathol 8:269–290
45. Ortmann M, Vierbuchen M, Koller G, Fischer R (1988) Renal oncocytoma. I. Cytochrome c oxidase in normal and neoplastic renal tissue as detected by immunohistochemistry – a valuable aid to distinguish oncocytomas from renal cell carcinomas. Virchows Arch [B] 56:165–173
46. Papotti M, Torchio B, Grassi L, Favero A, Bussolati G (1996) Poorly differentiated oxyphilic (Hürthle cell) carcinomas of the thyroid. Am J Surg Pathol 20:686–694
47. Polyak K, Li Y, Zhu H, Lengauer C, Willson JK, Markowitz SD, Trush MA, Kinzler KW, Vogelstein B (1998) Somatic mutations of the mitochondrial genome in human colorectal tumours. Nat Genet 20:291–293
48. Rainwater LM, Farrow GM, Hay ID, Lieber MM (1986) Oncocytic tumours of the salivary gland, kidney, and thyroid: nuclear DNA patterns studied by flow cytometry. Br J Cancer 53:799–804
49. Roncaroli F, Riccioni L, Cerati M, Capella C, Calbucci F, Trevisan C, Eusebi V (1997) Oncocytic meningioma. Am J Surg Pathol 21:375–382

50. Rosai J, Carcangiu ML, DeLellis RA (1992) Tumors of the thyroid gland. (Atlas of tumor pathology, 3rd ser) Armed Forces Institute of Pathology, Washington
51. Schark C, Fulton N, Jacoby RF, Westbrook CA, Straus FH, Kaplan EL (1990) N-*ras* 61 oncogene mutations in Hürthle cell tumors. Surgery 108:994–999
52. Shirafuji N, Takahashi S, Matsuda S, Asano S (1997) Mitochondrial antisense RNA for cytochrome C oxidase (MARCO) can induce morphologic changes and cell death in human hematopoietic cell lines. Blood 90:4567–4577
53. Silver MM, Burns JE, Sethi RK, Rowe RD (1980) Oncocytic cardiomyopathy in an infant with oncocytosis in exocrine and endocrine glands. Hum Pathol 11:598–605
54. Simon HG, Risse B, Jost M, Oppenheimer S, Kari C, Rodeck U (1996) Identification of differentially expressed messenger RNAs in human melanocytes and melanoma cells. Cancer Res 56:3112–3117
55. Soares P, Berx G, van Roy F, Sobrinho-Simões M (1997) E-cadherin gene alterations are rare events in thyroid tumors. Int J Cancer 70:32–38
56. Sobrinho-Simões M (1995) Tumours of thyroid: a brief overview with emphasis on the most controversial issues. Curr Diagn Pathol 2:15–22
57. Sobrinho-Simões M, Nesland JM, Holm R, Sambade MC, Johannessen JV (1985) Hürthle cell and mitochondrion-rich papillary carcinomas of the thyroid gland: an ultrastructural and immunocytochemical study. Ultrastruct Pathol 8:131–142
58. Takahashi MH, Thomas GA, Williams ED (1995) Evidence for mutual interdependence of epithelium and stromal lymphoid cells in a subset of papillary carcinomas. Br J Cancer 72:813–817
59. Tallini G (1998) Oncocytic tumors. Virchows Arch 433:5–12
60. Tallini G, Ladanyi M, Rosai J, Jhanwar SC (1994) Analysis of nuclear and mitochondrial DNA alterations in thyroid and renal oncocytic tumors. Cytogenet Cell Genet 66:253–259
61. Tallini G, Hsueh A, Liu S, Garcia-Rostan G, Speicher MR, Ward DC (1999) Frequent chromosomal DNA unbalance in thyroid oncocytic (Hürthle cell) neoplasms detected by comparative genomic hybridization. Lab Invest 79:547–555
62. Tamura G, Nishizuka S, Maesawa C, Suzuki Y, Iwaya T, Sakata K, Endoh Y, Motoyama T (1999) Mutations in mitochondrial control region DNA in gastric tumours of Japanese patients. Eur J Cancer 35:316–319
63. Torroni A, Stepien G, Hodge JA, Wallace DC (1990) Neoplastic transformation is associated with coordinate induction of nuclear and cytoplasmic oxidative phosphorylation genes. J Biol Chem 265:20589–20593
64. Tremblay G, Pearse AGE (1960) Histochemistry of oxidative enzyme systems in the human thyroid with special reference to Askanazy cells. J Pathol Bacteriol 80:353–358
65. Valenta LJ, Michel-Bechet M, Warshaw JB, Maloof F (1974) Human thyroid tumors composed of mitochondrion-rich cells: electron microscopic and biochemical findings. J Clin Endocrinol Metab 39:719–733
66. Van den Berg E, Dijkhuizen T, Storkel S, de la Riviere GB, Dam A, Mensink HJ, Oosterhuis JW, de Jong B (1995) Chromosomal changes in renal oncocytomas. Evidence that t(5;11)(q35;q13) may characterize a second subgroup of oncocytomas. Cancer Genet Cytogenet 79:164–168
67. Wallace DC (1986) Mitochondrial genes and disease. Hosp Pract 21:77–87, 90–92
68. Wallace DC (1992) Diseases of the mitochondrial DNA. Annu Rev Biochem 61:1175–1212
69. Wallace DC (1999) Mitochondrial diseases in man and mouse. Science 283:1482–1488
70. Wallace DC, Brown MD, Melov S, Graham B, Lott M (1998) Mitochondrial biology, degenerative diseases and aging. Biofactors 7:187–190
71. Warburg O (1956) On the origin of cancer cells. Science 123: 309–314
72. Watson RG, Brennan MD, Goellner JR, van Heerden JA, McConahey WM, Taylor WF (1984) Invasive Hürthle cell carcinoma of the thyroid: natural history and management. Mayo Clin Proc 59:851–855