Mitochondrial DNA and Human Thyroid Diseases

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Abstract. Cells of the thyroid tissue, either diseased or normal, can accumulate altered mitochondrial genomes in primary lesions and in surrounding parenchyma. Depending on the experimental approaches and the extent of the mutational process, it has been possible to demonstrate the occurrence of homoplasmic or heteroplasmic point mutations, presence of a common deletion and random large-scale mtDNA aberrations in various pathological states. Point somatic mutations documented in 5–60% of thyroid tumors do not concentrate in obvious hotspots but tend to cluster in certain regions of the mitochondrial genome and their distribution may differ between carcinomas and controls. Large-scale deletions in mtDNA are quite prevalent in healthy and diseased thyroid; however, the proportion of aberrant mtDNA molecules accounts for a very small part of total mtDNA and does not seem to correlate with pathological characteristics of thyroid tumors. Common deletion is most abundant in Hurthle cell tumors, yet it also occurs in other thyroid diseases as well as in normal tissue. The principal difference between the common deletion and other deletion-type mtDNA molecules is that the former does not depend on the relative mtDNA content in the tissue whereas in a subset of thyroid tumors, such as radiation-associated papillary carcinomas and follicular adenomas, there is a strong correlation between mtDNA levels and prevalence of large-scale deletions. Relative mtDNA levels by themselves are elevated in most thyroid tumors compared to normal tissue. Distinct differential distribution and prevalence of mutational mtDNA burden in normal tissue and thyroid lesions are suggestive of the implication of altered mtDNA in thyroid diseases, especially in cancer.

Key words: Mitochondrial DNA, Mutation, Thyroid cancer, Heteroplasmy

ALTERATIONS of mitochondrial DNA (mtDNA) have been implicated in the pathogenesis of a variety of human diseases and specific (patho) physiological states. A series of syndromic disorders referred to as degenerative mitochondrial diseases is causatively associated with point mutations or large-scale aberrations (deletions or insertions) of the mitochondrial genome [1–8]. Speculatively, mtDNA mutations and organelle dysfunction may also be involved in Parkinson’s and Huntington’s diseases, severe male osteoporosis and aging [4, 9–11].

Studies of mtDNA mutations in cancer is a rapidly expanding area that explores the possible links of neoplastic growth with DNA modifications within the organelle. Many types of human malignancy such as colorectal, liver, breast, lung and bladder cancer have been shown to harbor mtDNA mutations [12–15]. Studies of mtDNA in thyroid pathology are rather limited to date compared to the extensive investigations of nuclear DNA, therefore it is worthwhile to attempt to summarize the data available from the literature and to supplement them with those obtained in our laboratory.

In this article, we overview some relevant aspects of the molecular biology of mtDNA and the possible association of its mutation with tumors drawing special attention to the alterations of mitochondrial genome and thyroid neoplastic diseases.
Native human mtDNA is a 16568 bp \[16, 17\] compact circular double-stranded DNA molecule accommodating 37 genes; see Fig. 1. Of them, 13 encode peptide components of the respiratory chain and ATP synthase, and products of the 2 rRNA and 22 tRNA genes are essential for a mitochondrial protein synthesis. MtDNA does not have canonic introns and besides a 1.1 kb D-loop (pos. 16023/576) \[2\] that concentrates several regulatory sequences, nearly every nucleotide participates in coding. Transcription of mtDNA relies completely on nuclear DNA-encoded factors, and newly synthesized polycistronic units undergo extensive postranscriptional modifications.

The complementary DNA chains composing the mitochondrial genome, the heavy (H-) and light (L-) strands, have different specific gradient buoyant properties and distinct base compositions. The H-strand encoding 28 of 37 mitochondrial genes has more G bases, whereas the L-strand is richer in pyrimidine ones. The replication of mtDNA has several traits. According to the most recent findings, it may initiate in a strand-coupled manner from multiple sites across a zone that spans the cytochrome b and NADH dehydrogenase subunits 5 and 6 genes, and then proceeds bidirectionally (theta replication) until one side of the replication fork reaches the region containing the origin of heavy strand synthesis, O\(_H\), where it stalls \[18\]. After the fork arrest, replication continues in one

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Note that the Cambridge reference sequence \[16\] specifies human mtDNA length as 16569 bp. The size has been proposed for correction due to an eventual error revealed in the originally deposited sequence \[17\].

Here and hereafter base numbering refers to that of the Cambridge sequence mtDNA L-strand.
direction and the final stages are likely to be completed rapidly. DNA replication in the organelle is dependent on polymerase \textit{gamma} (POLG), which has a higher infidelity rate than the nuclear DNA polymerase \textit{alpha}. Importantly, some deficiencies in the DNA polymerase \textit{gamma} gap filling and proofreading activities may lead to base misincorporation when the enzyme encounters abasic sites or modified nitrogenous bases in the template [19].

The mtDNA repair machinery is largely preserved in the organelle. Substantial evidence has been accumulated to indicate that quite efficient base excision repair, mismatch and recombination repair and direct damage reversal occur within the mitochondrion [20]. At the same time, there are data suggestive of an underrepresented nucleotide excision repair system [21]. Additionally, some specific kinetic parameters of the mtDNA repair mechanisms differ from those of the nucleus, perhaps contributing to the increased mutational rate of mtDNA [22].

Regarding other factors making for excessive mtDNA damage, it is worth noting that the D-loop region which has an unusual triple strand organization [23] has been shown to be a place of a contact of mtDNA with the organelle membrane [24]. The latter circumstance may provide a mechanistic basis for higher probability of preferential exposure of this mtDNA segment to active chemical compounds such as membrane-associated lipid peroxides and other reactive oxygen species (ROS) capable of promoting the enhanced mutagenesis of the fragment [25–27]. Indeed, this region has been demonstrated to succumb to excessive mutational burden presumably because of its elevated susceptibility to DNA damage and insufficient mtDNA repair mechanisms [28].

Unlike nuclear DNA, mtDNA structure is not maintained by DNA-bound protein scaffolds like histones. The significantly smaller number of proteins [29] associated with mtDNA makes it more vulnerable to micro-environmental factors and provides less protection from DNA-modifying agents.

Taken together, a combination of exogenous and endogenous factors, such as the above mentioned aggressive milieu rich in ROS, error-prone POLG-dependent mtDNA replication, weaker DNA repair machinery and lack of the protective protein sheath, leads to an increased specific rate of mtDNA mutations, and to a longer accumulation and preservation time of those than is known for nuclear DNA. The estimated relative excess of mtDNA evolvement is approximately 10- to 100-fold for the mitochondrial genome as a whole and 100- to 200-fold for the D-loop fragment as compared to nuclear DNA [30–35].

Because of the important biological role of mitochondria as “power plants”, the elevated mutational load may seem to have dramatic consequences for proper cell functioning. However, the peculiarities of mitochondrial biology and genetics are such that they manage to protect and maintain the functioning of the cellular energy source.

Somatic cells usually contain several hundred mitochondria and each organelle may contain up to a dozen mtDNA copies. The smallest number of mitochondria, amounting to only a few, has been found in resting lymphocytes [36]. By contrast, mammal oocytes may have as many as $10^4$ of these organelles [37, 38]. Due to the structure of the gametes and the process of fertilization, only oocyte mitochondria are passed on to the embryo since in spermatozoa the mitochondria concentrate apart from the head [39]. Thus, mitochondrial genome is conferred matrilinearly, and the virtual lack of mitochondria of paternal origin creates the condition known as haploidy. As each organelle may contain a number of genomes, the mitochondrial genetic status is defined as polyhaploid, on which point mitochondrial genetics is at variance with the Mendelian’s [40].

Theoretically, in every individual, the maternally transmitted mtDNA should have an identical allelic structure, in so-called homoplasm. By contrast, simultaneous coexistence of more than one sequence variant of mtDNA within the organism is referred to as heteroplasm [41]. In view of the high mutational rate of mtDNA and the large number of mtDNA copies per organelle and per cell, the probability of the existence of mtDNA with alternate primary structure(s) in every individual is likely to approach to the level of 1. However, a necessary proviso would be that the determination of the homoplasmic or heteroplasmic state depends strongly on the technical approach employed for DNA analysis [42]. More sensitive and discrete techniques on the one hand and the prevalence of mtDNA sequence variants on the other hand would yield higher incidence of revealed heteroplasm [43, 44].

Genotype-phenotype correlation is an important issue directly linked to the mtDNA structure. How big can a potential threat from mutated mtDNA be? Clearly, in the case of homoplasmic mtDNA mutation, the
aberrant mitochondrial gene(s) involved will be the only ones that are expressed. The appearance of altered RNA and protein products may distort cell homeostasis and therefore be harmful and pathogenic as seen in mitochondrial diseases [45–48]. More obscure situations arise when the mtDNA mutation is heteroplasmic and occurs concomitantly with wild-type mitochondrial genome. Striking findings in this regard show that cells are quite tolerant to the presence of mutant mtDNA species [49]. The first line of defense lies probably in the decreased stability of altered RNA transcripts leading to the diminished accumulation of mutant products [50]. The next level of backup is the mitochondrial capacity for complementation, implying that the products of intact mtDNA genes can functionally compensate for the mutated ones within quite a broad range [51]. According to experimental estimates, the specific content of aberrant mitochondrial genomes would have to reach approximately 60% of total mtDNA before the onset of biochemical and phenotypic changes can take place [52–54]. It is therefore unlikely that low-level heteroplasmic mutations will significantly affect or result in clinical manifestations [55]. Along these lines, the problem of the biological relevance of mutated mtDNA-encoded genes has yet to be understood and further studies are necessary to clarify whether they indeed influence the course of a disease or merely contribute to the development of morphogenetic and pathological changes.

**Mutations of mtDNA in human cancers**

Most human malignancies display genome instability as seen in the appearance of non-inherited microsatellite markers or eventual loss of inherited ones [56–58]. The main reason for the nuclear genome instability is associated with the dysfunction of the mismatch repair system occurring as a result of possible genetic or epigenetic modifications of genes whose products are involved in the DNA repair machinery [59, 60]. Although such malfunctioning has yet to be directly associated with mtDNA mutations of most instances, mitochondrial genome instability has been shown to be correlated with nuclear genome instability in gastric cancer [58]. Therefore, at the moment there is not enough evidence to rule out common or partly overlapping mechanisms underlying genome instability in the nucleus and in the mitochondria [61].

Mitochondrial genome instability has been found in cancers at nearly every level of complexity: point mutations, mtDNA deletions and insertions, and multiple variations of microsatellite length tracts [62–66]. Note that different laboratories were able to find homoplasmic or heteroplasmic mtDNA mutations in virtually all types of tumors examined. Assuming somatic mutations of mtDNA mutations do take place in neoplastic cells, the mechanisms for achieving the homoplasm has been the subject of discussion for quite some time. Indeed, how is it possible for a mutation of any kind, that might have arisen in one or, less probably, in a few copies of mtDNA, to prevail in abundance against the background of a multitude of other non-mutated mitochondrial genomes? Several models have been proposed to explain this phenomenon: i) the defective mitochondrion takes over the replication control and is intensively amplified in attempt to compensate for the organelle’s functional inadequacy [67, 68]; ii) the mutated mitochondrial genome has a profound replicative advantage acquired as a result of mtDNA mutation [69–71]; and iii) a combined restriction/amplification effect associated with random genetic drift and clonal expansion of tumor cells [72, 73]. Relevant to the latter mechanism, the “bottleneck” hypothesis suggests that there is a severe limitation as to the number of maternal mitochondria that may be transmitted to descending daughter cells during each division cycle [74]. A computer modeling confirmed by experimental results, however, has provided evidence of the possibility that homoplasm may be generated in a cell by chance. In this scenario, no physiological advantage or enhanced tumorigenic potential of cells harboring mutated mtDNA is necessary to yield homoplasm in the tumor through the unbiased mtDNA replication and sorting during cell division [75]. Therefore, at least some cases of homoplasmic mutations can be explained without implication of cell selection or specific growth advantage.

Mutations of mtDNA are quite widespread in human cancer. Complete sequence analysis of the mtDNA genomes of primary ovarian cancers and control tissues have revealed somatic mtDNA mutations in 60% (6 of 10) of tumor samples [63] with their preferential accumulation in the mtDNA region spanning the D-loop region, 16S, 12S rRNA and cytochrome b genes. Most of these mutations were homoplasmic T to C or G to A transitions. Interestingly, in the D-loop
region only, single or multiple somatic point mutations were detected in 20% (3 of 15) of ovarian cancer samples. Considering that the D-loop accounts for approximately 1/16-th of the mitochondrial genome, the finding demonstrates that this region indeed carries point mutations more often than other mtDNA segments.

Prostate cancer tissue specimens and matched prostatic intraepithelial neoplastic lesions studied for the presence of mtDNA mutations harbored 20 mtDNA mutations in the tumor tissue of 3 patients in the D-loop region, 16S rRNA and the NADH 1 regions [64]. Examination of human bladder, head and neck, and lung primary tumors also revealed high frequency of somatic point mtDNA mutations [15]. A substantial part of these mutations was homoplasmic: 64% (9 of 14) in bladder cancer, 46% (6 of 13) in head and neck, and 43% (6 of 14) in lung cancer. The reported homoplasm in these cases is supportive of the view that the mutant mtDNA becomes dominant in tumor cells.

Analysis of the mtDNA mutational status in normal and tumor tissues from breast cancer patients showed that both types of tissues may carry heteroplasmic point mutations [14]. In contrast to this finding, 74% (14 of 19) of examined breast cancer DNA specimens have been demonstrated to possess at least one somatic point mutation with preferential accumulation in the D-loop region (22 of a total 27 somatic mutations detected upon sequencing of the entire mitochondrial genome) [65]. Taking these reports as an example of the examination of a similar type of human malignancy using different methods, it is necessary to bear in mind that not only different sample series, but also experimental techniques may lead to variant readings of the problem.

Sequencing analysis of a specific and highly polymorphic homopolymeric C stretch (also referred to as D310 or hypervariable region II) located in the D-loop between nucleotides 303 and 316–318 has revealed somatic mutations (1- or 2-bp deletions/insertions) in 22% of examined DNA samples from various human tumors [76]. The D310 region has been claimed to be a mutational hotspot for primary tumors, and in the proposed model, homoplasmic D310 alterations in the tumor were suggested to arise from a tumor progenitor cell through random genetic drift and clonal tumor growth.

Besides point mutations, several types of human cancers have been reported to harbor deletions in mtDNA. A somatic 50 bp mitochondrial deletion flanked by the 9-bp direct repeats in the D-loop was detected in gastric adenocarcinomas [66]. In renal cell carcinomas, a 264 bp deletion has been recovered from about half of the mtDNA molecules [77]. More characteristic for mtDNA is the so-called common deletion (CD), which comprises a mtDNA species with deleted 4977 bp region spanning the ATPase6, COIII, ND3, ND4, ND5 and 5 tRNA genes (between bases 8470 and 13447) of the mitochondrial genome. Its facilitated formation may perhaps be attributed to the 13 bp-long direct repeats flanking the deletion-prone region. Recent study has shown that CD was observed in 81% of gastric carcinoma [78]. Heteroplasmic CD was found in breast cancer, especially in post-menopausal patients, thus it may be more characteristic of aging cells [14]. Supportive of this notion, is a direct correlation of increased level of large-scale deletions with patient’s age found in a study of malignant prostate tumors [79].

**Mutations of mtDNA in thyroid diseases**

1. **Point mutations of mtDNA**

   Systematic analysis of the primary structure of mtDNA from a series of thyroid pathology tissues has demonstrated a difference in the incidence of mtDNA sequence variants between the diseased tissue and control specimens, especially between thyroid cancer cases and control population [80]. A statistical significance was found in the differential distribution of all variant types between carcinomas and controls but not in benign tumors and non-neoplastic diseases versus the controls. Three different somatic mutations have been identified in 23% (3 of 13) of papillary thyroid cancer (PTC) cases suggestive of possible involvement of mtDNA mutations in thyroid tumorigenesis.

   In a study of 79 benign and malignant thyroid tumors (43 Hurthle and 36 non-Hurthle cell neoplasms) and matched normal parenchyma, 57 somatic mutations, mostly transitions, were found in 34 tumors, and a total of 253 sequence variants were detected in 59 patients [81]. Follicular and papillary carcinomas displayed a significantly higher prevalence of non-silent mutations of complex I genes than follicular adenomas...
(FA). Also, significantly higher prevalence of complex I and complex IV sequence variants has been found in the normal tissue adjacent to malignant tumors implying that mtDNA variants and mtDNA somatic mutations of complex I and complex IV genes might be involved in thyroid tumorigenesis.

In spite of the fact that somatic mtDNA mutations in human cancers are often observed in the D-loop, and particularly in the D310 C-tract, the mutational rate of this area in thyroid cancers of various histological types and grade has been found to be quite low. Alterations in the D310 region were observed only in 5.7% (2/35) PTC, 5.6% (1/18) medullary carcinomas, 11.1% (1/9) anaplastic carcinomas and 11.1% (1/9) follicular thyroid carcinomas [82]. In an investigation of thyroid tumors diagnosed in children from Belarus after the Chernobyl accident and from 40 sporadic tumors in German patents, the mutation frequency correlated only with the age of patients at surgery, but not with radioiodine contamination [83]. In our experiments, we also examined the (CA)$_n$ microsatellite in the hypervariable region I between nucleotides 514–523, and the C$_6$TC$_n$ stretch of the D310 in the DNA extracted from surgical thyroid specimens from patients living in radiocontaminated zones in Russia. What we found were only heteroplasmic mutations, both in normal and tumor samples with no statistical evidence of any association with the level of radiopollutant contamination in the place of patient’s residency.

As thyroid is well-known to be a target for radiation as manifested in an increased incidence of tumor diseases of this organ in exposed individuals [84, 85], we attempted to address the question of whether possible exposure to radioiodine may elevate the mutational rate in other regions of mtDNA in people possibly affected by the radioactive Chernobyl fallout. Recent analysis demonstrates that Chernobyl-associated childhood thyroid cancer may have been induced by short-lived radioactive iodine at the time of accident [86], once again emphasizing the importance of radiation factor in thyroid carcinogenesis. Examination of the presence of point mutations in the three regions of mtDNA (D-loop, 12S RNA, ND1) in the DNA specimens derived from the tumor and corresponding normal thyroid tissue (17 cases, 11 PTC and 6 FA) revealed a variety of specimens carrying either homoplasmic or heteroplasmic sequence variants of mtDNA as summarized in Table 1. The mutations were observed in non-coding regions as well as in those coding for tRNA/rRNA and for mitochondrial proteins, in the latter case sometimes leading to the predicted amino acid change (missense mutations). Two samples had mutations in more than one region. In total, 10 sequence variants were found, all but one the transitions, of which 6 occurred in follicular adenoma (FA), and the remaining 4 in PTC cases.

Statistical analysis of data did not reveal any significant difference in rates of the occurrence of the mutations in FA versus PTC, in normal versus tumor DNA specimens. Similarly, no association with the level of

<table>
<thead>
<tr>
<th>Nucleotide position</th>
<th>Location</th>
<th>Nucleotide change</th>
<th>Heteroplasmy*</th>
<th>Aminocacid change</th>
</tr>
</thead>
<tbody>
<tr>
<td>149</td>
<td>D-loop</td>
<td>C→T</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>263</td>
<td>D-loop</td>
<td>A→G</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>303–315</td>
<td>D-loop</td>
<td>C$_6$TC$_n$→C$_6$TC$_n$</td>
<td>+N</td>
<td>–</td>
</tr>
<tr>
<td>303–315</td>
<td>D-loop</td>
<td>C$_6$TC$_n$→C$_6$TC$_n$</td>
<td>+N</td>
<td>–</td>
</tr>
<tr>
<td>398–399</td>
<td>D-loop</td>
<td>CC→GG</td>
<td>+N</td>
<td>–</td>
</tr>
<tr>
<td>636</td>
<td>tRNA Phe</td>
<td>A→G</td>
<td>+N</td>
<td>–</td>
</tr>
<tr>
<td>654</td>
<td>12S rRNA</td>
<td>T→C</td>
<td>+T</td>
<td>–</td>
</tr>
<tr>
<td>709</td>
<td>12S rRNA</td>
<td>G→A</td>
<td>+N</td>
<td>–</td>
</tr>
<tr>
<td>4164</td>
<td>ND1</td>
<td>A→G</td>
<td>–</td>
<td>Silent (Met)</td>
</tr>
<tr>
<td>4216</td>
<td>ND1</td>
<td>T→C</td>
<td>–</td>
<td>Tyr→His</td>
</tr>
<tr>
<td>4257</td>
<td>ND1</td>
<td>A→G</td>
<td>+T</td>
<td>Silent (Gln)</td>
</tr>
<tr>
<td>4334</td>
<td>tRNA Glu</td>
<td>A→G</td>
<td>+T</td>
<td>–</td>
</tr>
<tr>
<td>4336</td>
<td>tRNA Glu</td>
<td>T→C</td>
<td>–</td>
<td>–</td>
</tr>
</tbody>
</table>

*Sign “–” stands for homoplasmic polymorphisms or point mutations revealed in the tumor tissue compared to normal counterpart. “+” depicts heteroplasmic DNA samples with letters N or T indicating type of tissue that carried the point mutated mtDNA, Normal or Tumor, respectively.
radiiodine contamination in the place of the patient’s residency, pTNM stage of PTC or other pathological features of FA was found. There was a tendency of heteroplasmic sequence variants to occur more often in tumor tissue rather than in normal one as well as in coding than in non-coding regions; however, the limited set of detected mutations did not provide sufficient statistical power to attest these trends.

Taken together, of 34 DNA specimens each analyzed in 3 mtDNA regions, 8 samples displayed the presence of point mutations accounting for 23.5% mutational rate which is quite low compared to the largest series reported elsewhere (59.6%, 34/57) [81]. The index obtained in our experiments might though arise from the smaller size of mitochondrial genome subjected to PCR-SSCP and sequencing analysis in the work, only 4.8% compared to 70% in [81]. On the other hand, the results demonstrated that the regions studied were unlikely to be mutation hotspots in thyroid tissue.

All 8 heteroplasmic point mutations detected either in normal or tumor tissue-derived mtDNA occurred as non-prevalent forms as representatively shown in Fig. 2. The abundance of variant sequences varied within 5–10% by densitometry being in good agreement with results of sequencing of individual clones obtained from bulk PCR products displaying additional band(s) on SSCP gel. When 10 to 20 clones were sequenced, 1–5 of them usually contained a variant sequence. Therefore, mutated mtDNA comprised a minor portion of total mtDNA making it difficult to link it with active role in thyroid tumorigenesis.

A summary of point mutations in mtDNA in thyroid tumors is given in Table 2.

2. Large-Scale alterations of mtDNA

Hashimoto’s thyroiditis has been associated with the CD and defects of cytochrome-c oxidase in oxyphilic cells in affected but not in normal regions of a diseased gland as shown by Muller-Hocker et al. [87]. From the experimental design of the cited work it was unclear what kind of cells was the source of the mutated mtDNA; however, these were likely the Ashkanazy cells known to possess a large number of mitochondria [88, 89] thought to arise as a compensatory response to insufficient energy production [90, 91]. Occurrence of the CD has been demonstrated also in Hürthle cell carcinoma, multinodular goiter (MNG) and in FA with or without oxyphilic cell changes [92]. Normal thyroid tissue was usually devoid of this mutation irrespective of the presence of mild lymphocytic thyroiditis with the exclusion of one case of MNG out of total 8 cases studied [93]. In the most frequently occurring form of thyroid malignancy, papillary thyroid carcinoma, the CD has not been found. Somewhat at variance with these findings is a previous study of a series of 12 oncocyctic thyroid tumors which did not reveal increased frequency of mtDNA deletions in the tumors compared to controls [93]. An earlier report also showed no heteroplasmy or other gross alterations of mtDNA by restriction analysis of the DNA from a similar type of thyroid tumors [88].

In our study [94], prior to determination of the prevalence of large-scale deletions (LSD) in mtDNA, a real-time PCR measurement of the relative mtDNA level was performed in the three regions of the mitochondrial genome examined for the presence of point mutations described in the previous section and in an additional fragment localized to the mitochondrial cytochrome b gene. Normalized to nuclear DNA-
encoded b-actin gene, the four regions displayed finely concordant data with correlation coefficient of 94–99%. This finding was suggestive of the absence of highly abundant large deletions or gene amplification in the examined specimens. Relative mtDNA content was elevated in most of the tumor tissues studied. Both radiation-associated and spontaneous PTC and FA displayed higher mitochondrial to nuclear DNA ratio, which was clearly seen especially in the radiation-associated PTC series. Results of the measurement of relative mtDNA content in thyroid tumors were in line with the known increase of mitochondria number in various types of thyroid tumors, not only in Hurthle cell neoplasms [95], and also ruled out shortage of mtDNA as a possible reason of low rate of point mutations determined in the experiments described before. Statistical evaluation did not reveal any correlation between mtDNA levels and pathological characteristics such as the size and extent of the tumor or with the presence of oxyphilic cell changes in this set of cases.

Quantitative determination of the levels of CD and of random LSDs in matched normal and tumor thyroid tissues of radiation-associated and spontaneous PTCs and FAs was performed using real-time PCR and a sensitive fluorochrome-labeled asymmetric PCR, respectively, a typical readout of the latter being shown in Fig. 3. The CD was found in all tested samples, but its abundance was very low compared to that of wild-type mtDNA. This observation was supportive of a report claiming that CD may potentially account for maximal 7.4% and 4.3% of total mtDNA in the tumor tissue of Hurthle cell follicular cancer and Hurthle cell papillary cancer, respectively, and 0.2% in PTC [81].

Elevated LSD scores were found in most tumor tissues of radiation-associated PTC cases compared to matched normal thyroid specimens. In the FA, number of such cases was lower, and in spontaneous PTCs it accounted for less than one half of the series. The most important finding was a highly positive significant correlation between the number of LSDs and mtDNA content in radiation-associated tumor tissues of PTC and FA, but not in sporadic groups. In contrast to the tumor, in normal tissues there was a tendency for an inverse correlation to exist between LSD and mtDNA content. Thus, the synchronous elevation of the number of LSD and mtDNA level in the tumor tissue may be a specific feature of radiation-associated PTC, whereas discordance of these two parameters is more characteristic of spontaneous cases. Similar to the CD, the abundance of aberrant mtDNA molecules is very low as compared to non-mutated wild type mitochondrial genomes. Interestingly, the CD level, unlike LSD, did not correlate with mtDNA content in any subgroup of samples, implying that the mechanisms underlying CD and LSD formation might be different. Indeed, the generation of the CD has been attributed in part to the defects of mtDNA replication [96], whereas random LSDs are likely to arise after misrepair of double-strand mtDNA breaks. In model experiments with cultured human primary thyrocytes irradiated with 0.5–5 Gy of X-rays, the number of LSDs marginally significantly increased with the exposure dose, suggestive of the fact that namely this type of mtDNA aberrations may be associated with genotoxic stress, whereas changes in mtDNA and CD levels were not detected within two days after exposure. Taken together, the results of the studies demonstrate that simultaneous determination of a number of characteristics of mtDNA, including its mutational status and relative content, may be an informative approach to further understanding thyroid tumorigenesis.
and in discriminating various ethiopathogenic groups of PTC. A summary of large-scale deletions in mtDNA in thyroid tumors is given in Table 2.

As follows from the literature data and the results discussed, both normal and transformed thyrocytes possess a wide spectrum of structurally aberrant mtDNA molecules whose formation may be driven by a complex composition of internal mitochondria-, cell- and tissue-specific as well as external physical-chemical environmental factors. In spite of the multiplicity of the deletion-type sequence variants, aberrant mtDNA constitutes only a minor part of the total mitochondrial genetic material of a cell. Therefore, it is unlikely that mutated mtDNA plays an active and causative role in the pathogenesis of a disease. Rather its involvement may be at the level of modulation of the disease progression or it may arise as a “side byproduct” of the pathogenic processes serving in this case as a molecular marker, as demonstrated, for example, in radiation-associated thyroid tumors. Further studies employing thoroughly selected, rigorously correct functional models are warranted to improve our understanding of the place and role of mtDNA mutations in the disease.

Table 2. Summary of mtDNA mutations in thyroid tumors

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>mtDNA phenotype</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Intact mitochondrial DNA</strong></td>
<td></td>
</tr>
<tr>
<td>Elevated in most of thyroid tumors</td>
<td>16.6 kb</td>
</tr>
<tr>
<td><strong>Point mutations</strong></td>
<td></td>
</tr>
<tr>
<td>Homoplasmic or heteroplasmic</td>
<td></td>
</tr>
<tr>
<td>Heteroplasmy may be of low abundance</td>
<td></td>
</tr>
<tr>
<td>No evident mutational hotspots including the D310</td>
<td></td>
</tr>
<tr>
<td>Tend to cluster in the complex I and IV genes</td>
<td></td>
</tr>
<tr>
<td>In normal tissue may cluster in complex I genes – possible genetic predisposition to cancer</td>
<td></td>
</tr>
<tr>
<td>Differential distribution different from that of normal tissue</td>
<td></td>
</tr>
<tr>
<td><strong>Large-scale deletions</strong></td>
<td></td>
</tr>
<tr>
<td><em>Common deletion</em></td>
<td></td>
</tr>
<tr>
<td>Flanked by 13 bp direct repeats</td>
<td></td>
</tr>
<tr>
<td>Elevated level in most of tumor tissues</td>
<td></td>
</tr>
<tr>
<td>Especially high levels (up to 7%) in Hurthle cell cancer</td>
<td></td>
</tr>
<tr>
<td>Low abundance (0.2%) in papillary cancer</td>
<td></td>
</tr>
<tr>
<td>No association with total level of mtDNA</td>
<td></td>
</tr>
<tr>
<td>Does not change in irradiated primary thyrocyte culture</td>
<td></td>
</tr>
<tr>
<td><em>Random large-scale deletions</em></td>
<td></td>
</tr>
<tr>
<td>Usually flanked by short patches (2-7 bp) of microhomology</td>
<td></td>
</tr>
<tr>
<td>Elevated level in most of tumor tissues</td>
<td></td>
</tr>
<tr>
<td>Low abundance in follicular adenoma and papillary cancer</td>
<td></td>
</tr>
<tr>
<td>Correlation with total level of mtDNA in radiation associated follicular adenoma and papillary cancer – possible marker of ethiopathogenic groups</td>
<td></td>
</tr>
<tr>
<td>Change in dose-dependent manner in irradiated primary thyrocyte culture</td>
<td></td>
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</tbody>
</table>
Acknowledgements

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