

GENETIC OF THYROID CANCER
FAMILIAL NON MEDULLARY THYROID CANCER

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Abstract. Differentiated non-medullary thyroid cancer (NMTC) is mostly sporadic, but the recurrence of familial form of the disease has been reported. Short or dysfunctional telomeres have been associated with familial benign diseases and familial breast cancer. We aimed to study the telomere-telomerase complex in familial NMTC (FNMTC). The genetic analysis included the measurement in the peripheral blood of relative telomere length (RTL), telomerase reverse transcriptase (hTERT) gene amplification, hTERT mRNA expression, telomerase protein activity and search of hTERT or TERC (telomerase RNA component) gene mutations. We, also, studied telomeric fusions and associations as well as other chromosomal fragility features by conventional and molecular cytogenetic analyses, in phytohemagglutinin stimulated T-lymphocytes from familial patients, unaffected family members, sporadic PTC patients and healthy subjects. We found that, telomere length was significantly shorter in the blood of familial patients compared to sporadic PTCs, healthy subjects, nodular goiter and unaffected siblings. hTERT gene amplification was significantly higher in FNMTC patients compared to the other groups and, in particular, it was significantly greater in offspring with respect to parents. hTERT mRNA expression as well as telomerase activity were significantly higher in FNMTC patients compared to sporadic. In addition, we demonstrated that familial patients have a significant increase in spontaneous telomeric associations and telomeric fusions compared to healthy subjects and sporadic cases. Q-FISH analysis demonstrated that familial cases display a significant decrease in the telomeric PNA-FISH signal intensity in metaphase chromosome. Our study demonstrates that patients with FNMTC display an imbalance of the telomere-telomerase complex in the peripheral blood.

Keywords: Familial non medullary thyroid cancer, telomere instability, telomere length, telomere-telomerase complex.

Differentiated thyroid cancer (DTC), although mostly sporadic, may have a familial occurrence (familial non-medullary thyroid cancer, FNMTC), with a prevalence of up to 10% (1-2). The risk of developing FNMTC in first-degree relatives of subjects with DTC is significantly higher (between 3.2-8.6) than in the general population (3, 4). Several rare hereditary syndromes caused by germline mutations of known tumour suppressor genes, are associated with the occurrence of DTC, mainly of the papillary histotype, such as familial adenomatous polyposis, Cowden syndrome, Werner syndrome, and Carney complex (5-8). However, most of the FNMTC patients have thyroid cancer as the only disease manifestation and are not associated with a distinct phenotype. So far, no candidate gene(s) has been discovered for this form of FNMTC and only in a minority of cases a locus of susceptibility has been identified: the locus TCO on 19p13.2 (9), the locus PRN1 on 1q21 (10) and the locus NMTC1 on 2q21 (11).

Genetic integrity is partly maintained by the architecture of telomeres and it is gradually lost as telomeres progressively shorten with each cell replication due to incomplete lagging DNA strand synthesis and oxidative damage. Telomerase is a reverse transcriptase enzyme that counteracts telomere shortening by adding telomeric repeats to the G-rich strand. It is composed

of a telomerase RNA component (TERC), that serves as a template for the addition of repeats and a protein component, telomerase reverse transcriptase (hTERT) (12). In humans this enzyme is present and active in germ cells, in adult stem cells and in activated immune cells, while it is absent, or expressed at very low levels, in adult differentiated cells and resting immune cells. In the absence of telomerase or when the activity of the enzyme is low compared to the replicative erosion, apoptosis is triggered (12). Interestingly, patients who have inherited or acquired genetic defects in telomere maintenance seem to have an increased risk of developing familial benign diseases such as dyskeratosis congenital syndrome (13) and malignant diseases such as head, neck, lung, breast and renal cancers (14). Some studies have shown that relative telomere length segregates in families (15, 16) and that decrease in telomere length may play a role in age-related genetic instability (17). On these bases, we have studied the telomere-telomerase complex based on previous report of high telomerase activity and short telomeres in cancer development (14). In sporadic thyroid carcinoma telomerase activity is detectable in nearly 50% of thyroid cancer tissues and some authors proposed that the detection of telomerase activity may be helpful in differentiating between benign and malignant thyroid tu-

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mours (18-20). Our results demonstrated that FNMTc patients display shorter telomeres, increased amplification in hTERT gene copy number and higher telomerase activity compared to sporadic PTCs in the peripheral blood. The hTERT gene amplification positively correlates with hTERT mRNA expression which is translated into a functional protein. We have also observed that the relative telomere length found in FNMTc patients of the second generation was similar (and sometimes even shorter) to that of the parents and was significantly shorter compared to unaffected siblings. This result might be explained by the evidence that average RTL is partly transmitted throughout generations (15, 16) and an X-linked pattern of inheritance and a paternal transmission has been proposed. Our patients of the first generation may also have inherited short telomere from their parents (that we could not study) or might have acquired the abnormality following mutations/alterations of specific control mechanisms, such as shelterin complex or transcription factors. Whatever the mechanism(s), our results suggest that patients born with short telomeres might reach earlier in life, the threshold telomere length sufficient to trigger cancer development and/or progression. This is consistent with the observation that our patients of the second generation were always diagnosed with thyroid cancer at an earlier age compared to their affected relative in the first generation. An intriguing finding of our study is the association of short telomeres and elevated telomerase activity. We can speculate that the high telomerase activity found in our FNMTc patients represents a mechanism of telomere stabilization which precludes DNA-damaged cells from apoptosis and contributes to their genomic instability and immortalization, or might represent an ineffective tentative of telomere repair. Both hypotheses may be supported by the inverse correlation found between RTL and hTERT gene amplification. Alternatively, exaggerated hTERT activity may be expression of an increase in hTERT gene copy number (or gene amplification), which per se represents a genetic abnormality associated with genomic instability, as recently demonstrated. In addition, we expanded our observations, demonstrating that telomere instability, represented by the formation of telomeric fusions is an exclusive feature of FNMTc patients, suggesting their non-random occurrence and the possibility to predispose to the formation of telomeric fusions in proliferating cells as a consequence of breakage/fusion/bridge cycles. FNMTc patients have a frequency of spontaneous chromosome fragility slightly higher than healthy subjects and PTCs, and show a preponderance of acentric fragments carrying telomeric sequences, hence resulting from sub-telomeric chromosomal breakage. Taken together, our observations indicate that FNMTc patients show a predisposition toward spontaneous chromosome fragility indicated by increased telomere fusions and associations and shorter telomeres which may be linked to earlier cancer development.

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