Genetic variability of the catalytic subunit of telomerase (*TERT*) in the context of its expression and telomere length in an *in vitro* cell model and in cancer patients

ABSTRACT

Telomerase is a DNA polymerase involved in the maintenance of telomere length, proliferation of stem and cancer cells, apoptosis and regeneration of damaged tissues. Germline cells, intestinal crypts, hepatocytes, stem cells, activated T and B lymphocytes, and germ cells have high levels of telomerase activity, but expression of this enzyme in somatic cells has not been identified. The activation of telomerase is a feature of most cancer cells, which enables them to reach a state of unrestricted proliferation. Telomerase is a holoenzyme with reverse transcriptase properties, an integral component of which is the RNA template (TERC), the telomerase reverse transcriptase catalytic subunit (TERT), and a complex of six proteins called shelterin. The primary task of telomerase is to extend the 3' ends of chromosomes by adding a repeated sequence of six nucleotides (5'-TTAGGG-3')n, which form segments called telomeres. Chromosomes lacking telomeres may assemble abnormally and uncontrollably, causing genomic instability and changes in the karyotype. Telomerase also performs a number of non-canonical functions, including: control of metabolic processes, epigenetic regulation of chromatin structure, participation in stress response pathways and signalling pathways. While an increase in telomerase activity does not appear to be essential for the initiation of cancer development, it is certain that it stimulates the progression and expansion of oncogenic cells by maintaining telomere lengths above a critical number of repeats, thus preventing the initiation of aging or death.

The *TERT* gene is located on the shorter arm of chromosome 5 (5p15.33) and consists of 15 introns, 16 exons and a 260 bp promoter core. The most common genetic variations include somatic mutations and changes associated with the occurrence of single nucleotide polymorphisms (SNPs). Among the mutations in the area of the *TERT* promoter (*TERT*p), we distinguish mutations located at -124 bp (*C228T*) and -146 bp (*C250T*) from the transcription start site. The occurrence of these variants results in the formation of an 11 bp nucleotide fragment, which is a new binding motif for E-twenty-six (ETS) transcription factors.

The aim of the doctoral dissertation was: 1) to determine and compare the presence of *TERT*p somatic mutations, telomerase activity, telomere length and *TERT* expression in cell lines representing haematological malignancies, solid tumours and normal lines cultured *in vitro*; 2) to determine the relationship between *TERT* genetic variants and telomere length in the context of clinical parameters in patients with acute myeloid leukaemia (AML), chronic lymphocytic leukaemia (CLL) and breast cancer; 3) to study the relationship between the genetic variation of *TERT* and the expression of *MYC*, *SP1*, *TP53* genes in the blood of women with breast cancer and in cells derived from cancer tissue - organoids; 4) to demonstrate the role of genetic variability and *TERT* gene expression as well as telomere length as potential biomarkers of selected haematological and solid tumours.

The results of the study were presented in four subsequent scientific publications, which addressed the issues related to the regulation of the *TERT* expression level, telomere length and telomerase activity in an *in vitro* cell model and in patients with haematological diseases of the myeloid and lymphoid linkage, women with breast cancer, and cells isolated from breast cancer tumours - organoids.

The first publication analysed the presence of two *TERT*p mutations, *C228T* and *C250T* using a panel of 27 *in vitro* cultured cell lines. These mutations have been identified in 5 solid tumour cell lines: glioblastoma, epidermal carcinoma, melanoma, bladder cancer and breast cancer. Cell lines with *TERT*p mutation were characterized by shorter telomeres, and *TERT* expression associated with an increase in telomerase activity. It was also shown that haematological cell lines without mutations were characterized by the highest expression of *TERT* compared to solid tumour lines and normal lines, and by an association between *TERT* expression and telomere length.

Patients with haematological diseases, acute myeloid leukaemia (AML) and chronic lymphocytic leukaemia (CLL), had shorter telomeres compared to healthy controls. AML patients over 61 years of age had longer telomeres compared to younger patients. Overall survival (OS) analyses showed that patients with the *CC TERT*p rs2853669 genotype had a shorter OS than patients with the T allele. AML patients under 61 years of age who had mutation in the tyrosine kinase gene (FLT3) had shorter telomeres and poorer OS compared to patients without this mutation. AML patients with mutation in the nucleophosmin gene 1 (*NPM1*) and no *FLT3*-ITD mutation had longer telomeres than patients with *FLT3*-ITD mutation and no *NPM1* mutation.

In the group of patients with CLL in a less advanced stage of the disease (0-I according to the Rai criterion), telomeres were noted to be longer than in the group of patients with the advanced form of the disease (II-IV). Additionally, among CLL patients with the *C* allele of *TER*T rs2736100 (intron 2), telomeres were longer in the less advanced stage of disease (according to Binet A and Rai 0–I), compared to patients in more advanced stages of Binet B-C and Rai II–IV.

In the last paper, the genetic variability of *TERT*, telomere length and the expression of a panel of genes: *TERT*, *MYC*, *SP1*, *TP53* in a group of women with breast cancer and in organoids (cells isolated from a fragment of a breast tumour) were investigated.

The analysis showed a correlation between the expression of *TERT* and *TP53*, as well as between *SP1* and *MYC* in organoids. The studied group of patients was characterized by a relationship between the expression of *TERT* and *MYC* genes, as well as *TP53* and *MYC* genes. The next part of the study concerned the genetic variability of *TERT* in the context of telomere length and clinical parameters of women with breast cancer. Patients with the *TERT* rs10069690 allele *A* (intron 4) and the *TERT* rs2736100 genotype *GG* had longer telomeres than women with other genetic variants. Patients with the *TERT* rs2736100 *T* and *TERT*p rs2735940 *C* allele, had more invasive tumours (determined by histopathology) than women with the *TERT* rs2736100 *GG* and *TERT*p rs2735940 *TT* genotypes. Additionally, patients with the *A TERT* rs10069690 allele had less frequent amplification of the human epidermal growth factor receptor 2 (HER2) gene. Analysis of the variable number of tandem repeats polymorphism VNTR-MNS16A showed that women with VNTR-234 had less invasive tumours than those with other MNS16A genotypes.

The series of publications ends with a review paper on the regulation and role of the *TERT* gene in the development of cancers.

The results of the studies described in the above publications confirmed the importance of polymorphic variability of the *TERT* gene and telomere length in the pathogenesis of chronic lymphocytic leukaemia, acute myeloid leukaemia and breast cancer. Studies suggest that differences in telomere length, the presence of specific mutations and SNPs in the *TERT* gene, as well as *TERT* expression may be potential biomarkers in haematological malignancies and breast cancer.