7. SUMMARY OF THE RESULTS AND CONCLUSIONS

1. Significantly increased telomerase activity compared to healthy controls detected already in approximately 50% of patients with early forms of MDS (RA, RARS; P = 0.0006), indicates that misbalance of telomere-telomerase complex appears as an early event in pathogenesis of MDS, when clinical features of leukemogenesis, e.g. blastic cells in bone marrow are not present yet.
2. Progression of the disease, characterized also by increasing portion of blasts in bone marrow, was accompanied by elevation of telomerase activity, but its values in patients with advanced forms of MDS (RAEB, RAEB-t) were not significantly different to those detected in early forms of MDS. Highest values were obtained in samples of patients with secondary AML from MDS. Prognostic importance of telomerase activity were pointed out by its significant inverse correlation with telomere length and also by difference in surviving: in the group with telomerase negativity 75% of MDS patients survived more than 5-years, while only 30% in the group characterized by high levels of telomerase activity. As erosion of telomere length precedes increasing of telomerase activity, telomere length is supposed to be a better prognostic factor of MDS evolution (Sieglová et al., 2004).
3. Significantly higher levels of telomerase activity in comparison to those obtained in MDS, represent a characteristic feature of majority of hematopoietic cells of patients with acute leukemia before treatment (80% of AML patients, 86% of ALL patients). Levels of telomerase activity were significantly related to portion of blasts in studied tissue, and show decreasing trend in relation to induction treatment. On the other hand, surviving of patients with AML shows no relationship to telomerase activity. Also, there were no differences in telomerase activity between different FAB subtypes of AML, except of M5 FAB subtype (only three patients!) with

significantly higher telomerase activity in comparison to M2 FAB subtype. Therefore, telomerase activity may serve as an universal marker of acute leukemia important for monitoring of treatment efficiency and prediction of relapse, especially in patients without specific molecular characteristic of leukemic cells.

4. As increasing of telomerase activity represents an early event in pathogenesis of MDS, we have supposed that also expression of genes encoding proteins involved in its signaling pathways might be deregulated. In agreement with this hypothesis, up-regulation of studied genes was observed already in patients with early forms of MDS (*hTERT* 38%, *c-Myc* 26%, *POT1* 38%, *TEP1* 22%, *TNKS* 26%, *TRF1* 38%). The highest values and their variability during the course of the disease showed expression of *hTERT* and *c-Myc* genes, where higher levels of expression were found more frequently in patients with advanced forms of MDS. Those results, together with finding of significant positive correlation between *hTERT* and *c-Myc* expression, propose importance of knowledge of their expression dynamics in a course of MDS. Although, increased *hTERT* expression, found mainly in patients with a large portion of blasts, was always accompanied by positive levels of telomerase, no significant correlation coefficients were confirmed. Expression of *c-Myc* gene was also related to expression of positive regulators of telomerase activity encoded by *TNKS* and *POT1* genes.
5. Positive results of expression of studied genes were obtained always in more than half of patients with primary AML before treatment (*hTERT* 59%, *c-Myc* 81%, *POT1* 78%, *TEP1* 83%, *TNKS* 85%, *TRF1* 52%).
6. Our results indicate that regulation of telomerase activity and telomere length is more complex mechanisms as it was supposed earlier. It seems that under certain circumstances expression of positive regulators *hTERT* and *TNKS* might be even down-regulated. This presumption is based upon our finding of lacking relationship between telomerase activity and expression its main

regulatory gene *hTERT*. On the other hand, we have showed significant correlation between telomerase activity and expression of *POT1* gene, encoding another positive regulator playing role in protection of telomere ends. The lowest values of relative expression were characteristic feature of *TEP1* and *TRF1* genes encoding proteins participating only in assembly of telomerase ribonucleoprotein particle or in negative regulation of telomerase activity and telomere length. Up-regulation of *TRF1* gene and its significant inverse correlation with telomere length may be explained by its role in down-regulation of telomerase activity based on avoiding to bind telomerase to telomere sequences. Thus, increased expression of *TRF1* gene may be recognized as a part of cell protective mechanism leading to apoptosis of leukemic cells with the same effect as is anticipated to reach by anti-telomerase therapy.

Our results indicate that expression profiles of *hTERT* gene, encoding catalytic subunit of telomerase, and *c-Myc* gene, encoding transcriptional factor, represent promising prognostic factors in MDS, although more detailed evaluation of their prognostic significance requires further clinical studies. That is why, we are currently conducting further long-term examinations of patients involved in this study in the frames of the research project no. 0002373601 supported by Ministry of Health.

As change of *hTERT* gene expression foreruns telomerase deregulation, it seems to be better prognostic factor and nonspecific molecular marker of leukemic cells. Despite of this fact, in regard to more complex regulation of telomere-telomerase complex, monitoring of dynamics of telomerase activity brings also important information as it represents a main mechanism leading to stabilization of telomeres, while bringing to leukemic cells facility of unlimited proliferation. Moreover, precise quantification of telomerase activity by modified method based on TRAP

protocol, offers an advantage for routine clinical practices, due to small amount of cells required in comparison to other techniques used in this study.

Knowledge of telomerase activity, telomere length and expression profiles of *hTERT* and *c-Myc* gene represents an important tool for better characterization of patients, especially with early forms of MDS, with the aim to specify their individual risk and to choose an optimal treatment strategy.