Review report on the PhD Thesis of Kristýna Tomášová:

Role of Telomeres / DNA Repair in Solid Tumors

The presented dissertation of Mgr. Tomášová, which aims to explore the role of telomeres and DNA repair in solid tumors, is of high quality. It is written in English in a clear scientific style and provides an up-to-date overview of the topic as well as novel results.

Formally the thesis is structured into 7 chapters, logically divided into an introduction to the topic, aims and hypotheses, material and methods, results and discussion, conclusions, references and last chapter is dedicated to 7 publications, five published and two under review.

In the introduction part she explains the molecular basis of solid tumors, stressing genomic instability as a key factor in cancer development and through telomere length (TL) shortening fostering phenotypes such as oncogene activation, tumor suppressor gene inactivation, epigenetic alterations, immune system evasion, and cellular immortalization.

The Thesis consolidates information on TL in cancer patients and healthy individuals. It examines how TL relates to clinicopathological characteristics, such as disease stage, treatment response, and prognosis. Additionally, it reviews current knowledge on DNA repair regulation at telomeres and in the nucleus, presenting new findings on oxidative damage processing by DNA glycosylases in cancer. The thesis highlights the need for further research on the significance of TL in malignant diseases and the complex interactions in DNA damage and repair management, aiming to clarify these intricate processes.

The introductory part represents a summary of state of the art knowledge about the function of human telomerases, their role in cancer, the mechanism of their elongation, DNA damage response in health and disease and specifically in telomeres, as well as how oxidative stress influences telomere functions and processes at nuclear and mitochondrial levels. This part of the thesis clearly demonstrates the high level of knowledge of the PhD candidate and her ability to synthesise scientific facts, formulate questions and hypotheses.

The objectives of the work are clearly defined, testing the hypothesis that disruptions in telomere biology might increase susceptibility to certain cancers, influence disease progression, prognosis, and treatment response.

Material and methods: The PhD candidate performed human studies with several unique human cohorts of colorectal, breast and ovarian cancer patients. She applied currently most advanced molecular biology methods such as DNA sequencing, monochrome multiplex qPCR measurement of TL, qPCR duplex TaqMan assay for Mitochondrial DNA Copy Number, gene and protein expression analysis, fragment analysis of 5 microsatellite markers for detecting microsatellite Instability, gene mutation analysis, mRNA Sequencing, Genome-wide methylation profiling, etc. demonstrating her high scientific and experimental knowledge and skills and eagerness to learn new methods. From the scientific and methodological point of view the thesis is extraordinary.

Result and discussion: The results are described in six manuscripts and one review; 5 papers were already peer reviewed and published in very good journals, which confirms the high quality of the research results. The PhD candidate examined TL and DNA repair and their crucial roles in genomic instability and carcinogenesis. She focused on TL as a marker for tumor progression and survival in colorectal, ovarian, and breast cancer patients, studying TL in both tumors and peripheral blood

lymphocytes. The research investigated TL regulation by telomerase and shelterin proteins, changes in TL and mitochondrial DNA in precancerous adenomas and adenocarcinomas, and the effects of cancer therapy on telomeres. Additionally, it explored new telomerase gene variants affecting TL and alterations in BER glycosylases in sporadic cancers. A review article also discussed DNA repair pathways at telomeres and the DNA damage response, especially in colorectal cancer. She obtained many important results, several of which can be applied in therapy.

Main findings:-

- Adenomas and cancer tissues show telomere regulation changes, with shorter TL in adenomas indicating early carcinogenesis events.
- Tumor and leukocyte TL could provide insights into survival and therapy effectiveness, but larger studies are needed for validation.
- Epigenetic changes in cancer tissues, particularly in shelterin and telomerase genes, significantly regulate TL and affect telomerase gene expression. Telomerase expression in blood is not clearly linked to pathology.
- DNA oxidation damage and inadequate repair are less common in sporadic CRC than in inherited CRC syndromes with glycosylase defects. Sporadic cancer initiation involves more diverse factors.
- TL shows promise as a cancer-related marker, useful in diagnostics, prognosis, and treatment response, but its interpretation is complex and must be validated carefully.
- Dysregulation of TL and mitochondrial DNA copy number occurs in adenomas but not in tumors, suggesting that disrupted coordination between telomeres and mitochondrial function might trigger carcinogenesis.

I have several questions to stimulate discussion:

- How does oxidative damage to mitochondrial DNA contribute to carcinogenesis?
- Is reduction of telomere length a cause or effect of the carcinogenic process?
- Why should telomere length in blood cells reflect telomere length in (pre)tumorous tissue? And why should it decrease during treatment?
- TL was reduced in CRC (tissue and blood cells) – and yet cancer cells survive by restoring TL. An apparent contradiction.

Overall, the quality of the PhD Thesis is excellent, at a high scientific level, and it meets all the formal criteria for the defense. The candidate has demonstrated the ability to think scientifically and solve a scientific problem theoretically, methodically and experimentally. The quantity, but especially the quality, of the work exceeds the requirements for a PhD and I recommend its approval.

Fjellstrand Norway, May 4, 2024,

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