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Summary of the doctoral dissertation of Justyna Wiczk

According to the World Health Organization, the number of cancer patients will increase from 14 million to 19 million per year in 2025. Therefore, search for new, more effective ways of fighting cancer is one of the key directions of research in modern medicine.

Radiotherapy is one of the most frequently used methods for the treatment of localized tumors bringing, however, the risk of a wide range of serious complications like secondary tumors. One way to minimize the side effects of this modality is to sensitize tumor cells to ionizing radiation. It can be achieved by increasing the difference in radiosensitivity between healthy and cancer cells and reducing, at the same time, the radiation dose or replacing ionizing radiation with less harmful UV rays. All these can be accomplished by employing radio- or photosensitizing compounds which sensitize the cells, especially those cancerous, to radiation.

The idea of using sensitizers is not new. However, despite numerous theoretical, experimental and even clinical studies, the mode of their action is still unclear. Issues addressed in the context of my dissertation led to the better understanding of the sensitizing mechanism of halogen derivatives of nucleosides (Hal-Nucs) as well as to the development of analytical techniques dedicated to the studies of the irradiated systems. The results of my study could, therefore, be used to develop a new, effective anticancer therapy based on the modified nucleobases.

One of the problems undertaken in my doctoral thesis was to investigate the effect of the type of sensitizer on the damage caused by attaching an electron to a Hal-Nuc incorporated into DNA. The obtained results enabled to choose the most efficient Hal-Nuc (5-BrdU and 5-BrdC), among the studied bromonucleobases, that leads efficiently to irreparable DNA damage. In the same project I demonstrated that solvated electrons were not able to fragment native DNA.

As a part of my doctoral work I conducted a research on the photosensitivity of longer DNA fragments labeled with 5-BrdU. The development of the DHPLC-based methodology, which allows simple and fast analysis of DNA damage, enabled to demonstrate that the photochemically induced DNA breaks, considered so far as forming in a primary photochemical process, are in fact secondary breaks, caused by higher temperature. Moreover, the worked out DHPLC method not only made possible the detection of DNA strand breaks,

but also other types of biopolymer degradation, such as intra-strand dimers (covalent bonds between adjacent bases). By using enzymatic digestion coupled with mass spectrometry, it was possible to propose their structure. The ability to detect and assign such products provide a valuable information from the standpoint of future tumor therapy.

Although the digestion of the damaged material allows to analyze altered nucleosides relatively easy, it is a time consuming method and leads to the loss of information related to the DNA sequence dependency of the damage. Quantitative PCR (qPCR) is free of the mentioned above disadvantage. Indeed, the employment of qPCR allowed rapid and quantitative determination of the damage without the need of DNA degradation.

The research conducted in the current work provided data that qualitatively and quantitatively describe the formation of lesions in the modified DNA. These results may be, in turn, used for a rational selection of nucleobase derivatives for further experiments on tumor cell cultures, ultimately verifying the effects of the tested compounds in target environment. For example, after the analysis of gene sequences, which encode repairing enzymes of the BER (Base Excision Repair) type, it will be possible to identify the most frequent base triplets and then, using the knowledge about particularly reactive sequences gained from the above-described tests, to select the derivative, which after incorporation to DNA, will produce the most serious damage to these particular genes. In this way a cancer cell could be deprived of its natural repair mechanisms and then a relatively low radiation dose should lead to lethal effects. Thus, my research should contribute to the development of new, safer cancer therapy. Moreover, this dissertation expands our knowledge related to the application of techniques such as qPCR and DHPLC for the quantitative identification of damage to DNA.