



Szkoła Główna Gospodarstwa Wiejskiego
w Warszawie
Instytut Biologii

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**Opracowanie systemów bioczujnikowych
przeznaczonych do wykrywania mRNA
surwiwiny i monitorowania poziomu stężenia
ATP w komórkach nowotworowych**

Development of biosensing systems for survivin mRNA detection
and monitoring of ATP level concentration in cancer cells

Rozprawa doktorska

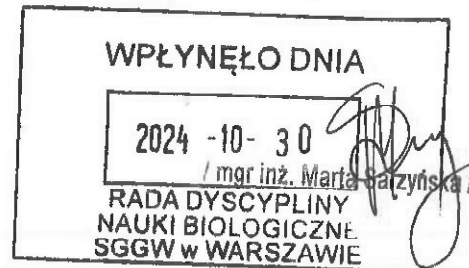
Doctoral thesis

Rozprawa doktorska wykonana pod kierunkiem

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Warszawa rok 2024

Abstract

Cancer is one of the most common causes of death worldwide, and the detection of new cases is constantly increasing every year. One of the main reasons for the high mortality rate is the late diagnosis of patients. Therefore, the potential cancer biomarkers as well as the new methods for diagnostics and treatment of this deadly disease are still being sought.

The aim of this thesis was the development of fluorescent biosensing systems based on single-stranded oligonucleotide probes, which were used for the detection of potential cancer biomarkers: (i) expression of survivin mRNA and (ii) monitoring the level of adenosine-5'-triphosphate (ATP) concentration in human cells. Two cell lines were used in the study: human colon adenocarcinoma cells SW480 and normal epithelial cells CCD841 CoN.

The conducted investigations carried out in this thesis aimed to assess the following hypotheses:

H1: Detection of survivin gene expression in cancer cells can be performed using a molecular beacon probe with a sequence complementary to the mRNA sequence responsible for survivin coding.

H2: Increased energy demand of cancer cells can be detected by the monitoring of ATP concentration level in cells using the biosensing systems based on the aptamer that binds to ATP.

The scope of the research included the development and testing of the biosensing systems attached with a fluorescent dye, based on the molecular beacon and aptamer probes. In conducted research the following methods were used: fluorescence spectroscopy, fluorescence resonance energy transfer and optical microscopy with fluorescent filters. Firstly, the biosensing systems were characterized by determination of the detection limit, selectivity and stability at various temperatures. Then, an effective method of transfecting cells with a nucleotide probe using two nanocarriers: graphene oxide and liposomes was developed. Finally, the expression of survivin mRNA was determined, the tumor cells were distinguished from the normal cells, and the concentration of ATP levels modulated by ATP synthase and glycolysis inhibitors was monitored.

The presented results are consistent with current research concerning the new methods for cancer detection and therapy. They indicate the potential of fluorescent biosensing systems utilizing for the cancer biomarker detection and application of nanomaterials as the carriers for drugs and oligonucleotides probes delivery into cells.

Keywords: oligonucleotide probes, ATP, survivin, ATP synthase and glycolysis inhibitors, fluorescence spectroscopy