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Searching for peptides interacting with the PD-1 and PD-L1 immune checkpoint proteins for cancer immunotherapies

Doctoral Thesis

Poszukiwanie peptydów oddziałujących z białkami immunologicznych punktów kontrolnych PD-1 i PD-L1 wykorzystywanych w terapiach nowotworowych

Doctoral dissertation conducted at the Department of Biomedical Chemistry
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Gdańsk 2023

Abstract

Cancer is a group of diseases that humanity is facing since the beginning of its existence. The number of cancer cases grows each year and it is estimated that it may reach around 60 million new cases by 2050. This forces the governments and scientists to work on new anticancer therapies, prevention and treatment programs. Nowadays, there are many different approaches to treating cancer. Often, the therapy combines more than one type of treatment; however, the results are not always effective. In the last two decades, a lot of attention has been devoted to the immune-oncology, especially immune checkpoints inhibition. For their work on this subject, the two scientist, Thasuku Honjo and James P. Allison, received the 2018 Nobel Prize in Physiology and Medicine. Immune checkpoints are responsible for the modulation of immune response through the regulation of T lymphocytes activity. One of the best-known and most characterized inhibitory immune checkpoint is PD-1 with its ligand PD-L1. Blocking the binding between those molecule may have many potential clinical applications and their antagonists can be applied in cases of cancer and infectious diseases.

Currently, there are many approaches to blocking the formation of the proteins belonging to the immune checkpoints bringing patients new hope and opportunities for recovery. However, FDA-approved cancer therapies are mostly based on mAbs which have many side effects and are not always effective. Moreover, the cost of the annual therapy per patient consisting of a single agent blocking the PD-1/PD-L1 complex can reach more than USD 100,000. Combination therapy, using at least two mAbs, brings better effects; however, it is even more expensive and, therefore, less available to oncology patients. All this make it necessary to search for new, better and more affordable therapies that block the PD-1/PD-L1 axis, capable of restoring the function of the immune system. In my research, I focused on finding the peptide inhibitors of the PD-1/PD-L1 complex formation, able to restore functions of the immune system. To design the peptides, I used the crystal structure of the PD-1/PD-L1 complex and information obtained from the MM/GBSA analysis.

In the first stage of my research, I obtained thirteen linear and cyclic peptides with disulphide bridges, derived from the PD-1 structure. Using the SPR technique, I determined their affinity for PD-L1. The strongest binding was received for peptides

(10) and **(7)** but six other compounds interacted with the protein. All peptides that bound to PD-L1 were chosen for further evaluation. I examined their stability in RPMI 1640 medium with 10% FBS, used in cell culture, and their influence on the viability of three cell lines used in the further experiments. For most of the tested compounds, I observed a decrease in their concentration. However, I did not observe any additional signals in the chromatograms received from the HPLC analysis, which may indicate that they were not a subject of the degradation process, but could interact with the medium components. Subsequently, I examined the effect of peptides on cell viability. I performed this assay on three cell lines - CHO-K1, Jurkat E6.1 and TCS Ctrl (modified BW5417). The Jurkat E6.1 cells were negatively affected only by the highest tested concentration of some of the examined peptides (150 μM). The CHO-K1 and TCS Ctrl were more sensitive to the compounds. The tested peptides showed a cytotoxic effect on these cell lines in a wider spectrum than in the case of Jurkat E6.1. I also examined the ability of the PD-1 derived peptides to compete with the PD-1 protein for binding to PD-L1 present on the cell surface and I determined whether their possess the ability to inhibit the formation of the PD-1/PD-L1 complex in a test by restoring NFAT mediated luciferase expression. The results of my research show that peptides **(7)** and **(10)** partially inhibit the formation of the protein-protein complex. Moreover, peptide **(7)** competes with PD-1 for binding to PD-L1.

The second approach to blocking the PD-1/PD-L1 complex consisted in designing peptides derived from PD-L1. I obtained seventeen linear and cyclic peptides with disulphide bridges. The PD-L1 derived peptides were tested in similar approaches to the PD-1 derived peptides. I examined their ability to compete with the PD-L1 protein for binding to PD-1 on the cell surface and their inhibitory properties against the PD-1/PD-L1 complex by measuring the expression of eGFP under the transcription factor NF-κB. In the competitive assay, peptides **(L1)** and **(L11)** prevent PD-L1 from binding to PD-1 in a concentration-dependent manner; moreover, peptide **(L11)** from all the PD-L1 derived peptides stimulates the eGFP expression by inhibition of the PD-1/PD-L1 complex formation in a concentration-dependent manner. This peptide also exhibits one of the strongest affinity for the PD-1 protein.

Based on the data received for the PD-1 derived peptides, I chose peptide **(10)** as parent peptide to design its analogues. I designed six compounds but only four of them were subject to further analysis due to problems arising during the synthesis. To all peptide

(10) analogues, I introduced modifications in the loop to stabilize their structure and thus improve their affinity for PD-L1. The modifications have not increased the binding strength of the peptides to PD-L1 compared to peptide **(10)**; however, they have influenced their stability and interaction with the components of the medium. These compounds also had a less negative effect on the cells used in the cell assays. In the cell functional assay, conducted to examine the competitive properties of peptide **(10)** analogues, it can be observed that changes introduced in the sequence of **A3** lead to the displacement of PD-1 from the complex with PD-L1 in a dose-depending manner. In the stimulation assay based on a reporter gene expression system (NF-κB::eGFP), peptide **(10)** and analogues **A3**, **A4** and **A5** restore the eGFP expression only at the highest concentration used.

The market of therapeutic peptides is growing annually and it is estimated to reach USD 50.60 Billion by 2026. The clinical trials focusing on the development of immune-oncology therapies using peptides and peptidomimetics in their approach, are becoming increasingly common. The molecules obtained in this work, after further modifications of their structure, may also be used as therapeutics in immuno-oncology.