Novel bispecific G-quadruplex conjugates as potential anticancer agents

Abstract

Cancer is one of the leading reasons for death in the 21st century and continues to grow. The explanations for this phenomenon may be several, however, the aging of the population can be one of the main factors for cancer. Cancer therapies can include surgery, chemotherapy, radiotherapy and one of the most promising approaches is the targeted therapy using e.g. aptamers. Aptamers are short, single-stranded DNA or RNA, that can fold in three-dimensional structures and bind to target molecules with affinity and specificity.

Since the development of aptamers through systematic evolution of ligands by exponential enrichment (SELEX) technology, G-rich aptamers has been standing out. G-rich aptamers comprise a large group of aptamers with the ability to fold into stable G-quadruplex structures. Under physiological conditions, they recognize different proteins and are capable of inhibiting the proliferation of cancer cells. Thereby, the attention for this G-rich structures, with antiproliferative activity in human cancer cells have increased is the last decade.

Tumor growth is accompanied by dysregulation of many biological pathways, so it's important for cancer therapy to develop a multifunctional therapeutic system with the ability to target more than one protein.

In this project we propose to investigate the biophysical and structural aspects of bispecific G-quadruplex constructs, with the capability of binding two different targets in cancer cells. We intend to design novel bispecific G-quadruplexes based on four single G-quadruplex units that already demonstrated anticancer properties in human cancer cell lines. The targets for the bispecific variants are, nucleolin, Stat3 and NOA1 proteins. These proteins are more expressed in cancer cells then in normal cells. The binding between proteins and single aptamers is the main mechanism of action for their anticancer properties. These facts led us to propose the research that will clarify the relationship between the structure when two G-quadruplex units targeted towards various proteins are conjugated, their ability to interact with the specific targets and the influence of conjugation on the anticancer properties of novel molecules. After determining the structural aspects of such constructs we also intend to test how various chemical modifications of the bispecific structures will improve their stability and biological activity. For this purpose, research assays will be performed *in vitro*. We are going to study the antiproliferative activity of all bispecific variants in the cervical carcinoma cell line and in breast cancer cell line. Additionally, for the most promising bispecific variants, it is also planned to analyse their stability under physiological conditions (resistance to degradation by cellular enzymes), how they are internalized by the cells and also their subcellular distribution.

This project will provide detailed knowledge about different structural aspects of bispecific G-quadruplex constructs, their interactions with specific targets and the influence of the conjugation on the antiproliferative activity of new compounds. The comprehensive study presented herein could indicate new, potent approach for effective cancer therapy and simplify the design of G-quadruplex-based drugs with promising anticancer properties in the future. Additionally, we expect to extend the knowledge about interactions between highly ordered structures of nucleic acids and proteins in general.

The results of this research work will be described in the international journals and presented at international conferences. Moreover, the realization of this project will be a significant part of PhD dissertation of PI.