ABSTRACT

Charles University, Faculty of Pharmacy in Hradec Králové, Department of Biochemical Sciences

University of Vienna, Faculty center for Pharmacy, Department of Pharmaceutical Chemistry, Laboratory of MacroMolecular Cancer Therapeutics

Candidate: Dajana Mihaličoková

Supervisor (Charles University): PharmDr. Anna Jirkovská, Ph.D.

Supervisor (University of Vienna): Univ.Prof. Dipl. Ing. Dr. Manfred Ogris

Co-supervisor (University of Vienna): Dr. Haider Sami, Ph.D.

Title of diploma thesis: *In vitro* assays for investigating nucleic acid assay

Keywords: transfection, splice correction, BCA assay, polyplexes

One of the most important tasks of biochemical research is to find out the right way how to cure cancer, genetic disorders and other illnesses which are still not curable. Towards this, gene therapy is emerging as a potential treatment owing to its ability to deliver genetic material inside the cell. Reporer gene based transfection process can be used to study gene expression. Transfection is mediated by vectors, either of viral or non-viral origin. Non-viral vectors offer several advantages over the viral counterparts like easier to synthesize, relatively cheap and the most important is their non-immunogenicity.

Cationic polymers based on polyethylenimine form complexes with plasmid DNA reffered to as polyplexes. In the present work, different types of polyethylenimine based polyplexes were employed to study gene expression and splice correction, as a measure of intracellular delivery of plasmid DNA and antisense oligos respectively. These transfection assays need protein normalization of the reporter gene data, which can be done by assays like bicinchoninic acid assay (BCA). BCA is an assay which belongs to the redox-based reactions and gives an estimate of total protein in a cell. The first goal of this thesis was to modify the currently used protocol for this assay. Both protocols can be performed but in the modified version we can directly skip one additional step during preparation of BSA standard curve. The main reason was to avoid additional step in the multi-well plate format.

The optimized protocol was then tested and used in transfection experiments. A further objective before transfection of B16 cell line with linear polyethylenimine and different types of plasmids was to confirm the healthy status of cells using Inverted Laboratory Microscope. Photos were taken before every passaging with 10x, 20x and 40x magnification. The morphology of the cells was proper in shape and colour as well. Further, the goal was to determine the expression of Thy-1.2 in cancer cells after transfection with Firefly luciferase based reporter genes under two different promoters.

In the last part of this thesis, polyplexes based on three different polyethylenimines such as linear polyethylenimine (LPEI), branched polyethylenimine (BPEI) and disulfide crosslinked polyethyleimine (c-LPEI) together with antisense oligonucleotides were investigated for splice correction *in vitro*. The aim of this part in my diploma thesis was to compare polyethylenimines and achieve splice correction by polyplex based transfections by measuring luciferase expression. BPEI and c-LPEI based polyplexes were the most efficient to correct splice switching with oligonucleotides. HeLa pLuc 705 cell line was used for the transfection and the activity was determined by Firefly luciferase assay.

In conclusion, polyethylenimine based polyplexes were efficient carriers for plasmid DNA and antisense oligonucleotide delivery in the tested cancer cells. Apart from *in vivo* experiments also *in vitro* assays can show us if insertion of nucleic acids into cancer cells were successful by measuring activity of Luciferase reporter gene assay.