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Development of an innovative drug delivery system targeted to adipose vessel utilizing novel nucleic acid aptamer for control of obesity [an abstract of dissertation and a summary of dissertation review]

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Mahmuda_Nargis_abstract.pdf (論文内容の要旨)
Obesity is one of the most serious public health problems of the 21st century worldwide. It is linked with several life threatening diseases like diabetes, cancer, cardiovascular diseases and so on. Unfortunately, still now there is no effective and safe drug in obesity treatment. Obesity i. e., growth of adipose tissue requires continuous remodeling of vascular network (angiogenesis), which play an important role for the growth of adipose tissue by supplying nutrients and oxygen. Angiogenic inhibitors can be used to inhibit angiogenesis but due to lack of active targeting it produce undesired toxicity to healthy tissues. As the primary stage of adipose tissue expansion is tightly associated with angiogenesis, therefore to inhibit angiogenesis and control obesity, ligand-based targeted drug delivery to adipose endothelial cell (EC) may introduce a highly effective and safe treatment. On the basis of this strategy, Kolonin et al. identified a peptide ligand (KGGRAKD) of prohibitin receptor expressed on the surface of vascular EC of adipose tissue and succeed to control obesity through targeted delivery with this ligand conjugated proapoptotic sequences (Adipotide). Previously, Hossen et al. have successfully developed a adipose vasculature-targeted nanocarrier referred to as prohibitin-targeted-nanoparticle (PTNP) utilizing the peptide ligand and demonstrated the effective therapy for obesity in mice via a proapoptotic peptide/protein-loaded PTNP. However, to date, no other targeting ligands to adipose vessels have been identified. Thus, in this study, to develop a novel adipose vasculature-targeted delivery system, we attempted to identify a nucleic acid aptamer, which can specifically recognize the adipose vascular endothelial cells, and to apply the identified aptamer as a ligand for targeted drug delivery system.

Generally, Systemic Evolution of Ligands by Exponential enrichment (SELEX) is the method to identify the aptamer that can bind to the target molecules, cells and tissues. It is an iterative process and continued until the saturation of targeted sequences. To identify the specific DNA aptamer against adipose endothelial cells, I had followed cell-based SELEX method. In the selection process, a library of single-strand DNA (labeled by a fluorescence dye, FITC) that contained 40-mer random sequence region flanked by two 18-mer PCR primer sequences was used. After 1st round SELEX, the binding of isolated DNA library to adipose ECs was assessed by flow cytometry. The fluorescence intensity of adipose ECs slightly increased by treatment with the 1st round library indicates that the recovered DNA molecules have the binding ability to adipose ECs. Thus, the process was carried out repeatedly. Binding assay through FACS analysis in different rounds confirms the progress of selection. The saturation of cell-SELEX was achieved after 9th cycle selection, confirmed from the merged fluorescence intensity of bound DNA between 8th and 9th cycle.
After saturation of targeted aptamers in cell-SELEX, it was necessary to eliminate the undesired aptamer candidates, which had possibility to bind to endothelial cells of other organs in the body. Thus, I followed in-vivo aptamer selection (10th cycle selection) by intravenous injection of the 9th round DNA library to mice. After isolation of in-vivo aptamers, cloning and sequencing was performed. Among 92 clones, I selected two sequences Seq1 and Seq2 having frequencies 31 and 21 respectively.

Targeting capability of selected aptamers was assessed by both in-vitro and in-vivo. For both experiments random library used as control. In-vitro evaluation was carried out through FACS analysis by the application of Seq1 and Seq2 on adipose ECs. For in-vivo targeting ability, Seq1 and Seq2 were injected into mice; bound DNA was extracted from adipose tissue and amplified. Appearance of strong DNA band in polyacrylamide gel electrophoresis revealed the homing ability of selected aptamers in adipose tissue. Usually primer regions not contribute in secondary structure formation of aptamer and target binding. But sometimes they act as functional moieties. To find out this issue for Seq1 and Seq2, we compared full-length and 40mer (Remove primer regions) Seq1 and Seq2. Similar binding pattern of full length and 40mer confirm that primers are not responsible for Seq1 and Seq2. Therefore, Seq1-40 and Seq2-40, were used for the following experiments and termed as Avecmer (Adipose vascular endothelial cell-targeted aptamer) 1 and Avecmer 2, respectively.

For the assessment of the feasibility of Avecmer1 and Avecmer as targeting ligands, I prepared the Avecmer-modified liposomes (Avecmer1-lip and Avecmer2-lip) by reverse-phase evaporation (REV) method. The rhodamine-loaded Avecmer1-lip and Avecmer2-lip was applied to adipose ECs, and then the cellular uptake of liposomes was observed by a confocal laser-scanning microscopy (CLSM). As a result, both of Avecmer1-lip and Avecmer2-lip showed the higher internalization into adipose ECs, compared to PEG5k-lip (negative control). These results demonstrated that nanoparticle modified with both Avecmer1 and Avecmer2 could be taken up into the ECs from EWAT.

Next, the feasibility of aptamer modified liposome as an in vivo carrier was assessed. After i.v.- injection of rhodamine-loaded Avecmer1-lip and Avecmer2-lip to normal mice, the accumulation of the liposomes to adipose tissue, liver and spleen was observed by CLSM. Clear fluorescence signal of Avecmer1-lip and Avecmer2-lip was appeared in the blood vessels of adipose tissue, whereas PEG5k-lip (negative control) was not. In addition, Avecmer1-lip and Avecmer2-lip was also detected in liver and spleen, however, the fluorescence intensity was similar with the PEG5k-lip. These results demonstrated that both aptamer-modified liposomes could efficiently target the adipose ECs.

Later both Avecmer1-liposome and Avecmer2-liposome were optimized for target binding based on the PEGylation of liposome surface with different density and length of PEG (polyethylene glycol). Study revealed the high density of longer PEG on liposome surface was the best composition for bind to target. From the study, it can be concluded that I could isolate high affinity DNA aptamers to adipose tissue vasculature through the combination of cell-based and in-vivo SELEX for the first time. By using the high affinity DNA aptamers, I successfully developed the targeted nanoparticle, which could efficiently internalize into adipose ECs in vitro and in vivo. In future, we would like to apply the aptamer-targeted nanocarrier system to an effective and safe nanotherapy for the control of obesity.