Studies on the regulation of gene expression and immune activation by stimuli responsive artificial nucleic acid

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(Abstract)

In recent years, tailor-made therapy that provides the optimum treatment for each individual has been attracting attention. Nucleic acids are becoming increasingly important as tailor-made therapeutic molecules mainly for the ease with which the specificity for a vast range of targets of these drugs is achieved. Therefore, many researchers in academia and pharmaceutical companies are paying attention to the development of nucleic acid based drugs. In particular, stimuli responsible nucleic acid drugs have a great potential for organ specific therapy. In this study, the author suggests the high photo-responsible antisense oligonucleotides and radiation trigger of artificial CpG oligonucleotide (CpG ODN).

Antisense oligonucleotides are valuable tools for selectively inhibiting target gene expression. Recently, Higuchi et al. reported that a photocrosslinkable oligonucleotide containing psoralen moiety at the 2′ position of adenosine effectively and specifically inhibits the expression of the codon 12 point-mutated K-ras (G12V) gene in living cells. Because of the specific and irreversible complexation between photoreactive AS-ODN and the target mRNA, the regulation of gene expression was achieved with a low concentration of AS-ODN (IC_{50} = 50 nM) and UV irradiation. This method has the potential to provide a location-specific drug that can regulate gene expression by photoirradiation at the desired location in the body; however, the requirement of relatively long photoirradiation because of the low photoreactivity of AS-ODN may cause undesired toxicity to healthy cells. In this study, the author tried to develop other photoresponsive AS-ODNs using a photoresponsive nucleoside analogue, 3-Cyanovinylcarbazole nucleoside (^{CNV}K), which can quickly photocrosslink to the pyrimidine base in complementary RNA strands with only a few seconds of photoirradiation. ^{CNV}K was incorporated into antisense oligonucleotides (^{CNV}K-ASs), and the author evaluated the photoreactivity and the sequence selectivity to mutated K-ras oligoRNAs, as well as the regulation of the function of K-ras mRNA. In this result, the ^{CNV}K-AS is quickly and selectively photocrosslinked to ORNs having mutated K-ras sequences by a few seconds of photoirradiation and the selectivity can be enhanced by adopting the mutated pyrimidine base as the photocrosslinking site of ^{CNV}K. The ^{CNV}K-AS clearly photocrosslinks to its target mRNA in a sequence selective manner and then the mRNA function is clearly regulated with only 1 second of photoirradiation suggesting that ^{CNV}K-AS have the potential to be effective photodynamic antisense drugs that act at desired locations around the body with a few seconds of photoirradiation. Moreover, the author successfully demonstrated that ^{CNV}K-AS that target GFP mRNA, effectively regulate GFP expression in a stable cell line expressing the GFP gene in a photo-responsive manner and that the expression level of GFP can be controlled temporally by 10 seconds of 366 nm irradiation. These photoreactive antisense oligonucleotides can be effective in the spatiotemporal regulation of endogenous gene expression in living cells.

CpG ODNs are short single-stranded synthetic DNA molecules that induce TLR9 mediated-immune activation. The author has synthesized disulfide crosslinking tethered CpG oligonucleotide (S-S CpG) as a radiation-trigger immune activator. The S-S CpG had an alkyl chain and two CpG DNA units were connected by an X-ray-sensitive disulfide bond. X-irradiation of Raw cells, to which S-S CpG was administered, resulting in an induced Ifnb mRNA expression because of the reductively-cleaved CpG immune activation. Thus, S-S CpG are promising as radiation-trigger immune activators that are applicable to drugs.

Key word : Tailor-made therapy, Stimuli responsible nucleic acid, Antisense method, CpG DNA