Construction of sonication responsive promoters to control
gene expression with ultrasound

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Promoters responsive to sonication could be a useful tool for efficient gene therapy since, with such promoters, expression of a therapeutic gene could be controlled by sonication. However, rational design and construct of artificial promoters of interest would be most difficult since relationship between nucleotide sequence and environment around the binding site (modification and structures of DNA, other transcription factor existence, etc.) affecting actual transcription factor binding has not been completely elucidated. I focused attention on oxidative stress condition of cells caused by sonication with a certain condition to construct sonication responsive promoters. Synthesized DNA oligomers containing binding motifs of transcription factors including AP-1, NF-κB, NF-Y and CBF-A, which are activated by oxidative stress, were randomly ligated and linked to a TATA box sequence to construct artificial promoters. These promoters were introduced upstream of the luciferase gene to control its expression. A plasmid with such a gene cassette was transfected into HeLa cells and sonicated with 1 MHz ultrasound at 1 W/cm\textsuperscript{2} and 10\% duty factor for 60 sec. Transfected cells with two out of eleven plasmids significantly increased luciferase activities compared with each of unsonicated controls 6 h after sonication. The enhancement of the best promoter peaked at 9 hr and subsided at 24 hr after sonication. The reactivity of the promoter to sonication was improved by random point mutations introduced into its nucleotide sequence. The most improved promoter was stably transfected into HeLa cells to establish a cell line, designated HeLa 11-9-37, showing about 8 fold enhancement 6 hr after sonication with 1 MHz ultrasound at 0.5 W/cm\textsuperscript{2} and 10\% duty factor for 60 sec. The established cell line was transplanted in the both flanks of a nude mouse. When a tumor was sonicated with 1 MHz ultrasound at 2.0 W/cm\textsuperscript{2} and 10\% duty factor for 120 sec, enhanced luciferase activity of the tumor to about 1.3 times was observed, suggesting possibility of gene therapy with ultrasound for controlling the expression of a therapeutic gene though the degree of enhancement was much attenuated.

\textit{Key words: promoter, cis-acting element, gene expression, transcription, TATA box.}