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学　位　論　文　要　旨

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学位論文題目

G-quadruplex structures as transcriptional regulators in *Dele* and *Cdc6* CpG islands, and as targets for DNA methylation detection

学位論文の要旨

DNA adopts the canonical double helix structure in most cases, but the formation of secondary DNA structures, such as G-quadruplex (G4) DNA and i-motif, have also been found in human genomic DNA. G4 structures can be formed by the folding of guanine (G)-rich sequences found in functional regions of the genome, such as telomere, gene promoters and cytosine-phosphatidyl-guanine (CpG) islands (CGIs). The existence of putative G4 sequences in such important regions suggests the involvement of G4 structures in several biological functions, such as the inhibition of telomerase, transcriptional regulation and epigenetic modulation and other regulatory mechanisms. In previous study, 1998 G4 forming sequences were identified using a fluorescent-labeled G4 ligand in mouse CpG islands DNA microarray. G4 formation was verified for 10 randomly selected sequences among the putative G4 forming sequences by CD spectroscopy and DMS footprinting analysis. The aim of this study is to investigate the biological function of G4 structures in the transcriptional regulation using luciferase reporter assay and to analyze effect of methylated G4 structures on the transcription.

Results have shown that the luciferase expression was activated when G4-forming sequences from the *Dele* and *Cdc6* genes have been cloned individually on reporter vectors carrying a minimal promoter and the luciferase gene, and also have been detected in experiments applying a promoterless reporter vector. Mutational analysis has proved the significance of guanine bases involved in G4 formation for the transcriptional activation. Furthermore, the transcriptional activity of *Dele*-F and *Cdc6* was decreased the activation by 35% to 37% in the presence of the telomestatin derivative L1H1-7OTD. Circular Dichroism (CD) spectroscopy was performed to analyze the effect on G4 structure after binding and

stabilization by the ligand L1H1-7OTD.

Sequences of *Dele*-F, *Dele*-R and *Cdc6* CpG islands, containing the G4-forming sequences, have been cloned in the promoterless reporter vector to study the role of G4 sequences within CGI regulatory region. Results demonstrated a high transcriptional activation by *Dele*-F. Mutational analysis reveals that the expression level was decreased by mutation on *Dele* G4; however, increased by mutation on *Cdc6* G4. These results indicated that *Dele* and *Cdc6* G4 formation is significant in the transcriptional regulation. It was concluded from the reporter assay results that *Dele* and *Cdc6* G4 DNAs individually possess enhancer and promotor function; however, when studied in more complex CpG islands *Dele* G4 also demonstrates promotor activity, whereas *Cdc6* G4 may possess a dual function of transcriptional regulation.

In mammalian genome, DNA methylation occurs in CGIs, which contain G-rich sequences candidates for G4 formation, causing epigenetic modification. In cancer cells, the DNA methylation level may raise and cause hypermethylation, which leads to genes overexpression of oncogenes, or hypomethylation that may speed up the tumorigenesis. Therefore, Several studies have proposed DNA methylation as potential biomarker for cancer diagnosis. Methylating G4 structure has been reported to stabilize G4 structure. In this study, to investigate the effect of methylated G4 on transcription, methylated and unmethylated G4-forming sequences of mouse *Dele* and human *DELE* were utilized in a reporter assay. Results have demonstrated an inhibition by 30% with methylated mouse *Dele*, and enhance of transcription by 290% with human *DELE* G4 DNA. DNA methylation has been generally known to suppress gene expression; therefore, the activation of transcription with *DELE* G4 needs furthermore study for it may contribute to elucidate a new transcriptional regulation mechanism using methylated G4 structures.

In addition, a new DNA methylation detection system was developed utilizing G4 and i-motif forming sequences. This detection system does not require sodium bisulfite treatment or methylated DNA ligands, which makes this system a remarkable time- and cost-saving method. Our hypothesis suggests that stabilized G4 and i-motif structures by DNA methylation may arrest DNA polymerase activity during quantitative polymerase chain reaction (qPCR). G4 and i-motif forming sequences from *VEGF* and *RET* were used as templates in qPCR, and PCR amplification efficiency was analyzed. The results showed decreased PCR amplification efficiency with increasing DNA methylation levels in the G4 and i-motif forming sequences. The difference in the amplification efficiency between unmethylated and methylated DNA templates was detected at 1.0×10^6 to 1.0×10^9 copies of *VEGF* DNA, and 1.0×10^5 to 1.0×10^9 copies of *RET* DNA. Quantitative PCR results have demonstrated that DNA methylation of the G4 and i-motif forming sequences can be detected by analyzing the amplification efficiency using qPCR.

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Title of thesis ：

G-quadruplex structures as transcriptional regulators in *Dele* and *Cdc6* CpG islands, and as targets for DNA methylation detection

G-quadruplex (G4) is a DNA secondary structure that has been shown to play an important role in biological systems. In this study, the biological functions of selected G4 sequences were studied using luciferase reporter assay. Results have shown that the luciferase expression was activated when G4-forming sequences from the *Dele* and *Cdc6* genes have been cloned in reporter vectors carrying a minimal promoter and the luciferase gene, and also have been detected in experiments applying a promoterless reporter vector. Furthermore, the activation was decreased by the telomestatin derivative L1H1-7OTD that can bind to G4 structure and stabilize it. When *Dele*-F, *Dele*-R and *Cdc6* CpG islands sequence were cloned in the promoterless reporter vector, the luciferase expression was activated with *Dele*-F CGI sequence and was inhibited by *Cdc6*, indicating that G4 formation is significant in these sequences for the transcriptional regulation. In conclusion, *Dele* and *Cdc6* G4 DNAs individually possess enhancer and promotor function; however, when studied in more complex CpG islands *Dele* G4 also demonstrates promotor activity, whereas *Cdc6* G4 may possess a dual function of transcriptional regulation.

DNA methylation has been considered to be a potential biomarker for cancer diagnosis. Methylated G4-forming sequences of mouse Dele and human DELE were studied to analyze the effect of methylated G4 on transcription. Additionally, a new DNA methylation detection system was developed utilizing G4 and i-motif forming sequences, based on the hypothesis that methylated G4 and i-motif structures may arrest DNA polymerase activity during quantitative polymerase chain reaction (qPCR). The results showed negative correlation between PCR amplification efficiency and methylation levels in the G4 and i-motif forming sequences. Results have demonstrated that DNA methylation of the G4 and i-motif forming sequences can be detected by analyzing the amplification efficiency using qPCR.