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Stabilization of G-quadruplex DNA by C-5-methyl-cytosine in *bcl-2* promoter: Implications for epigenetic regulation

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ABSTRACT

The C-5-methylation of cytosine in the CpG islands is an important pattern for epigenetic modification of gene, which plays a key role in regulating gene transcription. G-quadruplex is an unusual DNA secondary structure formed in G-rich regions and is identified as a transcription repressor in some oncogenes, such as *c-myc* and *bcl-2*. In the present study, the results from CD spectrum and FRET assay showed that the methylation of cytosine in the CpG islands could induce a conformational change of the G-quadruplex in the P1 promoter of *bcl-2*, and greatly increase the thermal-stability of this DNA oligomer. Moreover, the methylation of cytosine in the G-quadruplex could protect the structure from the disruption by the complementary strand, showing with the increasing ability to arrest the polymerase in PCR stop assay. This data indicated that the stabilization of the G-quadruplex structure in the CpG islands might be involved in the epigenetical transcriptional regulation for specific genes through the C-5-methylation modification pattern.

1. Introduction

The methylation of cytosine in gene CpG islands has been found to be a critical epigenetic modification pattern that regulates the gene transcription and decides the chromatin architecture [1]. So far, it has been widely accepted that the aberrations of DNA methylation are tightly correlated with the gene expression, and hypermethylation in the CpG islands of gene promoters can lead to gene silencing. However, how the methylation status of gene is involved in the regulation of the gene transcription or expression still remains unclear.

Bcl-2 protein belongs to a large apoptosis-related protein family [2]. By interferring the programmed cell death [3], Bcl-2 protein regulates the carcinoma growth in the early stage of tumour [4]. The previous studies have reported that the anti-apoptotic protein Bcl-2 is overexpressed in many solid tumors [5], and the overexpression also contributes to the resistance to the radio therapy or the chemotherapies by inhibiting the response of cells to the apoptotic signals trigerred by these agents [6]. Recently, it has been reported that the CpG islands in the *bcl-2* promoter are hypomethylated, that subsequently leads to the overexpression of Bcl-2 protein in colorectal cancer [5]. In addition, it has been shown that the *bcl-2* promoter will turn off if the dinucleotides in its CpG island are hypermethylated, while the mechanism has still been unknown [7].

Recently, researchers have mainly focused on the influence of the methylation of DNA on the chromatin structures and chromatin compaction, while the impact of hypomethylation or hypermethylation on DNA secondary structure in CpG islands has barely been studied. The CpG island of *bcl-2* promoter contains guanine-rich sequence possibly forming intramolecular G-quadruplex DNA, just like in the promoters of other oncognes, such as *c-myc* [8], and *c-kit* [9]. The G-quadruplex can act as an repressor on the transcription, that resulting in the decreased rate of transcription and expression. Moreover, the recent study has revealed that the G-quadruplex formed in the telomeric end is decreased by half if a guanine involved in the middle G-quartet is methylated at its 6-O position [10], indicating the introduction of methyl group has an impact on the stability of the G-quadruplex structure. There is another report showing that the methylation of cytosine greatly enhances the stability of intermolecular quadruplex formed in the oligomer $d(CGCG_3GCG)_4$ *in vitro* [11]. However, neither of these studies have revealed the real situation *in vivo*, since the 6-O-methylation does not occur naturally *in vivo* and the oligomer $d(CGCG_3GCG)_4$ is not a nature sequence in genome.

Thus, we were wondering whether the methylation of cytosine in the CpG island within the *bcl-2* promoter might influence on the stability of the G-quadruplex formed in the region, and ultimately

Abbreviations: CD, circular dichroism; FRET, fluorescence resonance energy transfer; PCR, reverse transcription-polymerase chain reaction; RMSD, root mean square deviation; FAM, 6-carboxyfluorescein; TAMRA, tetramethylrhodamine.

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lead to the activation or repression of the gene transcription. In the present study, CD spectrum, FRET melting assay, duplex-strand competition assay, PCR stop assay, and molecular dynamic simulation were applied to investigate whether the methylation of cytosine could influence on the conformation and stability of intramolecular G-quadruplex structure formed in *bcl-2* promoter. The results showed that the methylation of cytosine greatly enhanced the stability of G-quadruplex structure and therefore increased the protection ability from competitive disruption by its complementary strand. It is the first study on the relationship between the natural methylation pattern, the methylation on the 5-C of cytosine, with the switch of DNA secondary structures in human oncogene promoters, implicating a hypothesis that G-quadruplex structures in the oncogene promoters might be involved in the epigenetical regulation via the methylation of cytosine.

2. Materials and methods

2.1. Materials

All the DNA oligomers used in the present study were purchased from Sangon Biotech (Shanghai, China). For the fluorescence spectroscopy, the oligomers were labelled with 5′-FAM and 3′-TAMRA. The oligomers were dissolved using ddH_2O to the stock concentration of 100 μM and diluted to working concentration using different buffer. The oliogmers were heated at 95 °C for 10 min, and then slowly cooled to room temperature to form G-quadruplex.

2.2. CD spectrum measurements

CD spectrum measurements were performed on a chirascan circular dichroism spectrophotometer (Applied Photophysics) with a 0.2 mm pathlength cylindrical quartz cuvette. The oligomers S-G and S-M-G were diluted from stock to the correct concentration (5 μM) in Tris-HCl buffer (10 mM, containing 100 mM KCl, pH 7.4). The temperature of the sample was maintained using a Quantum (Northwest) temperature controller. Data were collected from 340 to 220 nm at 25 °C and substrated by the data of the buffer.

2.3. Fluorescence melting assay

Fluorescence melting curves were determined using a real-time PCR machine (Roche LightCycler 2®), with 0.2 μM of labelled oligomet in the 10 mM Tris–HCl buffer (pH 7.4) containing 100 mM KCl of a total reaction volume of 20 μL. Fluorescence readings with excitation at 470 nm and detection at 530 nm were taken at the intervals of 1 °C over the range of 37–99 °C, the samples were maintained for 30 s at specific temperature prior to each reading. Final analyses of the data were carried out using Origin 7.5 (OriginLab Corp.).

2.4. Fluorescence spectrum measurements

Fluorescence spectrum measurements were carried out on a Perkin Elmer LS55 fluorescence spectrometer at 25 °C. The excitation and emission slits were both 10 nm. Excitation was set at 480 nm, and emission was collected from 490 to 650 nm. A buffer blank was subtracted for all the data. In all experiments, 3 ml of fluorophor-labelled G-rich oligomers (S-G and S-M-G) were used at the concentration of 10 nM in Tris-HCl buffer (10 mM, pH 7.4) with or without 100 mM KCl, and 3 μl of unlabelled complementary C-rich oligomer (S-C) was titrated to reach a final concentration of 10 nM. The formation of duplex DNA was determined using the fluorescence intensity of the donor FAM at 518 nm. All the samples were incubated at 25 °C for 24 h to reach the equilibrium before measurement.

Table 1
Sequences of oligomers used in this study.

Oligomer	Sequence (from 5′ to 3′)
S-G	d(CGGGCGCGGGAGGAAGGGGGCGGGAGC)
S-M-G	d(CGGGCGCGGGAGGAAGGGGGCGGGAGC) C1,C5,C7,C21 were methylated
S-C	d(GCTCCCGCCCCCTTCCTCCCGCGCCCG)
S-G-rev	d(ATCGATCGCTTCTCGTGCTCCCGCCC)

The ratio of the remaining G-quadruplex for both S-G (the wild type oligomer) and S-M-G (the methylated oligomer) were calculated with the following equation:

$$\text{Ratio} = (F_1 - F_2)/F_1$$

*F*1 represents the fluorescence intensity after the addition of the complementary S-C at equal molarity in the buffer at the absence of KCl (which was set as a duplex full formation control). *F*2 represents the fluorescence intensity after the addition of the complementary S-C at equal molarity in the buffer at the presence of 100 mM KCl.

2.5. PCR stop assay

The sequences of the test oligomers used in the present study, including S-G, S-G-M and the corresponding complementary sequence S-G-rev, were listed in Table 1. The reactions were performed in 20 μl of 1× PCR buffer, containing 10 μmol of each pair of the oligomers, 0.16 mM dNTP, 2.5 U *Taq* polymerase, and KCl or $MgCl_2$ at the indicated concentrations. Reaction mixtures were incubated in the Eppendorf® thermocycler, with the following cycling conditions: 94 °C for 3 min, followed by 10 cycles of 94 °C for 30 s, 58 °C for 30 s, and 72 °C for 30 s. Amplified products were resolved on the 16% nondenaturing polyacrylamide gels in the 1 × TBE buffer and stained with Gel Red dye (Biotium).

2.6. Molecular modeling

The structure of mixed-type quadruplex was generated from the reported NMR structure (PDB code 2F8U[12]), by replacing the T15 and T16 with G15 and G16. While the structure of parallel quadruplex was generated from reported X-ray structure (PDB code 1KF1[13]), and necessary modifications were carried out including replacements and additions of bases. The methylated quadruplex structure was then generated by replacing the appropriate cytimidine residue with the methylated residue (C5-methylcytimidine). The partial charges of 5-C-methylcytimidine were computed using the HF/6−31G* basis set from GAUSSIAN 03 [14]. The other parameters for 5-C-methylcytimidine were assigned by analogy with the AMBER ff99 force field [15]. Two K^+ ions were placed manually in the central core of the complexes. Every ligand–receptor system was then solvated in a truncated octahedron box of TIP3P water molecules with a 10.0 Å buffer along each dimension. Additional positively-charged counter ions were added in the system to neutralize the negative charges on the DNA backbone. The AMBER ff99 force field [15] was applied for G-quadruplexes, ions, and water molecules. Periodic boundary conditions were applied to avoid the edge effect. The Particle Mesh Ewald (PME) method was used to calculate long-range electrostatic interactions with a 10 Å residue-based cutoff [16]. The hydrogen bonds were constrained using SHAKE algorithm [17]. The final systems were subjected to initial minimization to equilibrate the solvent and counter cations, and were then heated from 0 to 300 K in a 100 ps simulation, and followed with a 100 ps simulation to equilibrate the density. Afterwards, 4 ns constant pressure MD simulation was performed in an NPT ensemble at 300 K. All the calculations were carried out with the SANDER module. The MM/

PBSA method was used to calculate the free energy [18]. All the waters and counter ions were stripped off, but the K^+ ions present within the central channel were included. K^+ radius was set to 2.025 Å [19]. A total of 100 snapshots were taken from the last 2 ns trajectory with an interval of 20 ps.

3. Results

3.1. Methylation of the cytosine induced conformational change of G-quadruplex

The CD spectrum was applied to evaluate the G-quadruplex structures of the DNA oligomers after methylation of cytosine. The wild-type G-rich sequence in the *bcl-2* P1 promoter, S-G (Table 1), exhibited a small negative peak near 240 nm and two positive peaks at 270 and 295 nm at the presence of potassium (Fig. 1A), which represented a mixed parallel/anti-parallel conformation and was consistent with that in the previous reports [12]. However, the modified sequence S-M-G (Table 1) with four 5-methyl-cytosine substitutions displayed a conspicuous positive peak at 265 nm and a negative peak at 240 nm, together with a significant decrease of positive peak at 295 nm, indicating the vanishing or decrease of the anti-parallel G-quadruplex conformation, and the conversion of anti-parallel conformaion to a characteristic parallel conformation [20].

3.2. Methylation of the cytosine enhanced the stability of G-quadruplex

Since it has been known that the parallel conformation of G-quadruplex is more stable than the anti-parallel conformation, the conformational conversion observed in the CD measurements suggested that the methylation of the cytosine is likely to affect the stability of the G-quadruplex forming in the *bcl-2* promoter. Hence, the fluorescence resonance energy transfer (FRET) melting assay was applied to study the thermodynamic stability of the G-quadruplexes formed by the modified and wide-type oligomers. The oligomers were labelled with fluorophor FAM at the 5′ end as donor and tetramethylrhodamine (TAMRA) at the 3′ end as acceptor, and the emission intensity change at 530 nm was measured to identify the unwinding of the G-quadruplex in 10 mM Tris–HCl buffer containing 100 mM K^+. The melting curves were shown in Fig. 1B, and the calculated T_m value of the S-G oligomer was 46.9 °C and the T_m value of S-M-G was 55.8 °C. It is clear that the methylation of the cytosine raised the melting temperature of the G-quadruplex by about 9 °C, exhibiting a great activity on stabilizing the G-quadruplex structure.

To further investigate the effect of methylation on the G-quadruplex structures, molecular dynamics (MD) simulations were performed. Based on the CD results, the mixed-type and parallel quadruplex structures were built from the reported NMR structure (PDB ID: 2F8U [12]) and X-ray structure (PDB code 1KF1 [13]), respectively. Necessary modifications were carried out including replacements and additions of bases. The methylated quadruplex structure was then generated by replacing the appropriate cytosine residue with the methylated residue (C-5-methylcytosine). After an equilibration period, each starting structure had an unstrained production trajectory of 4 ns at 300 K (RMSD plots were shown in Fig. S1). Fig. 2 showed the average minimized structure of the wide-type sequence S-G and the modified sequence S-M-G over the last 2 ns trajectories with an interval of 20 ps. MM-PBSA method was further used to evaluate the free energy of the models (Fig. 2). For the mixed-type models, the estimated free energy of S-G was lower than that of S-M-G; while for the parallel-type models, the estimated free energy of S-G was significantly higher than that of S-M-G. Because of this free energy difference, the G-quad-

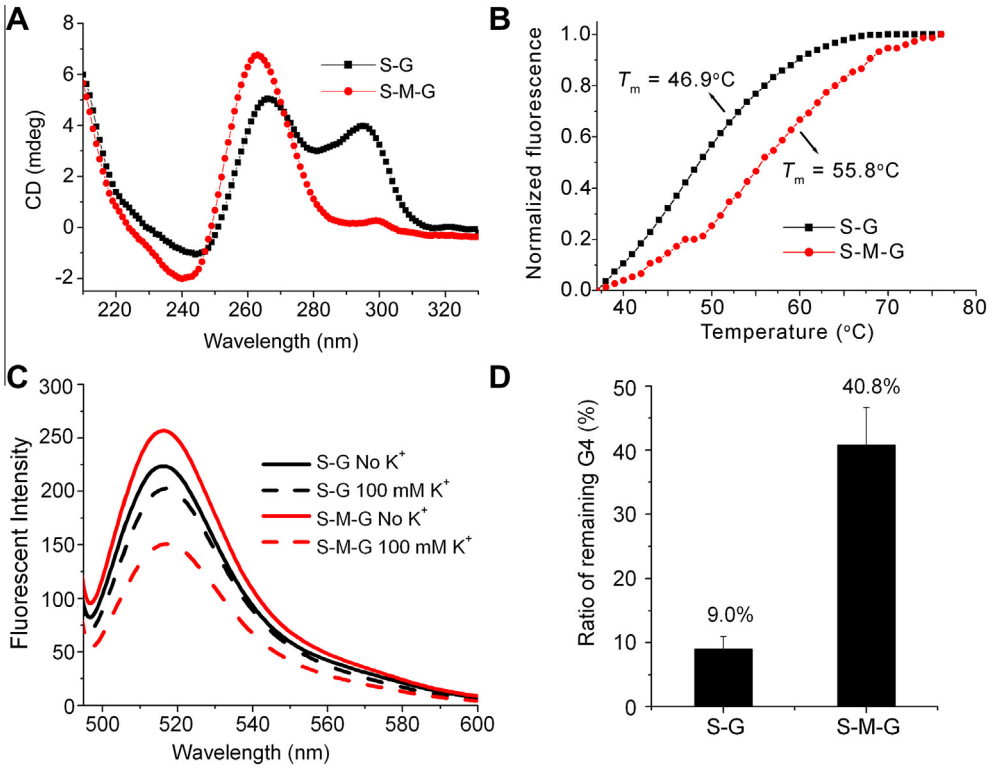

Fig. 1. (A) CD spectrum of the S-G (wild-type) and the S-M-G (methylated) oligomers in the 10 mM Tris–HCl buffer containing 100 mM K^+. (B) Fluorescence melting assay of the S-G and S-M-G oligomers in the Tris–HCl buffer containing 100 mM K^+ monitored at 530 nm. (C) FRET spectrum of the S-G or S-M-G at 10 nM with the S-C with equal molarity in the Tris–HCl buffer containing 100 mM K^+ or without K^+ at the equilibrium state (24 h after titrated with the complementary strand S-C). (D) The ratio of remaining G-quadruplex of the S-G and S-M-G oligomers at the equilibrium state. The ratio were caculated using the equation in methods section.

ruplex in the *bcl-2* promoter preferred to form the comparatively stable mixed-type model rather than the parallel conformation in the hypomethylated status. However, when the methylation happened, it was difficult for this sequence to retain the mixed-type conformation, and experienced a transition spontaneously to give a more stable parallel-type conformation. This hypothesis was further supported by the relatively higher backbone RMSD values in the mixed-type S-M-G and parallel-type S-G models compared to those in the mixed-type S-G and parallel-type S-M-G models (Fig. S1). Importantly, the lowest free energy was found for the parallel S-M-G, which was correlated with the experimental results, showing that the methylation of the cytosine made G-quadruplex structure more stable with a higher thermodynamic stability.

3.3. Methylation of the cytosine enhanced the ability on competition of G-quadruplex forming from duplex DNA

Since the stability of G-quadruplex was greatly enhanced by the methylation from the above data, whether the methylation of the cytosine could alter the competition between G-quadruplex and duplex formation in the presence of the complementary strands was further investigated using FRET duplex competition assay. The labelled oligomers, S-G and S-M-G, were titrated with the complementary strand S-C (Table 1) at the equal molarity. All the samples were incubated at 25 °C for 24 h to achieve the equilibrium state after the addition of S-C. As shown in Fig. 1C, the final fluorescence intensity of the modified oligomer S-M-G in a Tris–HCl buffer containing 100 mM K^+ was obviously lower than that in a Tris–HCl buffer without K^+, while the final fluorescence intensity of the wide-type oligomer S-G was almost the same in these two conditions. Further calculated data indicated that a considerable proportion of G-quadruplex formed by the S-M-G was retained (40.8%, Fig. 1D) in the presence of the S-C at the equilibrium state; while for the S-G, little G-quadrulex was preserved after the addition of the S-C (the calculated proportion of remaining G-qudruplex was only 9.0%, Fig. 1D). The proportion of the remaining G-quadruplex formed by the modified oligomer was significantly higher than that formed by the wild-type oligomer, indicating the methylation of the cytosine had profound impact on the competition by enhancing the competitive ability of the G-quadruplex from the disruption of the complementary strand.

3.4. The methylated oligomer exhibited a stronger inhibitory effect on DNA elongation process

PCR stop assay was performed to simulate the *in vivo* transcriptional process catalysed by DNA polymerase based on the assumption that DNA polymerase is incapable of traversing a stable DNA secondary structure [21]. The stable formation of G-quadruplex could block the 5′ to 3′ extension by the *Taq* polymerase and decrease the amount of the final double-stranded PCR products. Especially, the more stable the G-quadruplex is, the less PCR product is detected. As shown in Fig. 3, the methylated oligomer had a much stronger inhibitory effect on the DNA elongation process than the wild-type one in all three tested conditions (the PCR buffer, the PCR buffer containing K^+ at the physiological concentration, and the PCR buffer containing both K^+ and Mg^{2+} at the physiological concentration), which indicated that the stability of G-quadruplex induced by the methylation of the cytosine was possibly an indispensable factor for repressing gene transcription.

4. Discussion

In the present study, the effect of the methylation of the cytosine on the DNA quadruplex formed in the human oncogene *bcl-2* promoter was elucidated. We found that the methylation of the

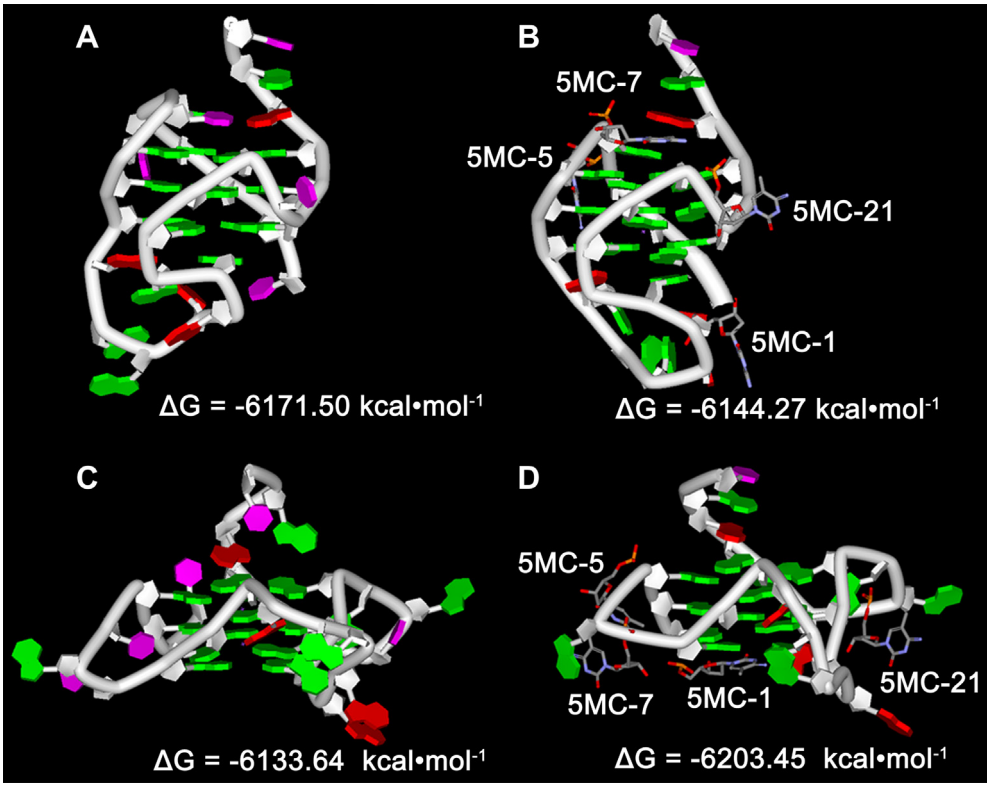

Fig. 2. Average structures from MD trajectories in K^+ buffer. (A) S-G, mixed-type. (B) S-M-G, mixed-type. (C) S-G, parallel-type. (D) S-M-G, parallel-type. The methylated residue was drawn in stick form and marked with '5MC-'. The G, A, and C residues were shown in green, pink, and red, respectively. (For interpretation of the references to colour in this figure legend, the reader is referred to the web version of this article.)

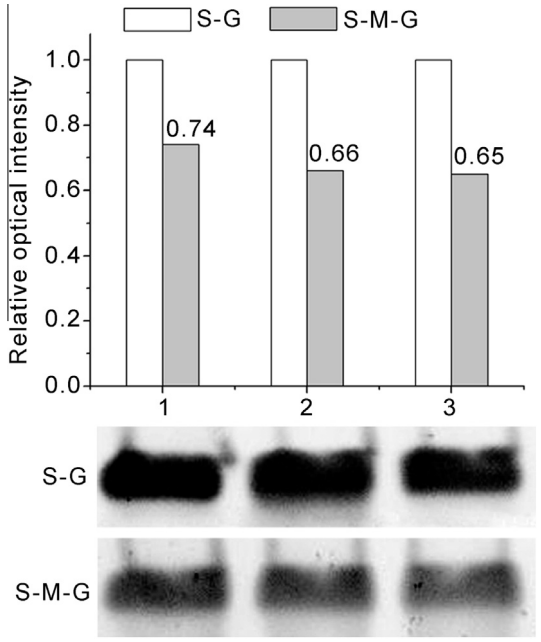

Fig. 3. The effect for methylation of the cytosine on PCR stop assay in various systems (1) PCR buffer (50 mM K^+ and 1.5 mM Mg^{2+}), (2) 100 mM K^+ and 1.5 mM Mg^{2+}, (3) 100 mM K^+ and 2.5 mM Mg^{2+}. The quantitative calculation of the results was shown in histogram, where the optical intensity of S-G was used as reference and considered to be 1.

cytosine in the quadruplex DNA of *bcl-2* promoter induced an obvious transition from mixed parallel/anti-parallel conformation to a more stable parallel conformation. The calculated T_m values in the FRET melting assay showed good correlation with the above finding. DM simulations were also carried out to evaluate the free energies of the four possible structures of the G-quadruplex formed by methylated or wild-type sequences, showing that when the *bcl-2* promoter was unmethylated, the DNA preferred to form the mixed-type G-quadruplex due to its comparatively lower free energy. However, the G-rich region in the *bcl-2* promoter exhibited an obvious conformational transition to the parallel form when the cytosine was methylated, which also showed good correlation with the experimental data. It has been reported that the methylation of the cytosine stabilized the complex primarily by producing favourable entropic (base–base stacking) interactions through dipole/induced dipole forces [11], which might explain our finding in the present study. In addition, due to the enhanced stability of the G-quadruplex, the proportion of unwinded DNA quadruplex formed by the methylated oligomer was much lower than that of the wild-type oligomer in the competition assay. Since the initiation for transcription *in vivo* involves the dissociation of the duplex DNA and subsequently the binding of polymerase to the single-stranded DNA, the results from our competition assay suggested that the methylation of the cytosine might increase the chance of G-quadruplex formation *in vivo* during transcription. PCR stop assay further showed an evidence for this hypothesis, that the methylated oligomer displayed a stronger inhibitory effect on DNA elongation process compared with the wild-type oligomer.

Basing on all the above data, we raised a hypothesis that the formation of G-quadruplex structure within the *bcl-2* promoter region could be an important step involved in the inhibition process on *bcl-2* transcription by DNA methylation. The previous studies have shown that the aberrations of DNA methylation have tight correlations with the tumour genesis. Specifically, the hypomethylation of CpG island occurs within the proto-oncogenes, notably in the promoter regions, while site-specific hypermethylation occurs within the tumour suppressor genes, indicating that the hypomethylation of CpG island promotes the expression of oncogenes while hypermethylation inactivates tumour suppressor genes [22]. Our results here showed that the C-5-methylation of the cytosine was capable of enhancing the stability of the intramolecular G-quadruplex structure formed in the human oncogen *bcl-2* promoter, thus increasing its protection ability from competitive formation by the duplex DNA. Fig. 4 showed a proposed mechanism for explaining the gene silencing induced by the DNA hypermethylation and gene activation caused by DNA hypomethylation. The methylated promoter is more likely to keep the non-canonical structure, G-quadruplex, instead of being converted to single stranded or duplex stranded, because the methylation of the cytosine of the G-quadruplex greatly enhances its stability. Subsequently, the G-quadruplex will act as a transcriptional obstacle resulting in an ineffective transcription, and vice versa.

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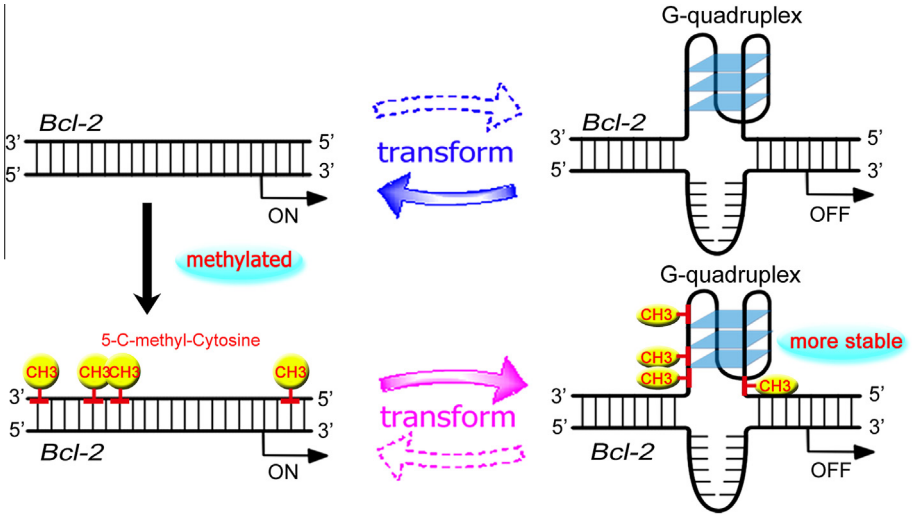

Fig. 4. The proposed mechanism for explaining the gene silencing induced by DNA hypermethylation and gene activation caused by DNA hypomethylation.

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Appendix A. Supplementary data

Supplementary data associated with this article can be found, in the online version, at http://dx.doi.org/10.1016/j.bbrc.2012.12.040.

References

[1] S. Sharma, T.K. Kelly, P.A. Jones, Epigenetics in cancer, Carcinogenesis 31 (2010) 27–36.
[2] V. Kirkin, S. Joos, M. Zornig, The role of *Bcl-2* family members in tumorigenesis, Biochim. Biophys. Acta 1644 (2004) 229–249.
[3] T.J. McDonnell, S.J. Korsmeyer, Progression from lymphoid hyperplasia to high-grade malignant lymphoma in mice transgenic for the t (14; 18), Nature 349 (1991) 254–256.
[4] T. Mikami, N. Yanagisawa, H. Baba, M. Koike, I. Okayasu, Association of Bcl-2 protein expression with gallbladder carcinoma differentiation and progression and its relation to apoptosis, Cancer 85 (1999) 318–325.
[5] Q. Zhu, Z. Jin, Y. Yuan, Q. Lu, D. Ge, M. Zong, Impact of MTHFR gene C677T polymorphism on *Bcl-2* gene methylation and protein expression in colorectal cancer, Scand. J. Gastroenterol. 46 (2011) 436–445.
[6] J.C. Reed, Apoptosis-based therapies, Nat. Rev. Drug Discov. 1 (2002) 111–121.
[7] A.R. Hoffman, J.F. Hu, Directing DNA methylation to inhibit gene expression, Cell. Mol. Neurobiol. 26 (2006) 425–438.
[8] V. Gonzalez, L.H. Hurley, The *c-MYC* NHE III(1): function and regulation, Annu. Rev. Pharmacol. Toxicol. 50 (2010) 111–129.
[9] S.T. Hsu, P. Varnai, A. Bugaut, A.P. Reszka, S. Neidle, S. Balasubramanian, A G-rich sequence within the *c-kit* oncogene promoter forms a parallel G-quadruplex having asymmetric G-tetrad dynamics, J. Am. Chem. Soc. 131 (2009) 13399–13409.
[10] C.S. Mekmaysy, L. Petraccone, N.C. Garbett, P.A. Ragazzon, R. Gray, J.O. Trent, J.B. Chaires, Effect of O6-methylguanine on the stability of G-quadruplex DNA, J. Am. Chem. Soc. 130 (2008) 6710–6711.
[11] C.C. Hardin, M. Corregan, B.A. Brown, L.N. Frederick, Cytosine–cytosine+ base pairing stabilizes DNA quadruplexes and the methylation of cytosine greatly enhances the effect, Biochemistry 32 (1993) 5870–5880.
[12] J. Dai, D. Chen, R.A. Jones, L.H. Hurley, D. Yang, NMR solution structure of the major G-quadruplex structure formed in the human BCL2 promoter region, Nucleic Acids Res. 34 (2006) 5133–5144.
[13] G.N. Parkinson, M.P. Lee, S. Neidle, Crystal structure of parallel quadruplexes from human telomeric DNA, Nature 417 (2002) 876–880.
[14] M.J.T., G.W. Frisch, H.B. Schlegel, G.E. Scuseria, M.A. Robb, et al., Gaussian 03, Revision E.01., Gaussian, Inc., Wallingford, CT, 2004.
[15] W.D. Cornell, P. Cieplak, C.I. Bayly, I.R. Gould, D.M. Ferguson, D.C. Spellmeyer, T. Fox, J.W. Caldwell, P.A. Kollman, A second generation force field for the simulation of proteins, nucleic acids, and organic molecules, J. Am. Chem. Soc. 117 (1995) 5179–5197.
[16] U. Essmann, L. Perera, M.L. Berkowtz, T. Darden, H. Lee, L.G. Pedersen, A smooth particle mesh Ewald method, J. Chem. Phys. 103 (1995) 8577–8593.
[17] J.P. Ryckaert, G. Ciccotti, H.J.C. Berendsen, Numerical integration of the Cartesian equations of motion of a system with constraints: molecular dynamics of n-alkanes, J. Comput. Phys. 23 (1997) 327–341.
[18] P.A. Kollman, I. Massova, C. Reyes, B. Kuhn, S. Huo, L. Chong, M. Lee, T. Lee, Y. Duan, W. Wang, O. Donini, P. Cieplak, J. Srinivasan, D.A. Case, T.E. Cheatham, Calculating structures and free energies of complex molecules: combining molecular mechanics and continuum models, Acc. Chem. Res. 33 (2000) 889–897.
[19] P. Hazel, G.N. Parkinson, S. Neidle, Predictive modelling of topology and loop variations in dimeric DNA quadruplex structures, Nucleic Acids Res. 34 (2006) 2117–2127.
[20] J. Kypr, I. Kejnovska, D. Renciuk, M. Vorlickova, Circular dichroism and conformational polymorphism of DNA, Nucleic Acids Res. 37 (2009) 1713–1725.
[21] H. Han, L.H. Hurley, M. Salazar, A DNA polymerase stop assay for G-quadruplex-interactive compounds, Nucleic Acids Res. 27 (1999) 537–542.
[22] P.A. Jones, S.B. Baylin, The epigenomics of cancer, Cell 128 (2007) 683–692.