Effect of DNA Methylation on 18S rRNA Gene Sequences during Culture of *Taxus chinensis* Cells

Fu Xiang, Liqing Li, Rui Yin, Wenwen Jin, and Longjiang Yu*

Institute of Resource Biology and Biotechnology, College of Life Science and Technology, Huazhong University of Science and Technology, Wuhan 430074, China.
E-mail: xiangfu@smail.hust.edu.cn

* Author for correspondence and reprint requests

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Cell suspension culture has rapidly become an alternative source of taxol, an anticancer compound. To investigate the role of DNA methylation in the cultural course of *Taxus chinensis* cells, analyses of 18S rRNA gene sequences of cultured *T. chinensis* cells and related species were conducted. The phylogenetic analysis of 18S rRNA gene sequences indicated that HG-1 (the cultured *T. chinensis* cells), like *T. mairei* (the natural variety of *T. chinensis*), should be a new variety of *T. chinensis*, and cell culture can change the 18S rRNA gene sequence at the level of species despite 18S rRNA is the most conserved gene. The analyses of the CpG and TpG+CpA relative abundance and GC content of the 18S rRNA gene sequences made clear that DNA methylation contributed to changes of the 18S rRNA gene sequence of HG-1 at the level of species, which can make HG-1 to become a new variety of *T. chinensis*.

Key words: 18S rRNA Gene, DNA Methylation, *Taxus chinensis* Cell

Introduction

Taxol is currently the best-known drug approved for the treatment of ovarian and breast cancer. For commercial use, taxol was first extracted from the bark of the yew *Taxus brevifolia*, which was threatened by extinction soon after the discovery of taxol. Cell cultures derived from different *Taxus* species are an important alternative source of taxol and other taxanes which can be converted chemically to taxol (Sohn and Okos, 1998). Recently, the vcommercial production of taxol by *Taxus* cell suspension cultures was established (Zhong, 2002). However, the difficulty of establishing cell cultures and cessation of cell growth and secondary metabolite production after a period of time in suspension culture limits the efficiency of taxol production from cell cultures. The instability of secondary metabolite production owes to changes in the genomic structure of cultured plant cells significantly (Baebler *et al.*, 2005). Interestingly, we had found that cell culture has an effect on the 18S rRNA gene sequences in the cultural course of *Taxus chinensis* cells (Xiang *et al.*, 2008). The 18S rRNA gene, which is highly conserved, can record the trace of biological evolution. As the most conserved gene, what caused the changes of the 18S rRNA gene sequence and what results were brought about in cell culture? DNA methylation may play a role in such a cultural course, and it prompted us to revisit the changes of 18S rRNA gene sequences in cell culture.

Material and Methods

Sequences

The 18S rRNA gene sequences of *T. chinensis* (AY544988), *T. media* (AY544989), and HG-1 (AY679156) were the same as the ones used previously (Xiang *et al.*, 2008). Of these, HG-1 are the cultured *T. chinensis* cells. The species *T. mairei*, a naturally occurring variant of *T. chinensis*, was from Wuhan arboretum. We sequenced the 18S rRNA gene sequence of *T. mairei* following the same protocol used for *T. chinensis*, *T. media*, and HG-1 (Xiang *et al.*, 2008), and it has been deposited at GenBank under accession number AY679157. According to the phylogenetic relationship reported previously (Xiang *et al.*, 2008), the 18S rRNA gene sequence of *Amentotaxus formosana* (D38248) was used as an outgroup.

Data analysis

Sequences were aligned by ClustalX (Thompson *et al.*, 1997), and low-scoring segments in the

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alignment output were realigned manually. The first and last 14 positions were excluded due to the artifacts in sequence AY544988, and 1836 nucleotide positions were obtained.

Neighbour-joining (NJ), maximum parsimony (MP), and maximum likelihood (ML) methods were used to reconstruct phylogenetic trees on these sites. The NJ and MP trees were reconstructed by the methods as implemented in MEGA4 software (Tamura *et al.*, 2007), and the pairwise distances in the NJ method were calculated using the Kimura 2-parameter model (Kimura, 1980). To assess support for the inferred relationships, bootstrap analysis with 2,000 replicates was conducted using the NJ and MP methods. The ML tree was constructed by the DNAML program in PHYLIP package (Felsenstein, 2004) with 1,000 replicates bootstrap analysis, and the transition/transversion ratio was 3.

The analysis of CpG relative abundance was conducted using DAMBE software (Xia and Xie, 2001).

Results and Discussion

The bootstrap consensus MP, NJ, and ML trees are shown in Figs. 1A, B, and C, respectively. It is interesting that HG-1 groups with *T. mairei* and not with *T. chinensis* with 100% bootstrap support in the MP tree, 64% in the NJ tree, and 69% in the ML tree, despite of HG-1 are the cultured *T. chinensis* cells. The phylogenetic position changes of the 18S rRNA gene sequences implied that HG-1, like *T. mairei*, should be a new variety of *T. chinensis*. This result is consistent with that reported previously (Xiang *et al.*, 2008). In other words, cell culture can change the 18S rRNA gene sequence at the level of species despite 18S rRNA is the most conserved gene. So, what caused such a result? In fact, we have found the methylation in the cultural course of *T. chinensis* cells, and it may contribute to the taxol content at some stage (to be published). Is it possible that DNA methylation caused the changes of the 18S rRNA gene sequence at the level of species?

Table I. Relative abundance (RA) of CpG and TpG+CpA and GC content.

Species	RA[a](CpG)	RA(TpG+CpA)	GC(%)
T. chinensis	0.9762	0.9440	0.4964
T. mairei	0.9762	0.9426	0.4972
HG-1	0.9621	0.9649	0.4936

[a] RA, odds-ratio measure, *i.e.*, $F(XY)/[F(X) \cdot F(Y)]$ for quantifying the relative abundance of dinucleotides. For nucleotide sequences, $F(X)$ and $F(Y)$ are the frequencies of X and Y, respectively, and $F(XY)$ is the frequency of dinucleotide XY.

To make sure the role of DNA methylation in the cultural course of *T. chinensis* cells, we analyzed the CpG and TpG+CpA relative abundance and GC content of the 18S rRNA gene sequences of HG-1 (the cultured *T. chinensis* cells), *T. chinensis*, and *T. mairei* (the natural variety of *T. chinensis*). CpG dinucleotides play an important role in cell differentiation and in the regulation of gene expression (Bestor, 1990). As shown in Table I, the CpG dinucleotide relative abundance is normal in all three sequences. It should be noted that the CpG relative abundance of HG-1 is lower than that of *T. chinensis*. Contrarily, *T. mairei*, which is the natural variety of *T. chinensis*, has the same CpG relative abundance as *T. chinensis*. Correspondingly, the TpG+CpA relative abundance has a higher value in the HG-1 sequence compared to sequences of *T. chinensis* and *T. mairei*, and the GC content in the HG-1 sequence is the lowest as shown in Table I.

In general, CpG methylation increases the mutation rate from CpG to TpG or CpA. As methylated CpG deaminates to TpG or CpA dinucleotides, the number of C and G decreases in

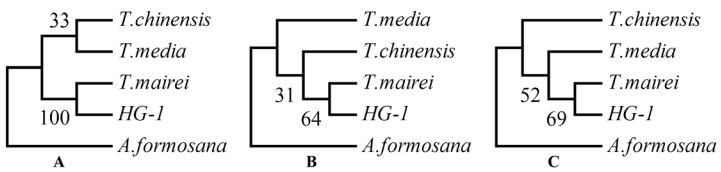

Fig. 1. The bootstrap consensus trees: (A) tree from maximum parsimony method; (B) tree from neighbour-joining method; (C) tree from maximum likelihood method. Numbers above branches are bootstrap values calculated from 2,000 replicates of bootstrapping (A and B) and 1,000 replicates of bootstrapping (C).

this process. This would lead to a lower expected number of CpG dinucleotides in the new sequence compared to the original sequence. In fact, in vertebrates and plants, CpG dinucleotides are massively methylated or demethylated in order to regulate the gene expression activity (Wang *et al.*, 2004), which is connected with a new developed function in evolution history (Yoder *et al.*, 1997). In the cultural course of *T. chinensis* cells, DNA methylation may contribute to changes of the 18S rRNA gene sequence of HG-1 at species level, which led HG-1, similar to *T. mairei*, become a new variety of *T. chinensis*. This is the reason why HG-1 clusters together with *T. mairei* and not with *T. chinensis* in all three consensus trees, as shown in Fig. 1.

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