International Congress Series 1239 (2003) 745–748

Application of restriction landmark genomic scanning for analysis of the postmortem phenomenon

T. Sawaguchi*, A. Kitamura, R. Shimada, T. Fujiwara, A. Sawaguchi

Department of Legal Medicine, School of Medicine, Tokyo Women's Medical University, 8-1 Kawada-cho, Shinjuku Ward, Tokyo 162-8666, Japan

Abstract

The restriction landmark genomic scanning method modified by methylation (RLGS-M) was applied for analyses of the postmortem time interval (PMI) using the methylation sensitive enzyme *Eco*52I and methylation insensitive enzyme *Eag*I as the landmarks. In the period between 4 and 8 h as a postmortem time interval, the methylation rate was slightly decreased and the gene activation seemed to be slightly increased. After that, the methylation rate was increased to the same level at the time of individual death. This fact suggested that the gene activation was decreased to the same level at the time of individual death. During the supra-vital period, which is defined as the period between the individual death and the cellular death in each organ, transient gene activation might occur even after the individual death.

Keywords: Postmortem; Restriction landmark genomic scanning (RLGS); Methylation; Gene activation; Supra-vital period

1. Introduction

Restriction landmark genomic scanning (RLGS) is a two-dimensional electrophoresis method with high accuracy that was developed 10 years ago in Japan [1,2]. "Genomic scanning" is defined as a high speed scan to simultaneously detect signals from many loci throughout a genome by one process. The purpose of this study is to investigate the possibility of a forensic application of this method [3,4], particularly for analyses of postmortem phenomena including the evaluation of postmortem time interval (PMI).

Abbreviations: RLGS, restriction landmark genomic scanning; RLGS-M, restriction landmark genomic scanning modified by methylation; PMI, postmortem time interval.

* Corresponding author. Tel./fax: +81-3-5269-7300.

E-mail address: tsawagu@research.twmu.ac.jp (T. Sawaguchi).

0531-5131/03
PII: S0531-5131(02)00481-8

In this study, the methylation sensitive enzyme *Eco*52I and methylation insensitive enzyme *Eag*I were used for DNA digestion as the landmarks in the process of the so-called RLGS-M method [5]. We used this method to screen the genome kinetics in the postmortem phenomenon, because DNA methylation relates to genomic development.

2. Materials and methods

The mice (C57/B6) were killed under anesthesia using diethylether and kept for 0, 4, 8, 12 and 24 h at 4 °C. Genomic DNA was extracted from the liver tissues of these mice, using the method of Blin and Stafford [6]. Methylation sensitive (*Eco*52I) and methylation-insensitive restriction enzymes (*Eag*I) were used for DNA digestion as the landmarks in the process of the so-called RLGS-M method. In the polyacrylamide gel electrophoresis of this study, *Eag*I or *Eco*52I was used as a landmark and *Pvu*II and *Pst*I were used for fragmentation of DNA in the two-dimensional electrophoresis.

One-dimensional electrophoreses were carried out in agarose gel and two-dimensional electrophoreses were carried out in polyacrylamide gel. *Eag*I or *Eco*52I were used as a landmark. *Pvu*II was used for the fragmentation of DNA treated by *Eag*I or *Eco*52I before one-dimensional electrophoreses. *Pst*I was used for the fragmentation of first fractionated DNA fragments in agarose gel after one-dimensional electrophoreses before two-dimensional electrophoreses.

First, to prevent non-specific labeling, 0.2 μM alpha-thio dCTP, 0.4 μM alpha-thio dGTP, 0.2 μM ddATP and 0.2 μM ddTTP were used as substrates and 1.75 U DNA polymerase I was used for blocking.

Second, Genomic DNA was cleaved by 20 U *Eco*52I or 20 U *Eag*I.

Third, the ends cleaved by 20 U *Eco*52I or 20 U *Eag*I were labeled by 0.37 MBq alpha-32P dCTP and 0.37 MBq alpha-32P dGTP as substrates and by the profiling activity of 1.3 U Sequenase ver2.0.

Fourth, the DNA previously cleaved by *Eco*52I or *Eag*I was treated by 20 U *Pvu*II.

Fifth, the DNA following treatment by restriction enzymes was electrophoresed in 0.8% Seakem GTGAgarose disc gel (60 cm (length)×2.4 mm (diameter)) containing 5% sucrose 0.2× Boyer buffer containing 100 mM Tris-acetate (pH 8.0), 40 mM sodium acetate, 3 mM EDTA−2Na, and 36 mM sodium chloride was used for electrophoresis. The conditions of electrophoresis were 100 V for 2 h and 200 V for 24 h.

Sixth, after the first dimensional electrophoresis the disc gel was treated by 750 U *Pst*I.

Seventh, the disc gel was connected with 5% polyacrylamide gel (480 cm×410 cm×1 mm) containing 50 mM Tris−HCl, 62 mM boric acid, and 1 mM EDTA−2Na, and electrophoresed in 1× TBE buffer under the condition of 150 V/90 mA for 24 h.

Eighth, autoradiography was carried out after two-dimensional electrophoresis.

3. Results

The rates of methylation were 21.56% (0 h), 20.28% (4 h), 20.93% (8 h), and 21.76% (12 h). The RLGS method could not be applied to the materials under a 24-h

Table 1
Results of kinetics of gene development analysed by RLGS-M method

Postmortem time interval (PMI)	0 h (Control)	4 h	8 h	12 h
Number of spots treated by EagI	218	217	215	216
Number of spots treated by Eco53I	171	173	170	169
Methylation rate(%)	21.56	20.28	20.93	21.76
Coincidence rate by *Eag*I to the control(%)		99.54	98.16	99.0
Coincidence rate by *Eco*52I to the control(%)		98.84	99.4	98.83

postmortem time interval because of DNA degeneration. Table 1 shows the total number of spots, the number of spots treated by *Eag*I, the number of spots treated by *Eco*52I, the methylation rates (%), the coincidence rates by *Eag*I to the controls and the coincidence rates by *Eco*52I to the controls.

4. Discussion

The gene activation reflected in the sensitivity of the methylation seemed to be changed slightly by the postmortem time interval. In the period between 4 and 8 h as a postmortem time interval, the methylation rate was slightly decreased and the gene activation seemed to be slightly increased. After that, the methylation rate was increased to the same level at the time of individual death. This fact suggested that the gene activation was decreased to the same level at the time of individual death.

"Supra-vital period" has been defined as the period between the individual death and the cellular deaths in each organ. From the above-mentioned results, during the supra-vital period, transient gene activation might occur even after the individual deaths.

Acknowledgements

This study was supported by the Yoshioka Hiroto Research Award by Tokyo Women's Medical University.

References

[1] I. Hatada, Y. Hayashizaki, S. Hirotsune, H. Komatsubara, T. Mukai, A genomic scanning method of higher organisms using restriction sites in landmarks, Proc. Natl. Acad. Sci. U. S. A. 88 (21) (1991) 9523–9527.
[2] Y. Hayashizaki, S. Hirotsune, Y. Okazaki, I. Hatada, H. Shibata, J. Kawai, K. Hirose, S. Watanabe, S. Fushiki, S. Wada, Restriction landmark genomic scanning method and its various applications, Electrophoresis 14 (4) (1993) 251–258.
[3] T. Sawaguchi, A. Kitamura, X. Wang, The possibility of forensic application of restriction landmark genomic scanning, Prog. Forensic Genet. 8 (2000) 422–425.
[4] T. Sawaguchi, X. Wang, A. Sawaguchi, Y. Okazaki, Y. Hayashizaki, Application of restriction landmark genomic scanning (RLGS) to the detection of DNA Polymorphism, DNA Polymorphism 3 (1995) 79–83.

[5] J. Kawai, S. Hirotsune, K. Hirose, S. Fushiki, S. Watanabe, Y. Hayashizaki, Methylation profiles of genomic DNA of mouse developmental brain detected by restriction landmark genomic scanning (RLGS) method, Nucleic Acids Res. 21 (24) (1993) 5604–5608.
[6] N. Blin, D.W. Stafford, A general method for isolation of high molecular weight DNA from eukaryotes, Nucleic Acids Res. 33 (9) (1976) 2303–2308.