Volume 255, number 2, 226–230 FEB 07602 September 1989

DNA fingerprints revealing common and divergent human DNA methylation patterns

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Received 25 July 1989

We compared DNA fingerprints of different cell populations from the same individuals, after separate digestion with the isoschizomers *Mbo*I and *Sau*3A. Methylation differences were observed within every individual when comparing fingerprints of *Sau*3A- with *Mbo*I-digested DNA, and of *Sau*3A-digested sperm with somatic DNA. In some cases, differences were also detected between fingerprints of *Sau*3A-digested somatic DNA originating from various cell sources. Methylation patterns common to all cell populations examined, including the germline, were observed with a higher frequency than divergent ones. These 'common methylations' are most likely to find their origin during early embryogenesis.

DNA methylation; DNA fingerprinting; Development

1. INTRODUCTION

Minisatellite DNA probes recognizing hypervariable regions reveal individual-specific DNA 'fingerprints' [1,2], with bands generally representing multiple unlinked regions throughout the human genome [3]. This makes the probe a suitable tool in the study of DNA methylation accompanying differentiation and development. We report here that DNA fingerprinting can be used to quantify the number of methylated sites. Moreover the number of DNA methylation sites identical in all tissues examined is higher than divergent methylation patterns, e.g. hypomethylation in one cell type vs another.

2. MATERIALS AND METHODS

Total blood, sperm and hair roots were obtained from young, healthy male volunteers. Granulocytes and T-lymphocytes were separated according to standard techniques [4,5]. High-molecular-weight DNA was extracted from the various cell sources as described elsewhere [6,7]. The DNA was digested overnight with 10 U *Sau*3A, *Mbo*I, *Hpa*II or *Msp*I (BRL) per μg DNA. 1 μg of digested DNA was separated by a 0.7% agarose gel electrophoresis, and transferred at 4°C by Southern blotting [8] to the nylon filter Hybond-N (Amersham).

The minisatellite probe 33.15 was labelled in its M13 vector by primer extension [1]. We adapted the reaction conditions, so that only the insert minisatellite sequence was labelled and not the M13 vector. This avoids the need to isolate the labelled insert by a low-melting-point agarose gel electrophoresis. (i) Annealing of the primer: 100 ng single-stranded M13 vector containing the 33.15 sequence + 3 ng of 17-mer sequencing primer (Pharmacia) in 10 μl Tris buffer (10 mM $MgCl_2$, 10 mM Tris-HCl, pH 8) at 65°C for 30 min. (ii) Primer extension: + 17 μl of 0.125 mM dATP, 0.125 mM dGTP, 0.125 mM dTTP, 10 mM Tris-HCl, pH 8, 1 mM EDTA + 2 μl (3 U/μl) Klenow polymerase (Amersham) + 2 μl [α-^{32}P]dCTP (3000 mCi/mmol) for 30 min at 37°C. (iii) Chasing reaction: + 2.5 μl 0.5 mM dCTP for 15 min at 37°C. The reaction is stopped by adding EDTA to a final concentration of 10 mM. The unincorporated radioactivity is separated from the labelled probe by Sephadex G50 gel filtration. Incorporation was usually 85%.

The nylon filter was prehybridized in 0.5 M sodium phosphate buffer, pH 7.2, 1 mM EDTA, 7% NaSDS [9], for 10 min at 68°C. The labelled probe was added to the prehybridization mixture after denaturing by boiling for 10 min. After overnight

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Published by Elsevier Science Publishers B.V. (Biomedical Division)
00145793/89/$3.50

hybridization, the filter was washed twice for 15 min at 68°C, once in 2 × SSC, 0.1% SDS and once in 1 × SSC, 0.1% SDS. Under the same conditions no M13-derived fingerprint patterns [10] were observed, when wild-type M13 phage DNA was used as a control. The described procedure results in reproducible DNA fingerprints with an improved resolution for the lower molecular fragments, due to reduced background labelling.

Statistical analysis was performed using the one-sided Student's *t*-test for paired observations.

3. RESULTS

We compared individual finterprints of sperm DNA with those of total blood (12 individuals), granulocyte and T-lymphocyte (10 individuals) and hair root DNA (7 individuals). With the exception of total blood, these cell populations are homogeneous both in composition and in differentiation stage. Moreover the somatic cells are derived from 2 different definitive fetal layers: the blood cells from mesoderm, the hair root cells from ectoderm. We compared fingerprints of *Hpa*II- with *Msp*I-digested DNA. The latter yielded an analyzable fingerprint pattern, the former a concentration of

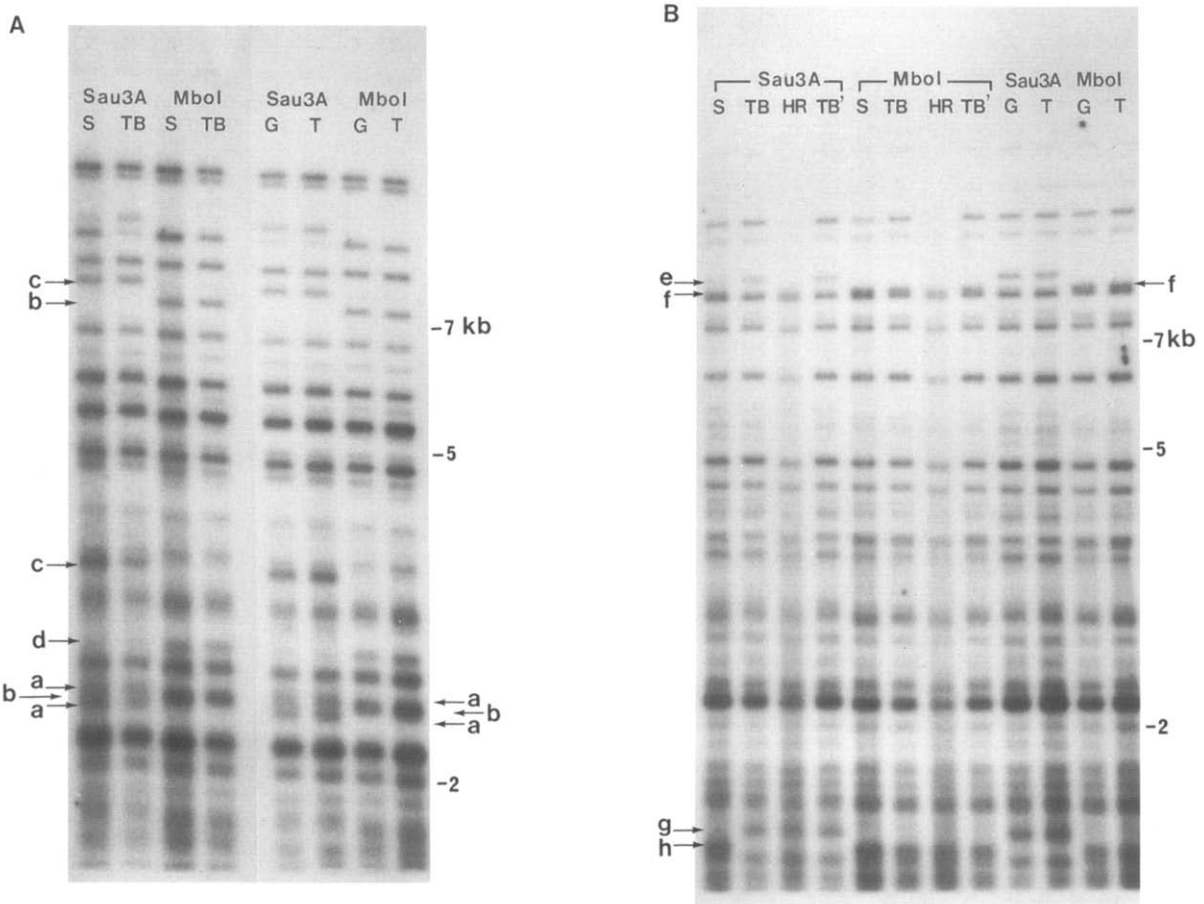

Fig.1. Individual-specific DNA fingerprints of different cell sources (individuals A & B). Lanes: S, sperm; TB, total blood; G, granulocytes; T, T-lymphocytes; HR, hair root; TB′, total blood of twin brother. Examples of methylation differences are indicated by arrows. (A) Examples of common methylation patterns (a–d): a, novel band in all *Sau*3A digests; b, novel band in all *Mbo*I digests; c, higher density band in all *Sau*3A as compared to *Mbo*I digests; d, higher density band in *Mbo*I as compared to *Sau*3A digests (the lower density bands are sometimes very faint). (B) Examples of tissue-dependent methylation differences (e–h): e, band present at higher density in *Sau*3A-digested total blood as compared to sperm DNA, but absent in hair root DNA; the same band is absent in all *Mbo*I digests; f, band present in *Sau*3A-digested sperm and hair root DNA, but absent in total blood DNA; the same band is present in all *Mbo*I digests; g, band present in *Sau*3A-digested DNA of all somatic cells examined, but absent in sperm DNA; this band is also absent in all *Mbo*I digests; h, band present in *Sau*3A-digested sperm DNA, but absent in all somatic DNA; this band is also present in all *Mbo*I digests.

the hybridization signals in a smear-like fashion in the high-molecular-weight region, making it impossible to resolve differences in terms of distinguishable and countable minisatellite bands. This problem did not arise when comparing fingerprints generated after separate digestion with a different pair of isoschizomeric restriction enzymes *Sau*3A and *Mbo*I, respectively, 5-methylcytosine-sensitive and -resistant. For this reason, we used these 2 endonucleases to quantify methylation differences by DNA fingerprinting.

No differences were detected in the fingerprint of each individual when the DNA, independent of its source, was digested with *Mbo*I. However, in all individuals, methylation differences, e.g. novel bands and/or density changes of existing bands were observed between *Sau*3A and *Mbo*I fingerprints. Enzyme-dependent methylation differences were noticed when comparing *Sau*3A and *Mbo*I fingerprints of either sperm or total blood DNA (fig.1A). The majority of the methylation differences were identical in sperm and total blood DNA, suggesting a common DNA methylation pattern for germline and somatic cells (table 1).

Table 1 also includes the number of tissue-dependent methylation differences observed between *Sau*3A fingerprints of total blood and sperm DNA. Statistical analysis indicates that the number of common methylations is significantly higher than the number of tissue-dependent methylations ($0.025 < p < 0.05$). Tissue-dependent methylation differences were detected in all individuals between *Sau*3A fingerprints of sperm DNA and all somatic DNA examined, e.g. total blood, granulocytes, T-

Table 1

Number of methylation differences in DNA fingerprints of 12 individuals

DNA fragment size (kb)	Mean number of novel bands ± SD	
	*Sau*3A vs *Mbo*I fingerprints of total blood DNA (enzyme-dependent)	Total blood vs sperm *Sau*3A fingerprints (tissue-dependent)
>7	1.3 ± 1.7 (0.8 ± 1.0/0.6 ± 0.8)	0.4 ± 0.9 (0.2 ± 0.4/0.3 ± 0.6)
7–5	1.4 ± 1.2 (0.8 ± 0.8/0.7 ± 0.7)	0
5–2	1.4 ± 1.8 (0.8 ± 1.2/0.6 ± 0.8)	0
<2	2.5 ± 0.8 (1.0 ± 0.4/1.5 ± 0.7)	2.2 ± 0.6 (1.0 ± 0.4/1.2 ± 0.4)
Total[a]	6.7 ± 2.4 (3.3 ± 1.5/3.3 ± 1.4)	2.6 ± 1.2 (1.2 ± 0.6/1.4 ± 0.9)
Common[b]	3.8 ± 1.8 (1.8 ± 1.2/2.1 ± 1.1)	
	Mean number of bands with higher density ± SD	
	*Sau*3A vs *Mbo*I fingerprints of total blood DNA (enzyme-dependent)	Total blood vs sperm *Sau*3A fingerprints (tissue-dependent)
>7	0.3 ± 0.5 (0.2 ± 0.4/0.1 ± 0.3)	0.6 ± 0.8 (0.3 ± 0.5/0.3 ± 0.5)
7–5	0.5 ± 0.7 (0.2 ± 0.4/0.3 ± 0.7)	0.2 ± 0.6 (0.1 ± 0.3/0.1 ± 0.3)
5–2	1.6 ± 2.0 (0.5 ± 0.9/1.1 ± 1.2)	0
<2	0.1 ± 0.3 (0.1 ± 0.3/0)	0
Total[a]	2.4 ± 1.8 (0.9 ± 1.0/1.5 ± 1.2)	0.8 ± 0.9 (0.4 ± 0.5/0.3 ± 0.5)
Common[b]	2.3 ± 1.9 (0.9 ± 1.0/1.3 ± 1.2)	

[a] Total number of differences over the complete fingerprint (the mean number of bands observed in a DNA fingerprint was 34.5 ± 2.2)

[b] Only those differences, as revealed by the *Sau*3A/*Mbo*I couple, where the same methylation pattern was found in all tissues examined (fig.1, arrows a–d)

When comparing fingerprints of total blood DNA, we counted the number of methylation differences observed in each size class, for both enzymes together or for each enzyme separately (*Sau*3A only, *Mbo*I only – between parentheses). The same strategy was used for the comparison of *Sau*3A fingerprints of total blood and sperm DNA, i.e. blood only/sperm only, given in parentheses

Table 2

Number of tissue-dependent methylation differences in *Sau*3A fingerprints of 7 individuals

Comparison set	No. of novel bands ± SD	No. of bands with density changes ± SD	Total no. of differences ± SD
TB vs S	2.3 ± 0.8 (7)	0.6 ± 0.8 (3)	2.9 ± 1.2 (7)
HR vs S	2.3 ± 0.8 (7)	0 (0)	2.3 ± 0.8 (7)
HR vs TB	0.3 ± 0.8 (1)	0.6 ± 1.0 (2)	0.9 ± 1.1 (3)

The number of methylation differences was counted between the *Sau*3A fingerprints of various cell sources (TB, total blood DNA; S, sperm DNA; HR, hair root DNA). The number of individuals, for whom differences were observed, is indicated between parentheses. The 7 individuals analyzed here are part of the 12 individuals examined in the complete study. The number of differences in the comparison set TB vs S were recalculated for the 7 individuals

lymphocytes and hair root (fig.1B). Most of these differences reflect hypomethylation of germline DNA relative to somatic DNA (fig.1B): absence (g) or decreased density (e) of a methylated fragment, or presence of an unmethylated fragment (f,h) in sperm DNA. Only once, hypermethylation of one fragment was noticed in sperm DNA as opposed to somatic DNA, namely in hair root DNA. In 3 out of 7 individuals, differences were observed when comparing *Sau*3A fingerprints of hair root with total blood (table 2). Statistical analysis was performed between the total number of differences of different comparison sets. No significant difference was found between the 2 comparison sets of somatic vs germline cells (e.g. hair root vs sperm/total blood vs sperm, $p > 0.05$). The 2 comparison sets of somatic vs sperm cells yielded significantly higher numbers than the comparison set of somatic cells (e.g. hair root vs sperm/total blood vs hair root, $p < 0.005$; total blood vs sperm/total blood vs hair root, $p < 0.0005$). These results indicate that the number of methylation differences between somatic and sperm cells is higher than between somatic cells. No cell-specific variations were seen when the finterprints of granulocyte and T-lymphocyte DNA were compared with total blood DNA (fig.1).

4. DISCUSSION

The main point of the present study is to demonstrate that the number of methylated sequences and the number of methylation differences can be quantified and compared in a statistical manner. The majority of methylation sites detected by DNA fingerprinting are common to all cell populations examined, including the male germline. The number of dissimilar, tissue-dependent methylation differences is significantly lower than the number of common methylations.

The most likely explanation for our methylation data is a programmed sequence of DNA modification steps during development. It is known from studies in mice that there is a progressive loss of methylation in the early pre-implantation embryo, followed by an extensive de novo methylation starting around the time of implantation [11–13]. Assuming similar developmental events in humans, the high level of identity between the DNA methylation patterns of germline and all somatic cells examined, could be the result of the de novo methylation in the early embryo, occurring before the establishment of the germline and before the formation of the three definitive fetal layers. The less frequent tissue-dependent differences could have their origin later during development as a result of additional methylations and/or demethylations. The higher number of methylation differences between sperm and somatic cells than between different somatic cells suggests that the (male) germline is set aside before the emergence of the different somatic lineages.

Acknowledgements: We thank Professor Jeffreys for providing us with the minisatellite probe.

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