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Genome-wide combination profiling of copy number and methylation offers an approach for deciphering misregulation and development in cancer cells

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Abstract

Copy number changes and DNA methylation alterations are crucial to gene regulation in mammals. Recently, a number of microarray studies have been based on copy number and DNA methylation alterations in order to find clinical biomarkers of carcinogenesis. In this study, we attempted to combine profiles of copy number and methylation patterns in four human cancer cell lines using BAC microarray-based approaches and we detected several clinically important genes which showed genetic and epigenetic relationships. Within the clones analyzed, many contained cancer-related genes involved in cell cycle regulation, cell division, signal transduction, tumor necrosis, cell differentiation, and cell proliferation. One clone included the *FHIT* gene, a well-known tumor suppressor gene involved in various human cancers. Our combined profiling techniques may provide a method by which to find new clinicopathologic cancer biomarkers, and support the idea that systematic characterization of the genetic and epigenetic events in cancers may rapidly become a reality.

Keywords: Copy number change; DNA methylation; BAC-microarray; Genetic and epigenetic relationship

1. Introduction

New techniques have been developed to perform genome-wide screening for alterations in copy number or DNA methylation patterns (Tollefsbol, 2004; Khulan et al., 2006; Schumacher et al., 2006). Recently, a number of studies based on copy number variation or DNA methylation profiling for finding new markers in cancer cells have been performed (Nygren et al., 2005; Wang et al., 2005; Ando and Hayashizaki, 2006; Ragoussis et al., 2006). The identification of genetic and epigenetic events involved in carcinogenesis has been facilitated through the accelerated application of high-resolution, high-throughput microarray platforms (Yan et al., 2001; Gitan et al., 2002; Shi et al., 2002; Yamamoto and Yamamoto, 2004; Ching et al., 2005; Hu et al., 2005; Weber et al., 2005). In addition to genetic abnormalities, epigenetic alterations such as abnormal DNA-methylation status changes are associated with many

Abbreviations: A, Adenine; bp, base pairs; BAC, Bacterial Artificial Chromosome; C, Cytidine; CGH, comparative genomic hybridization; CGI, CpG island; CpG, Cytidine–phophate–Guanosine Dinucleotide; Ct, threshold cycle; DNA, deoxyribose nucleic acid; G, Guanosine; GC, Guanoside–Cytidine Dinucleotide; IP, immunoprecipitation; kb, kilo base pairs; $\log_2$ T/R ratio, $\log_2$ transformed test:reference ratio; $\log_2$ IP/IN ratio, $\log_2$ transformed IP:Input ratio; Mb, mega base pairs; MeDIP, methylated DNA immunoprecipitation; MSP, methylation specific PCR; NCBI, National Center for Biotechnology Information; ng, nanogram; ObsCpG/ExpCpG, observed/expected CpG ratio; qPCR, quantitative polymerase chain reaction; T, Thymidine; TIFF, tagged image file format; μM, micromol/liter; μl, microliter.

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human tumor types (Jones and Laird, 1999; Jones and Baylin, 2002; Wilson et al., 2006). We attempted to combine profiles of copy number and methylation patterns in humans using array-based approaches. A platform technology which is achieved by coupling methylated DNA immunoprecipitation (MeDIP) with array-based comparative genomic hybridization (array CGH) has previously been reported (Weber et al., 2005). Here, we performed array comparative genomic hybridization (CGH) with 4030 human bacterial artificial chromosome (BAC) clones to assess copy number changes and methylation status in four cancer cell lines: SKBR3 (breast cancer), MCF7 (breast cancer), AGS (stomach cancer), and HT-29 (colon cancer). In this study, we identified clones with copy number gains, copy number losses, hypermethylation, and hypomethylation detected commonly in all four cancer cell lines analyzed. We then identified the clones with concomitant copy number gain and hypermethylation, those with concomitant copy number loss and hypomethylation, those with concomitant copy number gain and hypomethylation, and those with concomitant copy number loss and hypermethylation. These clones allowed us to explore the relationship between genomic and epigenomic events. Most of the clones identified contained several cancer-related genes, which have a significant role in cellular function and carcinogenesis. A comparison between four cancer cell lines and normal female blood using a MeDIP-array CGH revealed copy number variation and differential methylation patterns that link genetic and epigenetic instability, offering an approach for deciphering misregulation of genes in cancer. Genetic aberrations (Malkhosyan et al., 1998) and epigenetic alterations (Jenuwein and Allis, 2001) are good indicators of carcinogenesis and cancer prognosis. Furthermore, a relationship between global hypomethylation and genetic instability has been reported (Ehrlich, 2002; Eden et al., 2003). It has also been suggested that epigenetic alterations such as hypermethylation and hypomethylation affect some genomic sites, increasing the risk of errors in chromosome segregation, leading to genomic alterations and cancer development (Suzuki et al., 2006). Although until now the exact mechanisms integrating genetic aberrations with epigenetic alterations were unclear, it seems that there are both direct and indirect interactions between genetic damages and methylation pattern changes. Therefore, profiles linking genetic and epigenetic variations may be applied to find novel clinicopathological markers in the diagnosis and medical treatment of various cancers.

2. Materials and methods

2.1. Genomic DNA preparation and MeDIP assay

We prepared genomic DNA from four cultured cancer cell lines. The cells were first lysed overnight using a solution which included proteinase K. DNA was then extracted using a protein precipitation solution, followed by ethanol precipitation and RNase digestion. Before carrying out MeDIP, we sonicated genomic DNA to produce random fragments ranging in size from 300 to 1000 bp. We used 4 μg of fragmented DNA for a standard MeDIP assay (Weber et al., 2005). We denatured the DNA for 10 min at 95 °C and immunoprecipitation was carried out for 2 h at 4 °C with 10 μl of monoclonal antibody against 5-methylcytidine (Serotec) in a final volume of 500 μl IP buffer (10 mM sodium phosphate (pH 7.0), 140 mM NaCl, 0.05% Triton X-100). We then incubated the mixture with 30 μl of Dynabeads and M-280 sheep antibody to mouse IgG (Dynal Biotech) for 2 h at 4 °C and washed it three times with 700 μl of IP buffer. We then treated the beads with proteinase K for 1 h at 50 °C and recovered the methylated DNA by extracting using protein precipitation solution and ethanol precipitation.

2.2. Constructing BAC microarray

We obtained 96,768 clones through the KOGENOME project based on end sequencing and verified selected clones with FISH (multiple clones were eliminated). Basically, we divided the whole genome into 1 Mb sections based on physical maps and selected one clone per each Mb in a random manner except repeat sequence regions. We then added 1440 clones which represented cancer-related genes. Through two-step selection, we prepared 4030 BAC clones, of which 3030 clones contain a total of 5319 genes, and the other 1000 clones represent regions that do not contain genes. In addition, 371 BAC clones contain 258 cancer genes of a total 434 cancer genes and 1773 BAC clones include 1958 other genes from a total of 6,102 genes possibly implicated in cancer, which are annotated at http://atlasgeneticsoncology.org/Genes/Geneliste.html.

2.3. Array CGH

The BAC array used in this study consisted of 4030 human BACs; the space between the clones was approximately 1 Mb (Macrogen, Korea, http://www.macrogen.co.kr) (Cho et al., 2005; Park et al., 2006; Saito et al., 2006). The experiments were performed according to the manufacturer's protocol. Briefly, arrays were pre-hybridized with salmon sperm DNA to block repetitive sequences in the BACs. 500 ng of normal male DNA (reference) and cancer cell line gDNA (test) were labeled with Cy5-dCTP and Cy3-dCTP, respectively, by randomly primed labeling (Invitrogen). For methylation array CGH, 100 ng of input DNA (reference) and immunoprecipitated DNA (test) was labeled. The labeled probe and human Cot-I DNA were mixed and dissolved in hybridization solution. The probe mixture was denatured, cooled, and mounted on the array. Hybridizations were performed in a sealed chamber for 48 h at 37 °C. After hybridization, array slides were washed and dried. Scanning was carried out using a GenePix 4000B two-color fluorescent scanner (Axon Instruments); quantification was performed using MAC viewer software (Macrogen Instruments).

2.4. Statistical analysis for array-CGH

After scanning, the fluorescent intensities of the red and green channels were saved as two TIFF image files and the background was subtracted from these. The resulting values