Preliminary Results on Using Array Comparative Genomic Hybridization of Colorectal Cancer Tissues

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Abstract

Recent advances in cytogenetics have provided an opportunity for improvement in the measurement of aneuploidy by comparative genomic hybridization methods. In this study, chromosomal number variation(CNV) detection for 12 human colorectal cancer DNA samples was tried. Total 82 CNV was found, 19 gains and 63 losses, which including 326 chromosomal number variation regions. These results lead us to future studies that will be required to ascertain the meaning of gains and losses of chromosomes. (J Med Life Sci 2014;10(3):205-208)

Key Words : Array CGH, CNV, CNVR, Colon Cancer

Introduction

Worldwide, every year, more than 1 million individuals will develop colorectal cancer, and the disease-specific mortality rate is nearly 33% in the developed country[1,2]. During the past decades, significant progression of management in colorectal cancer patients has been made. In particular, the integration of monoclonal antibody drugs with conventional cytotoxic drugs has expanded the treatment of metastatic disease resulting in incremental survival gains. However, biomarker development is essential to aid selection of patients likely to respond to therapy, thereby rationalising treatments and improving outcomes[2].

Recent advances in cytogenetics have provided an opportunity for improvement in the measurement of aneuploidy by FISH or comparative genomic hybridization methods[3].

But it is not easy to exam the chromosomal number changes directly on the cancer tissues by FISH methods. Histomorphological characteristics of colorectal cancer, such as closely packed cells, nuclear overlapping and presence of mucinious material, can make FISH signals difficult to score and preclude cells for FISH evaluation[4].

The rationale for this approach stems from preparing next dual colour FISH experiment. Thus, the next step is to compare the performance of dual color FISH versus array CGH in colorectal carcinoma tissues.

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Materials and Methods

1) Patients

A total of 12 patients with histologically confirmed colorectal cancer who had undergone surgery at the Jeju National University Hospital between 2012 July and 2012 December were included in this study. The patients received chemoradiation therapy before surgery were excluded. This study was approved by the institutional review board of the Jeju National University Hospital.

2) Array CGH

Test and reference gDNAs were independently labeled with fluorescent dyes, co-hybridized to a NimbleGen Human CGH 2.1M or 385K or 135K Whole-Genome Tiling array, and scanned using a 2 μm scanner. Log2-ratio values of the probe signal intensities (Cy3/Cy5) were calculated and plotted versus genomic position using Roche NimbleGen NimbleScan software. Data are displayed in Roche NimbleGen SignalMap software. Scanned images(*.tif) were processed by NimbleScan S/W with default analysis settings. Extracted data was processed with SegMNT. Rearrangement breakpoints were determined by automated segmentation analysis of data sets after normalization of signal intensities. The test versus reference log2 ratios were averaged at window sizes corresponding to 1Xand 10Xthe median probe spacing.

Results

Copy number variation regions were found in 12 samples using reference sample(NA10851). The average CNV number was 81.7 which include 18.7 gains and 63.0 losses by 135K probe. Chromosomal number variation region was defined as overlapping more than 30% of CNV segments(table 1). The number of CNVR was total 326 and the genes which were included in those regions was recognized. The genes can be classified by individual chromosome(Table2).

Table 1. The gains and losses of chromosomes

Sample	Gain	Loss	Number of *CNV	Number of †CNVR
1	13	10	23	21
2	37	64	101	86
3	7	115	122	107
4	8	160	168	137
5	40	61	101	74
6	21	27	48	41
7	17	148	165	139
8	32	115	147	119
9	7	11	18	17
10	19	14	33	33
11	9	12	21	19
12	14	19	33	28
Average	18.7	63.0	81.7	68.4

*Chromosomal Number Variation †Chromosomal Number Variation Region

Table 2. Chromosomal Number Variation and Chromosomal Number Variation Region on each Chromosomes

‡Sam. § Chr.	1	2	3	4	5	6	7	8	9	10	11	12	*CNV	†CNVR
1	1	4	20	21	9	4	20	18	1	5	0	2	105	28
2	1	0	11	5	3	1	4	12	0	1	2	2	42	19
3	1	0	5	5	2	0	6	10	0	3	0	1	33	17
4	0	2	1	5	2	12	12	7	1	0	1	0	43	22
5	2	1	5	6	2	0	3	0	0	0	0	1	20	8
6	0	1	4	5	2	1	3	17	1	1	2	1	38	20
7	1	9	8	13	6	1	10	10	1	1	0	3	63	27
8	1	9	5	11	11	0	12	23	0	11	0	0	83	27
9	0	1	6	2	4	0	11	1	1	1	4	1	32	14
10	2	0	8	6	1	0	8	4	0	1	2	0	32	12
11	0	0	5	8	2	0	6	2	1	0	1	0	25	10
12	0	2	6	7	1	0	9	2	1	0	1	7	36	16
13	2	34	1	0	13	2	2	0	2	0	0	5	61	34
14	1	10	3	5	11	7	11	5	2	0	2	2	59	12
15	1	0	5	8	0	0	5	6	1	0	1	1	28	2
16	3	2	6	21	6	1	2	2	0	1	1	5	50	21
17	2	4	7	13	4	3	8	4	2	3	1	0	51	13
18	1	7	0	0	4	3	5	9	1	0	0	0	30	5
19	0	1	4	14	1	0	16	4	0	1	1	1	43	9
20	4	11	6	7	15	10	7	8	2	3	0	0	73	6
21	0	1	3	2	0	1	1	2	0	0	0	0	10	1
22	0	2	3	4	2	2	4	1	1	1	2	1	23	3

*Chromosomal Number Variation †Chromosomal Number Variation Region ‡Sample § Chromosome

Discussion

In 1990, Vogelstein et al[5] has demonstrated that tumorigenesis proceeds through a series of genetic alterations involving oncogenes and tumor suppressor genes. During the past 3 decades, there has been a significant evolution in the field of oncology. Recently, two kinds of pathways have been shown to drive the process of colorectal neoplasia. The first one contains oncogenes and tumor-suppressor genes that directly regulate cell birth and cell death. When a particular growth-controlling pathway gene is altered through mutation, the rate of cell birth exceeds that of cell death, and a tumor is initiated. When several such pathways are altered by mutation, a malignancy is likely to form. The other one is the genes that participate in the second kind of pathway, called stability genes, do not directly control cell birth or cell death, but rather control the rate of mutations of other genes, including growth-controlling genes. When stability genes are genetically altered, the cell accumulates mutations at a high rate and the tumorigenic process is accelerated[6,8].

In 1902, Theodor Heinrich Boveri asserted carcinogenesis was the result of aberrant mitoses and uncontrolled growth[9]. After this, researchers are increasingly aware how aneuploidy affects metabolic control and cause cancers. On aneuploidy basis Peter H. Duesberg et. al[10,11], now offer a coherent two-stage mechanism of carcinogenesis. In stage one, carcinogens cause aneuploidy which destabilizes the karyotype, and in stage two, aneuploidy evolves autocatalytically generating ever new and eventually tumorigenic karyotypes, ie. "genetic instability".

There were several previously well-defined arm-level changes, including gains of 1q, 7p and q, 8p and q, 12q, 13q, 19q, and 20p and q. Significantly deleted chromosome arms were 18p and q (including SMAD4) in 66% of the tumours and 17p and q (including TP53) in 56%. Other significantly deleted chromosome arms were 1p, 4q, 5q, 8p, 14q, 15q, 20p and 22q[12]. Other group assessed copy-number alterations in 74 tumour-normal pairs by applying GISTIC17 to the circular binary segmented (CBS)18 copy-number data. In addition to the IGF2 amplifications, They found known amplifications involving KRAS (13%; 10 out of 74) and MYC (23%; 17 out of 74) located in a broad amplicon on chromosome 8q[13].

In conclusion, there were frequently changed chromosomes in colorectal cancer tissues(chromosome 1, 7, 8, 13) were the next target to investigate correlation between clinical outcomes and chromosomal number changes with dual color FISH.

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