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Chromosomal Alterations in Colorectal Carcinomas Detected by Fluorescence *in situ* Hybridization and Comparative Genomic Hybridization and Their Relationship with Adenoma-Carcinoma Sequence: Review Analysis of Experience at a Single Japanese Cancer Unit

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To clarify the relationship with development of colorectal cancer, we investigated chromosomal aberrations in 715 specimens of the colorectal neoplasm by cytogenetic analysis. A gain of chromosome 17 was observed in the transitional epithelium around non-polypoid carcinomas, although the normal epithelium exhibited diploidy. Most tubular adenomas were diploid, however, loss of chromosome 11 and gain of chromosome 17 were increased in adenomas in association with an increased villous component. DNA aneuploidy, aneusomy and p53 deletion were predominantly observed in carcinomas, even in early cancers. Alterations of chromosomes 11 and 18 reflected different tumor morphologies in the early carcinomas. Gains of chromosomes 11, 17 and 18, and deletion of chromosomes 11 and 17p and p53 became more frequent following an increase in the depth of invasion. Aneusomy of chromosome 11 was a risk factor for patient survival after operation. Gains of chromosome 20 and 20q13.2 were associated with liver metastasis. Aneusomy and translocations of chromosome 17 and the p53 locus were predominantly observed in patients with multiple cancers and hereditary non-polyposis colorectal cancer. Our results indicate that in the process of development of colorectal carcinomas, specific chromosomal aberrations might be related to each step of development, or an alternative pathway of *de novo* carcinogenesis.

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Introduction

Colorectal carcinoma is one of the most common malignancies in the world. Most colorectal carcinoma develops from polypoid adenomas, a progression referred to as the adenoma-carcinoma sequence.[1,2] Macroscopically, sequential development of colorectal adenocarcinoma following cancer invasion has been elucidated and the regional or distant metastatic potential has been shown to increase.[3-5] Specific genetic events, such as k-ras and p53 mutations and loss of heterozygosity (LOH) of the DCC gene, are associated with carcinogenesis, the adenoma-carcinoma sequence or metastasis, which was first described by Fearon and Vogelstein[6] and Fearon.[7] On the other hand, karyotypic abnormalities were identified in colorectal adenomas and carcinomas in the 1970s by Mitelman et al.[8] and Sonta and Sandberg.[9] Recently, many random and non-random chromosomal alterations have been reported following analysis of karyotype or by restriction fragment length polymorphism (RFLP).[10]

In addition to the classical chromosome banding techniques, two promising new interphase analysis methods have been developed: fluorescence *in situ* hybridization (FISH)[11] and comparative genomic hybridization (CGH).[12] Both techniques have provided useful information about cancer genetics and recently have challenged chromosome banding as initial screening tests for chromosomal aberrations.[13] During the past decade, our group has also elucidated the chromosomal alterations in digestive tract malignancies, including colorectal carcinoma, using FISH and CGH techniques. Based on these studies, we have identified non-random alterations associated with the progressive development of colorectal carcinoma and its

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metastasis and patient prognosis.

While studies concentrating a single issue related to colorectal cancer are important, it is sometimes equally important to discuss the overall changes involved in the development and progression of colorectal cancers. The present report is a summary of the results of previous studies of our group, in addition to those of other investigators, on FISH and CGH analysis of colorectal cancers conducted over the past five years. Based on our results, we describe the relationship between numerical and structural alterations of chromosomes and development of the adenoma-carcinoma-metastasis sequence in colorectal neoplasm.

Materials and Methods

Materials

total of 715 specimens comprising 670 adenocarcinomas and 45 adenomas of the colorectum were obtained by endoscopic or surgical resection performed at the Division of Surgical Oncology in the Nagasaki University Graduate School of Biomedical Sciences between 1984 and 2002. Specimens were retrospectively and specifically selected for each purpose. Histology of adenomas, definition of early carcinoma and classification of cancer morphology were based on *The general rules for clinical and pathological studies on cancer of the colon, rectum and anus.*[14] A representative section of each cancer tissue fixed in 10% formalin, fresh frozen tissue, and primary cultured cancer cells obtained from fresh cancer tissue were used for the investigation. A contiguous section was subjected to hematoxylin-eosin staining. Fresh cancer tissue samples were obtained from viable sections of the tumor. Chromosome spreads of cancer cells at metaphase for total chromosome painting were obtained by primary culture as described by Nanashima et al.[15] The study design was approved by the Ethics Review Board of our institution and a signed consent was obtained from each subject.

Techniques

1) Flow cytometry

The nuclear DNA content of 10,000 nuclei per specimen was determined using a FACScan flow cytometer (Becton Dickinson, San Jose, CA). Frozen tissues were cut into very small pieces and treated with 0.1% Triton X-100 (Sigma Chemical Co., St. Louis, MO). The paraffin-embedded tissue was deparaffinized, rehydrated and isolated by citrate-buffered trypsin by the method of Schutte et al.[16] and Tagawa et al.[17] Isolated cells were stained with 50 μg/ml propidium iodide (Sigma). All samples with a DNA index not equal to one were considered to exhibit DNA aneuploidy.

2) Fluorescence in situ hybridization

a) Cell preparation: For paraffin-embedded tissues, tissues were first treated with 0.25% citrate-buffered trypsin for 18-24 hours after deparaffinization and rehydration.[16,17] Fresh single cells were treated with 0.1% citrate-buffered trypsin or hypotonic solution (75 mM KCl) for 30 minutes and were dropped onto a glass slide. The samples on a glass slide were fixed in a 3:1 mixture of ethanol-acetic acid solution at -20°C.[15]

b) FISH: FISH was performed using the method reported by Pinkel et al.[18] and modified by Yasutake in our department.[19] FISH using paraffin-embedded tissue (single cells and 5-mm thick sections) from the largest part of the specimen was conducted by the technique developed in our department.[20-24] Briefly, the sample on the glass slide was subjected to enzymatic treatment in 0.01-0.5% pepsin/0.2 N HCl at 37°C for 10-25 minutes and then acetylated in 0.25% acetic anhydrate for 10 minutes. After washing using 2× standard saline citrate (SSC), the target DNA was denatured with 70% formamide/2× SSC at 70°C for 2-10 minutes. After dehydration in ethanol, 20 μl of hybridization mixture was added containing biotinylated DNA probes as follows: DNA probes used were chromosomes 7, 8, 11, 17, 18, 20, 14/22 (combined)-specific alpha-satellite DNA probes (D7Z1, D8Z2, D11Z1, D18Z1, D20Z1, D14Z1/D22Z1), chromosome 11- and 17-specific total chromosome DNA probes, and a p53 cosmid DNA probe biotinylated by nick-translation (Oncor Inc., Gaithersburg, MD). A 20q13-locus specific DNA probe including the novel zinc finger gene ZNF217 was labeled with Spectrum Orange by the vendor (Orange: Vysis, Downers Grove, IL) and this probe was mixed with the chromosome 20-specific alpha-satellite DNA probe (D20Z1, Oncor) for two-color FISH.[25] The samples were incubated in a moist chamber at 37°C to induce hybridization. Hybridized samples were washed using 60% formamide and then conjugated with fluorescein-avidin DCS (green: Vector Laboratories, Burlingame, CA). The nucleus and metaphase chromosomes were counterstained with propidium iodide (red) or 4',6-diamidino-2-phenylindole (DAPI) (blue). The fluorescein signals were observed and counted under a fluorescence microscope with a 100-400× oil immersion lens (Olympus, Tokyo).

c) Evaluation of chromosome abnormality and scoring criteria: The number of FISH spots per nucleus was counted for 200 non-overlapping nuclei. Chromosomal aneuploidy was designated in cases in which the aneusomal population exceeded 15% (3SD).[26] In analysis using tissue sections, the chromosome index (CI) calculated as the total number of FISH spots per number of nuclei, was determined, using the procedure described by Dhingra et al.[23] A deletion of p53 (17p13) represented a copy number of p53 signals lower than that of chromosome 17.[27] For counting 20q13.2, the relative copy number was expressed as the mean level of amplification (the ratio of the number of signals from the 20q13.2 probe to the number of signals from the reference probe of chromosome 20 per individual cells).[25] Deletion of chromosome 17 or p53 was considered to have occurred when the nuclei had gross structural deletions or losses of the p53 signal in three or more metaphase spreads.[15] The presence of nuclei with translocations in three or more spreads was considered to represent translocation of the chromosome and the p53 locus.

3) Comparative genomic hybridization

a) Cell preparation: The 20-μm sections from frozen tissues were

digested with 0.6-μg/ml proteinase K, and DNA was extracted by a standard phenol-chloroform-isoamylalcohol procedure.[19,20] Tumor and normal (matched by sex) reference DNA samples were labeled with fluorescein-12-dUTP or Texas Red-5-dUTP (Du-Pont, Boston, MA), respectively, by nick translation. The double standard-labeled probe products were 500 to 2,300 bp in size.

b) CGH: CGH was performed using the method described by Kallioniemi et al.[12] and Korn et al.[28] Fluorescein-labeled tumor DNA (200 ng), 200 ng of Texas Red-labeled normal reference DNA, and 20 μg of unlabeled Cot-1 DNA (GIBCO-BRL, Gaithersburg, MD) were co-precipitated with ethanol and dissolved in 10 μl of 50% formamide, 10% dextran sulfate, and 2× SSC (pH 7.0). This probe mixture was denatured at 74°C for 5 minutes and hybridized to normal male metaphase cells for 2-3 days at 37°C. Hybridized samples were washed with 50% formamide and PN buffer (pH 8.0). Nuclei were counterstained with DAPI. The fluorescence CGH signals were analyzed by the Quantitative Image Processing System (QUIPS). Images were collected using a CCD camera mounted on a fluorescence microscope (Olympus) equipped with a single band pass filter. The 5-8 images of metaphases with good hybridization were analyzed using a digital image analysis system (FineStar™ FISH-CGH analysis system; Toyobo Inc., Tokyo).[29]

c) Evaluation of CGH images: Green-to-red fluorescence intensity ratio profiles were calculated for each chromosome. The total green and red fluorescence intensity for each metaphase image was normalized to 1.0 before calculation. The average ratio profiles for each chromosome and one standard deviation were plotted for analysis. Increased DNA sequence copy number was defined as an average tumor/normal ratio greater than 1.2 and decreased DNA copy number was defined as an average ratio of <0.8.[28]

Statistical analysis

For univariate analysis, categorical data were analyzed by the chi-square test or Fisher's exact test. Continuous data were expressed as mean ± standard deviation [SD]. Data of different groups were compared using one-way analysis of variance (ANOVA) and examined by Student's *t*-test or Dunnet's multiple comparison test. The overall patient survival was calculated according to the Kaplan-Meier method, and differences between groups were tested for significance using the log-rank test. The prognostic relevance of a single factor was determined by multivariate Cox's regression analysis. A two-tailed p-value less than 0.05 was considered significant. Statistical analyses were performed using the computer software STATISTICA™ (StatSoft, Tulsa, OK).

Results

Analysis of colorectal neoplasms

1) Normal colonic epithelium

All epithelia exhibited DNA diploidy in our series (data not published). Less than 5% of all nuclei showed monosomy of chromosome 7,[30] 8,[26] 11, 17,[30-33] 18,[34] 20,[26] or 14/22,[30] which was considered to be the limit error of hybridization. Whole chromosome spreads in all specimens showed normal structure and two signals of p53 locus[27] and 20q13.2.[25] In the transitional epithelium around the flat-type (non-polypoid) early carcinomas, nuclei with a gain of chromosome 17 (2.23±0.13) were observed and the mean number of copies of chromosome 17 was significantly increased compared to normal epithelium (1.93±0.12) ($p<0.05$).[35]

2) Adenomas

Although DNA aneuploidy was rarely detected in adenomas (2/45 or 4.4%),[20,31,33] alteration of each chromosome was observed as follows. Monosomy of chromosome 11 was mainly detected in single cells of adenomas (17/40 or 42.5%).[20,21,31,33] FISH analysis of tissue sections revealed a significantly higher frequency of decrease of chromosome 11 in adenomas in villous (1.67±0.05) than in tubular adenomas (1.86±0.11).[35] An increase of chromosome 17 was predominantly observed in adenomas with a villous component (2.15 ±0.14) compared to tubular component (1.90±0.13).[35] Therefore, the villous change of adenoma cells might be caused by aneusomal changes of chromosomes 11 and 17. Numerical changes of chromosome 18 were rare in all adenomas.[35] The mean frequency of p53 deletion was 7.8% in five adenomas.[27]

3) Colorectal carcinomas

The incidence of DNA aneuploidy was high in carcinomas (63/100 or 63%) than in adenomas (2/45 or 4.4%).[20,31,33] Gains of chromosome 11 and 17 were also significantly higher in colorectal carcinomas (52/109 or 48%, and 99/150 or 66%, respectively) compared to adenomas (0/13 or 0%, and 3/22 or 14%) ($p<0.01$).[30,33,35] The mean frequency of p53 locus deletion was significantly higher in carcinomas (57.0%) than in adenomas (7.8%).[27]

FISH analysis of early carcinomas showed that the mean number of copies of chromosome 17 in both polypoid and flat-type (non-polypoid) carcinomas (2.09±0.19) was significantly higher than in normal mucosa (1.93±0.12) ($p<0.05$).[35] The mean number of copies of chromosome 11 in polypoid carcinomas (1.70±0.15) tended to be lower than in adenomas (1.74±0.13), whereas that in flat-type carcinomas (1.90±0.15) was higher than normal mucosa, adenomas and polypoid carcinomas.[35] Thus, alterations of chromosome 11 were significantly different reflecting different tumor morphology in the early stage carcinomas.

Gains of chromosomes 17 and 18 in invasive T2 carcinomas (15/17 or 88%, and 10/11 or 91%, respectively) were significantly more common than in T1 carcinomas (8/18 or 44%, and 1/7 or 14%, respectively) ($p<0.05$).[31,34] FISH analysis using tissue sections revealed that the mean number of copies of chromosome 11 in both protruding and ulcerative carcinomas invading the muscularis propria layer was higher (2.01±0.10) than early carcinomas (1.79±0.18) ($p<0.05$). The mean number of copies of chromosome 18 in protruding carcinomas (1.54±0.10) was significantly lower than in that of ulcerative carcinomas (1.82±0.23) ($p<0.01$).[35] The incidence of numerical gain and arm deletion of chromosome 11 in T3

carcinomas (29/59 or 49%, and 12/20 or 60%, respectively) was significantly higher than in T1 and T2 carcinomas (7/35 or 20%, and 0/5 or 0%, respectively) ($p<0.05$).[30,31,36] The prevalence of p53 deletion and deletion of chromosome 17p were greater in T1 and T2 carcinomas (8/12 or 66.7%, and 3/5 or 60%, respectively), but was equivalent to that in more advanced carcinomas.[15,27] Translocation of chromosome 17 and the p53 locus was observed in 7/29 (24%) and 4/29 (14%), respectively, which did not differ with the T factor of the carcinomas.[15] With respect to chromosomes 8, 18 and 14/22 in T3 and T4 carcinomas, numerical chromosomal alterations were randomly observed, but no correlation with any factors could be observed.[26]

Postoperative prognosis and liver metastasis

In patients who underwent surgical resection for colorectal carcinomas, the 5-year survival rates of patients with loss or gain of chromosome 11 (70 and 41%, respectively) was significantly poorer than that of patients with chromosome 11 disomy (95%) by the Generalized-Wilcoxon test ($p<0.05$).[31] The risk ratios of monosomy and gain of chromosome 11 in the overall survival by multivariate analysis were 5.5 ($p=0.042$) and 10.4 ($p=0.020$), respectively.[31]

FISH analysis showed that gain of chromosome 20 in primary colorectal carcinomas and liver metastasis in patients with liver metastasis (14/18 or 78%, and 13/18 or 72%) were significantly higher than in colorectal carcinomas which did not metastasize to the liver (5/15 or 33%) ($p<0.05$).[26] Furthermore, the copy number of 20q13.2, which is the locus of the *ZNF217* oncogene associated with metastasis in breast cancer, was frequently higher in primary colorectal carcinomas and liver metastasis (2/18 or 11%, and 7/18 or 39%, respectively) in patients with liver metastasis than in those without liver metastasis (0/17 or 0%) ($p<0.05$).[25] Numerical aberration of chromosome 18 was not associated with liver metastasis.[36]

Malignant potential of double primary cancer and hereditary colorectal cancers

The prevalence of chromosome 17 aneusomy, the mean frequency of p53 deletion, and the frequency of translocations of chromosome 17 and the p53 locus in colorectal carcinomas in patients with multiple primary malignancies (64%, 70%, 67%, and 67%, respectively) were significantly higher than in patients with single colorectal carcinomas (23%, 53%, 13% and 0%, respectively) ($p<0.05$).[15,27,38] Furthermore, the prevalence of chromosome 17 aneusomy in hereditary non-polyposis colorectal cancer (HNPCC) (9/14 or 64%) was significantly higher than in sporadic colorectal carcinomas (11/42 or 26%) ($p<0.01$).[39] These results indicate that numerical and structural aberrations of chromosome 17, including the p53 locus, might be associated with multiple and hereditary carcinogenesis in the colorectum.

Discussion

Adenomas

DNA diploidy was predominant in adenomas but not in carcinoma by flow cytometric analysis with DNA diploidy.[20,31,33] However, alterations in each chromosome occurred even in such benign adenomas.[20,21,31,33] Mitelman et al.[8] were the first group to report numerical aberrations in colorectal adenomas using a karyotyping method, including gains of chromosomes 8 and 14. Reichmann et al.[40] and Murelis et al.[41] reported that gains of chromosome 7 and 13 were the dominant aberrations. Bomme et al.[42] conducted FISH analysis and reported that gains of chromosome 7, 13 and 20 were common in colorectal adenomas. To our knowledge, the relationship between chromosome alterations and histological findings of adenomas has not been reported. Our studies indicated that the incidence of alterations in chromosome 11 and 17 increased following histological changes from tubular to villous adenoma.[35] With increasing villous architecture, nuclear atypia, dysplasia and in situ carcinoma increased in frequency.[43] Therefore, alterations of specific chromosomes might be associated with the potential for adenomas to evolve into carcinomas.

Colorectal carcinomas

Our studies revealed that DNA aneuploidy was markedly increased in carcinomas, and that 37% of colorectal carcinomas showed DNA diploidy. Meling et al.[44] reported that one-third of colorectal carcinomas did not have an aneuploid peak as measured by DNA flow cytometry; each cancer had a complex karyotype, however. Our study also showed numerical aberrations of chromosomes 11 and 17 in cancers with DNA diploidy.[31] Meijer et al.[45] reported that gains of 13q, 20q and 8q were involved in the progression of adenomas to carcinomas by CGH analysis. Others reported that the most common karyotypic changes were rearrangement or gain of chromosome 17 and loss of chromosome 18.[46-48] Loss of 18q was frequent in Dukes C colon cancer by CGH analysis.[49] Our studies also revealed that numerical and structural aberrations in chromosome 17 and numerical aberrations in chromosome 8 and 18 were frequent in carcinomas.[15,26,30,33,35,36] These aberrations increased following development of cancer. Furthermore, numerical and structural aberrations of chromosome 11 might be associated with tumor progression although this event had not been reported previously. Other studies indicated that numerical aberrations or rearrangements of chromosomes 1, 7, 8 and 14 were also non-random alterations in the large intestine by karyotyping.[10] Thorstensen et al.[50] reported that 14q loss was an alteration in advanced colorectal cancer with metastatic ability. Thus, it is conceivable that certain chromosomal changes are related to tumor invasiveness in colorectal carcinomas.

In early colorectal carcinomas, morphological differences and their clinical influence have been noticed during the last decade, and flat-type cancers often invade the deeper layer when they are small in size.[51,52] Absence of K-ras mutation and low expression of cancer-associated sialomucin were more remarkable in flat-type

carcinoma compared to polypoid carcinomas.[53,54] Differences in the biological characteristics of cancer cells may reflect such morphological differences. Chromosomal analysis of flat-type colorectal carcinoma has not been reported so far. Our study revealed that numerical aberrations of chromosomes 11 and 18 were significantly associated with morphological differences between Tis, T1 and T2 colorectal carcinoma.[35] Our study indicated that flat-type carcinoma might have different pathway of cancer development, accompanied with different genetic and chromosomal events compared to polypoid carcinoma.

In addition to p53 mutation or overexpression,[55] our results also showed frequent aberrations of p53 locus in colorectal carcinomas compared to adenomas. These aberrations were observed in T1 or T2 carcinoma and, therefore, they might be the early genetic event in colorectal carcinomas.

Postoperative prognosis and liver metastasis

Our results suggest that numerical aberrations of chromosome 11 might be a useful predictor of prognosis of patients with colorectal carcinomas,[31] a finding not reported previously. With respect to prognosis, loss of 2p21-16.3,[56] 1p and 8p,[57] LOH and microsatellite instability at 17q21-23 including the nm23 gene,[58] and LOH at 18q[59] were found to be independent predictors of poor prognosis in patients with colorectal cancer.

Korn and Yasutake et al.[28] were the first group to report that loss of 18q and gain of 20q were the most frequent aberrations in metastatic liver tumor from colorectal carcinoma detected by CGH analysis (24/27 or 89%, and 23/27 or 85%, respectively). Recent studies also demonstrated a high incidence of gain of chromosome 20q in colon cancers with liver metastasis by CGH analysis.[60-62] Following this report, our studies also demonstrated that gain of copy number of centromere in chromosome 20 and of 20q13.2 were associated with liver metastasis from colorectal carcinomas.[25,26] Gain of 8q was also hypothesized to be a risk factor for liver metastasis in colorectal cancers.[28] These chromosomes might have genes associated with the metastatic potential of colorectal carcinomas.

Malignant potential of double primary cancer and hereditary colorectal cancers

Our studies demonstrated that numerical and structural aberrations of chromosome 17 and p53 locus were more frequent in colorectal cancer in patients with multiple primary carcinomas,[15,27,38] and in patients with HNPCC.[39] Accordingly, we hypothesize that instability or fragility of genes or chromosome 17 might influence carcinogenesis. No differences in the distribution of specific chromosomal abnormalities were discernible between sporadic adenomas and adenomas occurring in patients with familiar adenomatous polyposis (FAP) or HNPCC.[10] Aaltonen et al. reported that tumor cells of HNPCC exhibited a replication error phenotype (RER+)[63] and subsequently, mutations of the mismatch repair loci of MSH2 and MLH1 were identified by Fishel et al.[64] Planck et al.[65] reported that gains of chromosome 7 and 12, and structural aberrations of chromosome 20 and 22 were frequently detected by cytogenetic analysis in patients with HNPCC. Loss of chromosome 1,[66] deletion of chromosome 4[67] and LOH on 11q23[68] were important early events in the pathogenesis of colorectal carcinoma.

Relationship between adenoma-carcinoma sequence in colorectal neoplasm and chromosomal alteration (instability)

The above reports based on cytogenetic studies performed by our group and other investigators confirmed the specific relationship between chromosomal instabilities and development of colorectal neoplasms, as well as being consistent with the genetic model of colorectal carcinogenesis outlined by Fearon and Vogelstein[6] and Fearon.[7] In the most widely accepted model, the APC gene, DNA hypomethylation, K-ras mutation, and loss of DCC and the p53 gene typically mark the transformation to a malignant tumor. These relationships with our observations are summarized in Figure 1. From the very start of carcinogenesis to the development of advanced cancer in the colorectum, specific alterations of chromosomes may be related to alterations of tumor characteristics. The potential for carcinogenesis in normal colorectal epithelium may reflect not only genetic changes but also chromosomal instability.[69] The APC gene, K-ras gene, p53 gene and DCC gene were located on chromosome 5, 12, 17 and 18, respectively. In these chromosomes, deletion of p53 loci and chromosome 17 gain were frequently observed at the stage from adenoma with highly dysplasia to early carcinoma as well as abnormality of p53 gene by the Vogelstein's model. Chromosomal numerical instability may be related to gene mutation or other abnormalities. Furthermore, the numerical loss of chromosome 18 was related to development from early to advanced carcinomas as well as loss of DCC gene on chromosome 18. Therefore, monosomy of chromosome 18 may be associated with loss of DCC gene function in the colorectal carcinoma. By the present cytogenetic study, concept of the adenoma-carcinoma sequence in the colorectum might be also demonstrated. Further study concerning chromosome 5 or 12 should be necessary to clarify the relationship with abnormality of APC and K-ras gene. In the process of development of colorectal carcinomas, we have identified alternative pathways, such as *de novo* carcinogenesis and development of flat adenomas.[51,70,71] This type of carcinoma invades deeply despite the small tumor size.[72] The different characteristics of such tumors may reflect differences in genetic or chromosomal abnormalities compared with those in basic adenoma-carcinoma sequences in the colorectum.[36,73]

Conclusions

We reviewed our results of cytogenetic analyses of colorectal carcinomas from 1993 to 2000, as well as those of other investigators. Similar to the previously reported model of Fearon and Vogelstein[6] in which mutation or loss of specific genes was related to the development of colorectal carcinomas, we observed non-random

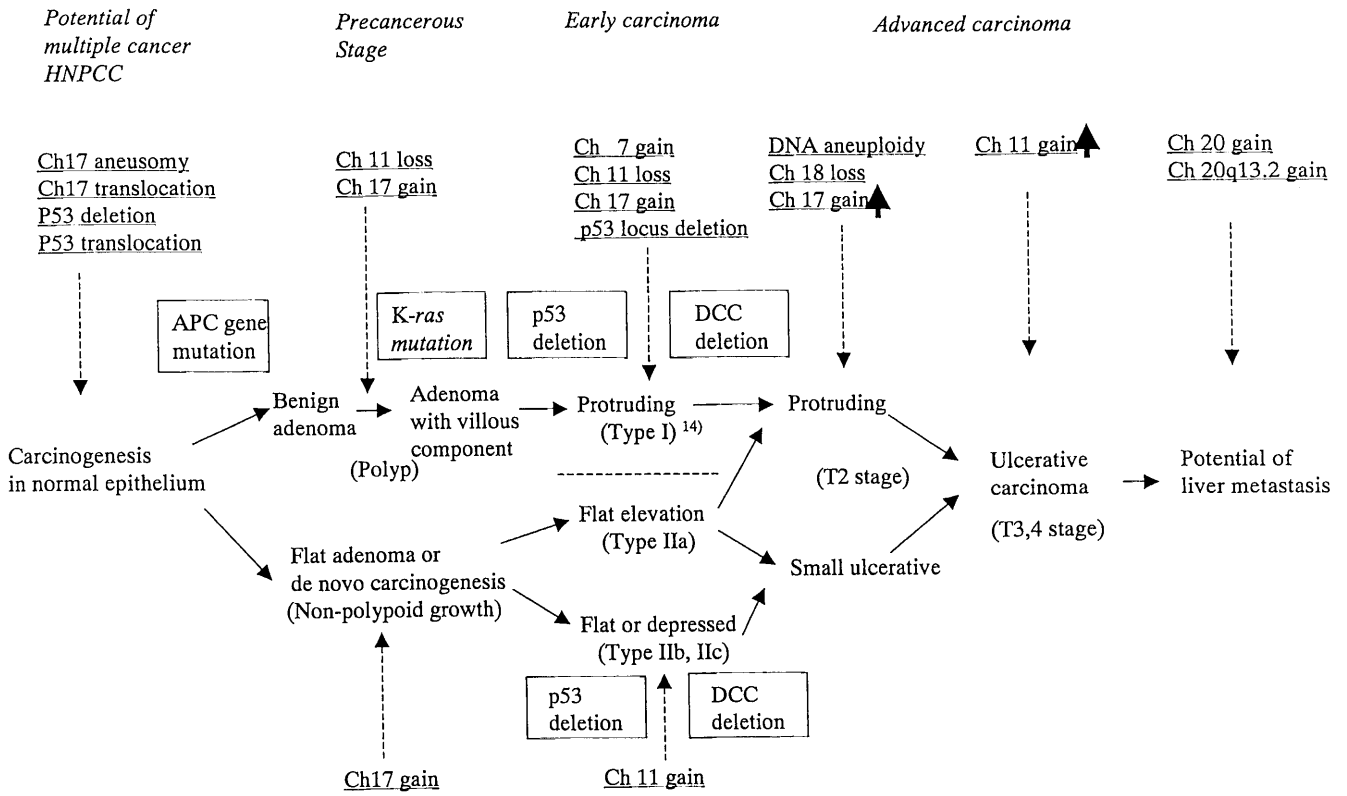

Figure 1. Specific chromosomal alterations based on the adenoma-carcinoma sequence. *Italic parts* showed the stages of colorectal carcinoma. Underlined parts showed the chromosomal alterations demonstrated in the present series. Parts surrounded by rectangle are genetic events associated with the adenoma-carcinoma sequences reported by Fearon and Vogelstein.[6] Dotted arrow shows the relationship between chromosomal alterations and cancer development. Thick arrow shows an increase of incidence of chromosomal aberration. HNPCC: hereditary non-polyposis colorectal cancer.

chromosomal aberrations at each step of tumor development from adenoma to advanced cancers with metastatic potential. These new data obtained by cytogenetic analyses of a large number of tumors should facilitate the construction of a detailed picture of the chromosomal events occurring during the development of colorectal neoplasms.

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