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Correlation Between DNA Content and p53 Deletion in Colorectal Cancer

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ABSTRACT

Objective: To find out whether tumour DNA content correlates with allelic loss of p53 and other pathological features in primary colorectal carcinomas.

Design: Ongoing prospective study.

Setting: University hospital, Italy.

Subjects: 128 patients who had undergone radical resections for colorectal carcinoma.

Interventions: Flow cytometric measurement of tumour DNA content and detection of allelic loss on the short arm of chromosome 17 by Southern blot (restriction fragment length polymorphism) analysis in fresh tumour specimens.

Main outcome measures: Correlation between DNA ploidy and deletion of p53, as well as between these two genetic events and clinicopathological variables.

Results: Interpretable DNA histograms were obtained for 122 tumour specimens. Forty-three tumours (35%) were diploid and 79 (65%) aneuploid. The diploid tumours were significantly more common in the proximal colon (from the caecum to the splenic flexure) than in the distal colon (from the descending colon to the rectum) ($p = 0.002$). The allelic state on the short arm of chromosome 17 was evaluated in 80 heterozygous patients. Forty-four tumour specimens (55%) showed deletion of 17p. Allelic loss of p53 was significantly more common in the distal and rectal tumours than in the proximal ones ($p < 0.0001$). Aneuploidy was more common among those tumours which had shown deletion of p53 than in those that had not ($p = 0.0008$).

Conclusions: DNA aneuploidy was significantly associated with the deletion of the p53 gene. This suggests that the functional loss of p53 may favour the growth and establishment of an aneuploid cell population within tumours. Tumours of the proximal and distal colon differ in their genetic nature.

Key words: colorectal cancer, DNA ploidy, tumour suppressor genes.

INTRODUCTION

The relationship between histopathological features and prognosis has been extensively investigated in colorectal carcinoma. Even if pathological staging still remains the most valuable prognostic factor, it cannot accurately identify patients at risk of recurrence, particularly those who fall into intermediate staging categories. In recent years, some new biological features of colorectal cancer such as tumour DNA content (as measured by flow cytometry) have received a great deal of attention. Several studies have dealt with the biological and prognostic significance of DNA ploidy in colorectal tumour; however, there have been conflicting results (2, 3, 10, 20, 21, 23, 27–29, 31, 36). This may be the result of lack of standardization in technical procedures and lack of homogeneity in the preparation of the analysed specimens (fresh/frozen or formalin-fixed paraffin-embedded). Cytogenetic studies have shown that the amount of DNA reflects the total chromosomal content of tumour cells (26). Nevertheless, the mechanisms that lead to aneuploidy are still not clearly understood.

More recently, DNA technology has improved our knowledge of the molecular genetic alterations that underlie the tumorigenesis of solid tumours. Changes in oncogenes, such as k-ras (4) and c-myc (1), and in tumour suppressor genes, such as p53 (11, 24, 25), adenomatous polyposis coli (APC) gene (6), and "deleted in colorectal cancer" (DCC) gene (12), have often been noted in colorectal carcinoma. The progressive accumulation of these alterations seems to be the basis for either the progression of the tumour from adenoma to carcinoma or the successful metastasising capacity of the tumour cells. In addition, DNA aneuploidy may be the result of genetic changes that

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can alter chromosomal number, such as the mechanism of allelic deletion.

The purpose of this segment of this ongoing study was to correlate DNA content and the deletion on the short arm of chromosome 17, where the p53 gene is located, in primary colorectal carcinomas. Any association of these two genetic events with the clinical and pathological features of colorectal cancer was also evaluated.

PATIENTS AND METHODS

Patients

Fresh tumour specimens were obtained from 128 patients (median age 65 years, range 32–75; 63 (49%) men and 65 (51%) women) who had undergone radical resections for colorectal carcinoma. Exclusion criteria from the study were: age over 75 years, synchronous metastases or residual tumour postoperatively or previous history of neoplasm. None of the patients had been given preoperative chemotherapy or radiotherapy.

Clinical and histopathological data

Age. Patients were divided into five age groups: 30–39, 40–49, 50–59, 60–69, and 70–75.

Tumour site. Tumours were classified as follows: proximal tumours (from the caecum to the splenic flexure), distal tumours (from the splenic flexure to the end of the sigmoid), and rectal tumours.

Histological type. Tumours were divided histologically into adenocarcinoma and mucinous carcinoma.

Tumour differentiation. Adenocarcinomas were divided into three types: well, moderately and poorly differentiated. This division was based on the World Health Organization classification (19).

Lymph node involvement. The number and level of metastatic lymph nodes were classified as follows: none (N0), 1–3 pericolic or perirectal lymph nodes (N1), more than 4 pericolic or perirectal lymph nodes (N2), and any vascular lymph node (N3).

Staging. All patients were staged according to both the Dukes' classification (modified by Gabriel et al.) (15), and the Jass system (17).

Flow cytometry DNA study

Multiple fragments of tumour were minced with a scalpel in a citrate-buffered solution to obtain a single nuclei suspension. A suspension of the standard cell population was obtained from scrapings of the mucosa, which had been cut 10 cm above the neoplasm. Both suspensions were filtered through a 50 μm nylon mesh to remove cell clumps. The concentration was adjusted to 106 cells/ml. Samples were stained with propidium iodide as described by Vinedelov and Christensen (34). Samples of tumour, normal mucosa, and a mixture of tumour and normal epithelial cells were analysed using a FACScan flow cytometer (Becton Dickinson, San Jose, Ca, USA) to give a single variable integrated fluorescence histogram. DNA ploidy was defined as the DNA index (DI), that is, the ratio between the mean channel number of the G0/G1 peak of the tumour cells and that of the normal epithelial cells. Tumours with DI = 1 were defined as diploid and tumours with lower or higher values as aneuploid. Cases with $1.90 \leq DI \leq 2.10$ were classified as tetraploid tumours when this peak reflected more than 20% of the cell population.

Allelic deletion analysis

Southern blot (restriction fragment length polymorphism) analysis was used to detect allelic loss on the short arm of chromosome 17. Briefly, DNA from tumour, normal colonic mucosa, and peripheral blood were digested with the restriction endonuclease BamH1, subjected to agarose gel electrophoresis and transferred to a nylon membrane (Hybond-N, Amersham Life Science). The 32P-pYNZ22 probe was used for the mapping of the gene region of chromosome 17.

Statistics

The significance of differences in ploidy and the incidence of the deletion of 17p among the various clinicopathological variables were assessed using the chi-square test. The relation between ploidy state and deletion of 17p was analysed using the chi-square test. Probabilities of less than 0.05 were accepted as significant.

RESULTS

Interpretable DNA histograms were obtained from 122 tumour specimens. Forty-three tumours (35%) were diploid and 79 (65%) were aneuploid. Among the specimens of the latter group, 12 cases (10%) showed a DNA tetraploid pattern. The mean coefficient of variation of the G0/G1 peak was 2.9 for the tumours and 2.5 for the normal mucosa. The relationships between ploidy and clinicopathological variables are shown in Table I. A strong correlation was found between ploidy and the site of the tumour. Aneuploid tumours were significantly more common in the distal colon and rectum than in the proximal colon ($p = 0.002$). A significant relationship was also noted between ploidy and the degree of tumour differentiation. All well-differentiated tumours were diploid.

Table I. *Correlation between DNA ploidy and clinicopathological variables. Data are expressed as number (%) of tumours*

	Diploid ($n = 43$)	Aneuploid ($n = 79$)
Sex:		
Male	21 (34)	40 (66)
Female	22 (36)	39 (64)
Age (years):		
30–39	2 (100)	0*
40–49	3 (33)	6 (67)
50–59	13 (52)	12 (48)
60–69	11 (22)	38 (78)
70–75	14 (38)	23 (62)
Tumour site:		
Proximal	19 (54)	16 (46)**
Distal	5 (14)	30 (86)
Rectum	19 (36)	33 (64)
Histological type:		
Adenocarcinoma	35 (35)	66 (65)
Mucinous carcinoma	8 (38)	13 (62)
Tumour differentiation:		
Good	5 (100)	0***
Moderate	24 (28)	63 (72)
Poor	6 (67)	3 (33)
Lymph node involvement:		
N0	31 (36)	56 (64)
N1	8 (35)	15 (65)
N2	3 (37)	5 (63)
N3	1 (25)	3 (75)
Dukes' stage:		
A	12 (48)	13 (52)
B	20 (32)	42 (68)
C1	10 (32)	21 (68)
C2	1 (25)	3 (75)
Jass system:		
I	10 (50)	10 (50)
II	9 (37)	15 (63)
III	15 (30)	35 (70)
IV	9 (32)	19 (68)

* $p = 0.03$; ** $p = 0.002$; *** $p = 0.001$.

Aneuploid tumours tended to be more common in the 60–69 age group than in the other groups.

The allelic state on the short arm of chromosome 17 was evaluated in 80 heterozygous patients. Forty-four tumour specimens (55%) showed deletion of 17p. The correlation between the deletion of p53 and clinicopathological variables are shown in Table II. There was a positive correlation between allelic loss and tumour site. In fact, deletion of p53 was more common in the distal colon and rectal tumours than in the proximal ones ($p < 0.0001$). The deletion of 17p was found less often in mucinous carcinoma than in adenocarcinomas ($p = 0.002$).

When DNA ploidy and allelic loss on 17p were compared, aneuploidy was more common in those tumours that had shown deletion of p53 ($p = 0.0008$) (Table III).

Table II. *Correlation between the deletion on the short arm of chromosome 17 and clinicopathological variables. Data are expressed as number (%) of tumours*

	Deletion of 17p	
	Absent ($n = 36$)	Present ($n = 44$)
Sex:		
Male	17 (47)	19 (53)
Female	19 (43)	25 (57)
Age (years):		
30–39	1 (100)	0
40–49	3 (60)	2 (40)
50–59	7 (41)	10 (59)
60–69	11 (34)	21 (66)
70–75	14 (56)	11 (44)
Tumour site:		
Proximal	22 (82)	5 (18)*
Distal	2 (8)	22 (92)
Rectum	12 (41)	17 (59)
Histological type:		
Adenocarcinoma	24 (37)	41 (63)**
Mucinous	12 (80)	3 (20)
Tumour differentiation:		
Good	3 (100)	0
Moderate	19 (34)	37 (66)
Poor	2 (33)	4 (67)
Lymph node involvement:		
N0	25 (45)	31 (55)
N1	7 (44)	9 (56)
N2	3 (60)	2 (40)
N3	1 (33)	2 (67)
Dukes' stage:		
A	5 (42)	7 (58)
B	20 (45)	24 (55)
C1	10 (48)	11 (52)
C2	1 (33)	2 (67)
Jass system:		
I	5 (50)	5 (50)
II	3 (21)	11 (79)
III	21 (54)	18 (46)
IV	7 (41)	10 (59)

* $p < 0.0001$; ** $p = 0.002$.

Table III. *Correlation between the deletion on the short arm of chromosome 17 and DNA content. Data are expressed as number (%) of tumours*

	DNA content		
	Diploid ($n = 30$)	Aneuploid ($n = 50$)	
Deletion of p53:			
Absent ($n = 36$)	21 (58)	15 (42)	
Present ($n = 44$)	9 (20)	35 (80)	$p = 0.0008$

DISCUSSION

The clinical use of DNA ploidy as a prognostic variable

in colorectal cancer is still controversial. Several studies have shown that ploidy correlates well with both histopathological features that are effective indicators of prognosis such as tumour staging (3, 20, 27), and the clinical outcome (2, 3, 10, 20, 21, 36). On the other hand, because this correlation was not seen in a number of other studies (23, 28, 29, 31), some doubts have arisen as to the importance of the DNA content as a predictor of the prognosis of colorectal carcinoma. These conflicting results may be the result of technical factors. For example, the different types of tumour material used for flow cytometric analysis, that is, paraffin-embedded or fresh-frozen specimens, may affect results. Most of the studies on the prognostic value of ploidy have been retrospective and used paraffin-embedded material. Today it is generally accepted that flow cytometric histograms derived from fresh-frozen specimens are of better quality with a lower coefficient of variation and less debris (9, 14). In the present study flow cytometric analysis was done on fresh or frozen specimens. Our results showed that DNA ploidy is not dependent on any of the histopathological variables known to affect prognosis, as expressed in the Dukes' and Jass systems or the number or level of metastatic lymph nodes. A significant correlation was found only between ploidy and the degree of differentiation. This is in contrast to findings reported in other studies (13, 18, 32). As the number of patients in the extreme categories (well and poorly differentiated tumours) was rather limited, we cannot say that our results constitute definite proof.

In any case, even if the prognostic significance of tumour DNA content has not yet been definitely established, flow cytometric findings have improved our knowledge of some of the biological mechanisms that underlie the pathogenesis of large bowel cancer. The most consistent finding that has emerged from the previous published studies of DNA analysis has been the significant correlation between ploidy and tumour site (5, 8, 11, 22, 25, 33). The present study also confirms this correlation given that the distal and rectal cancers were significantly more likely to be aneuploid than were the proximal ones. These flow cytomteric data may reflect a different type of biological susceptibility to neoplastic transformation at the different sites of the large bowel. The hypothesis that distinct mechanisms are involved in the aetiology of left and right-sided large bowel cancers has already been proposed on the basis of epidemiological findings. Some studies have shown that right-sided tumours predominate in geographic regions in which there is a low overall incidence of colorectal cancers, whereas left-sided tumours predominate in those areas where there is a high incidence (35). In addition, the site of the tumour differs in those subjects with the two hereditary syndromes of large bowel cancer — familial adenomatous polyposis and hereditary nonpolyposis colorectal cancer. In the former, the cancers arise mainly on the left side, whereas right-sided tumours predominate in the latter. Recent advances in molecular genetics seem to confirm the involvement of different site-related mechanisms in the pathogenesis of colorectal cancer, and several studies have shown that alterations in tumour suppressor genes and oncogenes occur more often in distal and rectal cancers than in proximal ones (11, 16, 25, 30). Our results are in agreement given that the deletion on the short arm of chromosome 17, where the suppressor gene p53 is located, was more common in tumours of the distal colon and in the rectum than in those of the proximal colon. Moreover, the comparison between DNA ploidy and allelic loss on 17p showed that aneuploidy was more common in those tumours that showed deletion of p53. These data are consistent with those previously reported by Offerhaus et al. (25) and suggest that the deletion of the p53 gene on 17p may be a structural chromosomal abnormality with growth promoting effects. This deletion could therefore promote the necessary growth advantage for the establishment of an aneuploid cell population within the tumour.

In conclusion, there is a relationship between DNA ploidy, molecular genetic alterations, and tumour biology. Our findings suggest that allelic loss of the gene p53 on 17p represents a genetic change that may be strictly related to DNA aneuploidy. This finding may give us some insight into understanding the mechanisms involved in aneuploidy. Recent DNA and molecular genetic studies also seem to support the hypothesis that two distinct biological categories of colorectal cancer can be identified by tumour location (7).

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RÉSUMÉ

But: Savoir si le contenu tumoral en DNA est corrélé avec la perte de l'allèle p53 et d'autres caractéristiques pathologiques des cancers primitifs colorectaux.
Type d'étude: Etude prospective en cours.
Provenance: Hôpital universitaire, Italie.
Patients: Cent vingt huit patients ayant eu une résection d'un cancer colorectal à visée curatrice.

Méthodes: Détermination du contenu tumoral en DNA par cytométrie de flux et détection de la perte de l'allèle sur le bras court du chromosome 17 par Southern Blot (FRLP) sur des fragments tumoraux frais.
Principaux critères de jugement: La corrélation entre la ploïdie et la délétion du p53, ainsi qu'entre ces deux événements génétiques et les caractéristiques clinicopathologiques.
Résultats: Des histogrammes de DNA interprétables ont été obtenus pour 121 tumeurs. Quarante deux tumeurs (35%) étaient diploïdes et 79 (65%) aneuploïdes. Les tumeurs diploïdes étaient significativement plus fréquentes sur la portion proximale du côlon (du caecum à l'angle splénique) que sur sa portion distale (du côlon descendant au rectum) ($p = 0{,}009$). La configuration allélique du bras court du chromosome 17 a été évaluée chez 80 patients hétérozygotes. Quarante quatre fragments tumoraux (55%) montraient une délétion du 17p. La perte de l'allèle p53 était significativement plus fréquente dans les tumeurs du côlon distal et du rectum que dans les tumeurs proximales ($p < 0{,}001$). L'aneuploïdie était plus fréquente parmi les tumeurs qui étaient le siège d'une délétion du p53 que parmi celles qui ne présentaient pas cette caractéristique ($p = 0{,}0008$).
Conclusions: L'aneuploïde du DNA était associée de façon significative à la délétion du gène p53. Ceci suggère que la perte fonctionnelle du p53 pourrait favoriser la croissance et l'établissement d'une population cellulaire aneuploïde au sein des tumeurs. Les tumeurs des portions proximales et distales du côlon ont une nature génétique différente.
Mots-clés: cancer colorectal, ploïdie DNA, gènes suppresseurs de tumeur.

ZUSAMMENFASSUNG

Ziele: Untersuchung zur Frage, ob der DNA-Gehalt primär kolorektaler Karzinome mit dem allelischen Verlust von p53 oder anderen pathologischen Eigenheiten korreliert.
Studienanordnung: Derzeit noch laufende prospektive Studie.
Studienort: Universitätskrankenhaus, Italien.
Patienten: 128 Patienten mit einem primären kolorektalen Karzinom mit radikaler Resektionsbehandlung.
Methoden: Bestimmung des Tumor-DNA-Gehaltes mit der Flow-Zytometrie, Nachweis des allelischen Verlustes auf dem kurzen Arm von Chromosom 17 aus Tumor-biopsien mit Southern Blot (FRLP).
Endpunkte: Korrelation zwischen der DNA-Ploidie und der Deletion von p53 sowie Korrelation dieser beiden genetischen Ereignisse mit klinikopathologischen Variablen.
Ergebnisse: Auswertbare DNA-Histogramme wurden bei 121 Tumorbiopsien erhalten. 42 dieser Tumoren (35%) waren diploid und 79 (65%) aneuploid. Die diploiden Tumoren wurden häufiger im proximalen Kolon (Zäkum bis linke Flexur) als im distalen Kolon (Descendens bis Rektum) gefunden ($p = 0{,}009$). Die Beurteilung des kurzen Armes von Chromosom 17 in 80 heterozygoten Patienten zeigte bei 44 Tumorbiopsien (55%) eine Deletion von 17 p. Der allelische Verlust von p53 wurde signifikant häufiger bei distalen Kolon- und rektalen Tumoren gefunden ($p < 0{,}001$). Aneuploidie wurde signifikant häufiger in den Tumoren nachgewiesen, in denen eine Deletion von p53 festgestellt worden war ($p < 0{,}001$).
Zusammenfassung: Es besteht eine signifikante Assoziation zwischen DNA-Aneuploidie und der Deletion des p53-Gens. Dies läßt vermuten, daß der funktionelle Verlust von p53 die Entstehung und das Wachstum aneuploider Zellpopulationen innerhalb kolorektaler Tumore begünstigt. Die Tumore des proximalen und distalen Kolons unterscheiden sich im Hinblick auf ihre genetischen Ursachen.
Schlüsselwörter: Kolorektale Karzinome, DNA-Ploidie, Tumorsuppressorgene.

РЕЗГОМЕ

Цель: Изучить, коррелирует ли содержание опухолевого ДНК с аллельной утратой p53 и другими патологическими признаками первичных колоректальных карцином.
Характер исследования: Онкологическое проспективное исследование.
Клиника: Университетский госпиталь, Италия.
Пациенты: 128 пациентов, у которых было произведено радикальное хирургическое лечение колоректальных карцином.
Методы: Цитометрическое определение содержания опухолевого ДНК и определение аллельных потерь в коротком рукаве 17-хромосомы посредством Southern Blot в свежих опухолевых образцах.
Задачи исследования: Корреляция между ДНК-плойдией и утратой части хромосомы 53, также как корреляция между двумя генетическими событиями и клинико-патологическими данными.
Результаты: ДНК-гистограмма была получена из 121 опухолевого образца. 42 опухоли (35%) были диплоидными и 79 (65%) анойплоидными. Диплоидные опухоли встречались статистически достоверно чаще в проксимальных отделах толстой кишки (от слепой кишки до левого угла толстой кишки) чем в дистальной части толстой кишки (от колон дисценденс до ректум) ($p = 0.009$). Аллельный статус короткого рукава хромосомы-17 был обследован у 80 гетерозиготных пациентов. 44 опухолевых образца (55%) показали выпадение 17p. Потеря аллели p53 отмечалась статистически достоверно больше в дистальных отделах толстой кишки и в прямой кишке чем в проксимальных отделах $p < 0.001$. Анойплойдия отмечалась более часто среди опухолей, которые показывали утраты части p53 чем у тех, у которых не было утраты части хромосомы $p = 0.0008$.
Выводы: ДНК-анойплойдия статистически достоверно чаще ассоциируется с утратой p53 гена. №то позволяет предположить, что функциональная потеря p53 может приводить к росту и развитию анойплоидных клеточных популяций внутри опухоли. Опухоли проксимальных и дистальных отделов толстой кишки имеют различную генетическую природу.
Ключевые слова: Колоректальный рак, ДНК-плойдия, тумор-супрессорный ген.

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