TÜBİTAK

ORIGINAL ARTICLE

Turk J Med Sci
2009; 39 (2): 209-213
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E-mail: medsci@tubitak.gov.tr
doi:10.3906/sag-0709-38

The Significance of DNA Ploidy in the Malignant Potential of Colorectal Adenocarcinomas

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Aim: The aim of this study was to evaluate the association between flow cytometric DNA ploidy and the potential for invasiveness and lymph node metastasis in colorectal adenocarcinomas.

Materials and Methods: The study included 40 colorectal carcinoma cases that were examined with flow cytometry and light microscopy. DNA ploidy in 4 different areas (deep tumor tissue, superficial tumor tissue, normal adjacent mucosa, and lymph node metastasis) was analyzed with flow cytometry and compared with invasiveness and lymph node metastasis

Results: Statistically significant differences in DNA content were observed between the deep and superficial layers of tumor tissues. The rate of aneuploidy was similar in the deep region of the tumors and lymph node metastases.

Conclusions: The results suggest that the level of aneuploidy observed in the deep invasive region of tumors was associated with lymph node metastases.

Key Words: Colorectal adenocarcinoma, DNA ploidy

Kolorektal Adenokarsinomların Malignite Potansiyellerinin Belirlenmesinde DNA Ploidinin Önemi

Amaç: Bu çalışmada kolorektal adenokarsinomlarda akım sitometri ile saptanan DNA kapsamının, tümörün invazyon derecesi ve lenf nodu metastaz potansiyeli ile karşılaştırılması amaçlanmıştır.

Yöntem ve Gereç: Kolorektal adenokarsinom tanısı almış 40 olgunun tümör dokusu ışık mikroskopik ve akım sitometrik olarak değerlendirildi. Dört farklı alandan (derin tümör dokusu, yüzeyel tümör dokusu, komşu normal mukoza ve metastatik lenf nodu) alınan doku örneklarinden akım sitometrik DNA ploidi analizi yapıldı. DNA kapsamları invazyon derecesi ve lenf nodu metastaz potansiyeli açısından karşılaştırıldı.

Bulgular: Derin ve yüzeyel tümör dokularının DNA kapsamında istatistiksel olarak anlamlı farklılık saptandı. Metastatik lenf nodu ve derin tümör dokusunda saptanan anöploidi oranları benzerlik göstermekte idi.

Sonuç: Bulgular, özellilkle derin tümör dokusunda saptanan anöploidinin lenf nodu metastazı ile ilişkili olduğunu desteklemektedir.

Anahtar Sözcükler: Kolorektal adenokarsinom, DNA ploidi

Received: September 24, 2007
Accepted: November 13, 2008

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Introduction

Colorectal carcinoma is one of the major causes of cancer death in most countries with a Western-type diet. Significant variability in rates of survival highlights the need for other biological indicators of behavior. Clinicopathological parameters, including age, and tumor location, size, histologic differentiation, and invasiveness, have been used in the evaluation of malignant potential (1).

The prognostic value of flow cytometric DNA ploidy in patients with colorectal carcinoma is not fully understood. Most investigators agree that the presence of aneuploidy based on flow cytometry has prognostic value and is associated with reduced patient survival (2-6); however, a limited number of studies reported that DNA ploidy status could be an independent prognostic variable with multivariate analysis that

includes traditional prognostic parameters (1,5,7-10). Moreover, some investigations reported that there isn't a significant relationship between prognosis and tumor DNA content (11-13).

The present study investigated DNA ploidy using flow cytometry. The study aimed to determine the correlation between DNA ploidy and the depth of invasiveness and lymph node metastasis.

Materials and Methods

Patients and Tumor Specimens

We studied the tumor specimens of 40 patients (23 males and 17 females) diagnosed with colorectal adenocarcinoma between 2002 and 2004 at the Department of Pathology, Karadeniz Technical University Medical Faculty Hospital. Mean age of the patients at the time of surgery was 63.5 years (range: 39-78 years). Tumor locations were as follows: sigmoid colon (12 cases), rectum (17 cases), and ascending colon (11 cases). Tumor sizes ranged between 2.5 and 14 cm. The patients were staged according to modified Astler-Coller classification: 6 patients (15%) were stage B1, 14 (35%) were stage B2, and 20 (50%) were stage C2. In all, 20 patients had positive nodes and 20 had negative nodes. In addition, the tumors were graded as well, moderately, or poorly differentiated based on the World Health Organization (WHO) criteria proposed by Jass and Sobin in 1989 (14). Of the 40 tumors, 25 were well differentiated, 12 were moderately differentiated, and 3 were poorly differentiated.

Flow Cytometry

Flow cytometric DNA ploidy analysis was performed on cell suspensions prepared from 50-µm sections from 4 different paraffin-embedded tissues: superficial (above the muscularis propria) and deep (below the muscularis propria) layers of tumor tissue, adjacent normal appearing mucosa, and metastatic lymph node. For analysis, after deparaffinization, tissue samples were minced with scalpels in phosphate buffered saline. Then, 100-ml cell suspensions were processed in a Coulter DNA Prep, including DNA-prep stain and DNA-prep LPR solutions. After 20 min of incubation a computerized multi-cycle DNA analysis program based on Hedley's method was used for calculating the DNA content and percentage of DNA aneuploidy.

Statistical Analysis

The Kruskal-Wallis variance analysis and Mann-Whitney U test with Bonferroni correction post hoc were used for analysis of DNA aneuploidy ratio average values.

Results

DNA Ploidy

We investigated the DNA content of paraffin-embedded tumor tissue samples. The relationship between flow cytometric DNA patterns and MACC was analyzed. The proportion of aneuploidy was 16.2% in 20 cases of MACC C_2 (Figure), 16.9% in 14 cases of MACC B_2, and 19.6% in 6 cases of MACC B_1. Kruskal-Wallis variant analysis revealed that the proportions of aneuploidy did not correlate with MACC stage ($P > 0.05$).

We investigated variations in the DNA content of paraffin-embedded samples from superficial and deep layers of tumor tissue and non-neoplastic adjacent mucosa. The proportion of DNA aneuploidy was 16.9% in the deep layers of tumor tissue, 5.68% in the superficial layers of tumor tissue, and 1.84% in non-neoplastic adjacent normal mucosa; the differences were statistically significant ($P < 0.05$) (Table 1).

We compared the patterns of aneuploidy in superficial and deep layers of tumor tissue to corresponding lymph

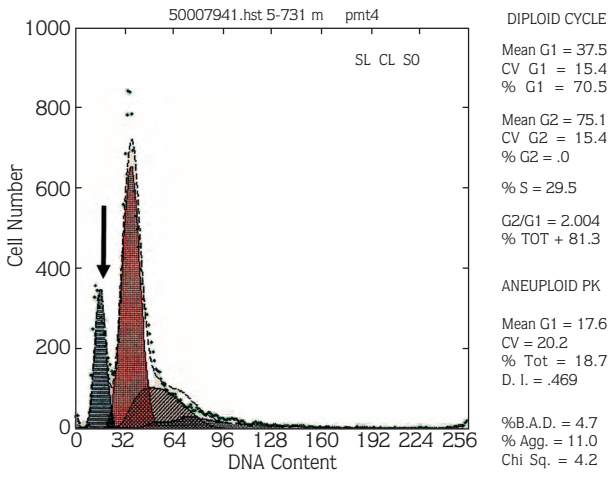

Figure. DNA content of MACC C_2 adenocarcinomas (left sided peak (arrow) demonstrating hypodiploid aneuploidy peak).

Table 1. Comparison of the proportion of DNA aneuploidy of superficial tumor tissue, deep tumor tissue, and non-neoplastic adjacent mucosa.

Tissue sample	DNA aneuploidy proportions % (mean ± SD)
Non-tumoral mucosa	1.84 ± 1.02
Superficial tumor tissue	5.68 ± 1.31
Deep tumor tissue	16.90 ± 4.00
Chi-square	54.924
Df	2
P	< 0.0005

node metastasis in 6 cases of MACC C_2. In these cases the proportion of DNA aneuploidy was 15.3% in the deep layers of tumor tissue, 6.2% in the superficial layers of tumor tissue, and 14.61% in lymph node metastases. The differences between the DNA proportion in lymph node metastases and deep tumor tissue layers, according to the Mann-Whitney U test, were not statistically significant ($P > 0.05$); however, a statistically significant difference was observed between lymph node metastases and superficial tumor tissue layers ($P < 0.05$).

Of the 40 carcinoma cases examined, 12 (30%) were classified as DNA diploid. Among the diploid cases, 7 (58%) had lymph node metastasis. Twenty carcinoma cases (50%) had hyperdiploid aneuploidy, in which 8 cases (40%) had lymph node metastasis. Eight cases (20%) had hypodiploid aneuploidy, in which 5 cases (62%) had lymph node metastasis. According to Kruskal-Wallis variant analysis, there was no significant relationship between DNA content and lymph node metastasis ($P > 0.05$) (Table 2).

Table 2. Comparison of DNA ploidy patterns with lymph node status.

DNA Ploidy	LNM negative (n)	LNM positive (n)	Total
Diploid	5	7	12
Hyperdiploid	12	8	20
Hypodiploid	3	5	8

LNM: Lymph node metastasis; $P > 0.05$.

Discussion

It has been reported that clinical and histological parameters have prognostic significance in colorectal carcinoma (15); however, it is difficult to estimate the individual prognosis of every patient due to the high variability of tumor growth. Stage and histologic grade of colorectal tumors are considered among the most relevant prognostic factors (16). Traditional morphological features, such as histologic grade, are subject to inter-observer and intra-observer variation, whereas staging may be influenced by critical factors such as care in pathologic examination and the extent of lymph node dissection during surgery (17).

Additional prognostic factors must be identified. Objective criteria, such as analysis of nuclear DNA content in cancers of the gastrointestinal tract, have contributed much to our understanding of prognostication. Numerous reports of the prognostic influence of DNA content have been published. There is considerable variation in the reported incidence of the DNA aneuploid pattern in colorectal carcinoma. DNA aneuploidy, whose incidence in colorectal carcinoma varies from about 40% to 80%, was shown to be an index of poor prognosis, with exceptions (14,16,18,19). We observed that 62.5% (25/40) of our cases had aneuploid DNA content, in agreement with the findings of previous studies (20,21). In the present study, as well as in some previous studies (21-23), no relationship between DNA ploidy and pathologic stage (MACC) was noted; however this contradicts the findings of other studies (24,25). These contradictory results may be related, in part, to different factors, such as intratumoral heterogeneity, sampling methods, interpretation of results, patient selection, and the number of cases studied.

Intratumoral heterogeneity is reported to be present in 5%-49% of patients with colorectal adenocarcinoma (18,26). Sampling methods, such as fresh and paraffin-embedded tissue, may also affect the results of DNA analysis. Emdin et al. reported that fresh and paraffin-embedded tissue produced comparable results (19). In contrast, Armitage et al. and Jones et al. noted less agreement between fresh and paraffin-embedded tissue (27,28). In the present study we used paraffin-embedded tissue.

The nature of specimens (i.e. superficial or deep) might be more important than the number of specimens when seeking an accurate DNA histogram. Kim et al.

compared the results of superficial and deep biopsies from the same tumor and reported differences (9). Giovagnoli et al. observed that DNA ploidy differences existed between the superficial and deep part of the same tumor, and that the majority of aneuploid cell populations were close to the serosa (29). In the present study we determined the ploidy of superficial and deep layers of tumor tissues, normal appearing mucosa, and lymph node metastases. The ploidy of superficial biopsies differed from that of deep biopsies, but the ploidy patterns of deep tumor tissues and corresponding lymph node metastases was similar in terms of the proportion of aneuploidy. This finding suggests that deeper parts of invasive tumors may be responsible for metastases of lymph nodes. The small proportion of aneuploidy observed in non-neoplastic mucosae supports the idea that this alteration may influence carcinogenesis (30).

In conclusion, the results of the present study show that the ploidy of the deep specimens reflected the ploidy of lymph node metastases. These data suggest that lymph node metastases arise primarily from deeply invasive tumor cells; therefore, samples taken from the deep invasive portion of a colorectal tumor may be more indicative of the prognosis than superficially obtained specimens.

Acknowledgements

This study was supported by the Research Fund of Karadeniz Technical University (2002.114.001.13).

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