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• BRIEF REPORTS •

Quantitative study of multiple biomarkers of colorectal tumor with diagnostic discrimination model

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Abstract

AIM: To evaluate the multiple biomarkers of colorectal tumor and their potential usage in early diagnosis of colorectal cancers.

METHODS: Multiple biomarkers (DNA contents, AgNOR, PCNA, p53, c-erbB-2) in 10 normal colorectal mucosae, 37 colorectal adenomas and 55 colorectal cancers were analyzed quantitatively in the computed processing imaging system. Discrimination patterns were employed to evaluate the significance of single and multiple indices in diagnosis of colorectal cancers.

RESULTS: The mean values of the analyzed parameters increased in order of the normal mucosa, adenoma and adenocarcinoma, and this tendency reflected the progression of colorectal malignancy. The parameters including DNA index, positive rates, densities of AgNOR, c-erbB-2, and p53, shape and density of nucleus were relatively valuable for diagnoses. Then a diagnostic discrimination model was established. The samples were confirmed with the model, the sensitivity rates in cancer group and adenoma group were 96.36% and 89.19%, respectively. The value of proliferating cell nuclear antigen (PCNA) in early diagnosis of colorectal cancers was uncertain.

CONCLUSION: The quantitative evaluation of some parameters for colorectal tumor can provide reproducible data for differential diagnosis. The established diagnostic discrimination model may be of clinicopathological value, and can make the early diagnosis of colorectal cancer possible.

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INTRODUCTION

Colorectal carcinoma is one of the most common malignant tumors worldwide. Quantitative changes in nucleus, DNA content, PCNA, AgNOR were observed in the progression of tumor[1-6]. Genes such as p53, *cerbB-2* might play a significant role in carcinogenesis[7-13]. Although the biologic parameters of tumors have been assessed extensively, measurement of these parameters has little impact on histological diagnosis. Furthermore, analysis of a single parameter is insufficient to evaluate tumor malignancy. Meanwhile, distinguishing benign from malignant lesions has traditionally been subjective; a quantifiable test is useful for the diagnosis of colorectal cancer. In this study, we used quantitative analysis to examine the potential usage of multiple biomarkers (DNA contents, AgNOR, PCNA, p53, c-erbB-2) in early diagnosis of colorectal cancer.

MATERIALS AND METHODS

Materials

Pathological specimens were obtained from Department of Pathology at Fujian Medical University from 1991 to 1996, including 55 cases of colorectal carcinoma, 37 cases of colorectal adenoma and 10 cases of normal colonic mucosae. The diagnosis was confirmed pathologically. The patients included 35 males, and 20 females with a mean age of 57±12.83 years (range 31 to 84 years). Histologically, there were 15 highly differentiated types, 19 moderately differentiated types, 21 poorly differentiated types, and 13 cases had a local lymph node metastasis and 1 case had the liver metastasis.

Reagents and methods

Schiff reagent, AgNOR staining fluid, antibodies against PCNA, c-erbB-2 and p53 and SP immunohistochemical reagent were purchased from Fujian Maxin Co.Ltd.

Formalin-fixed, paraffin-embedded specimens were cut in to 5 μm in thickness. The slices were stained with Feulgen, AgNOR and the detected antigen, and those in the control group were stained without primary antibody.

Semi-quantitative evaluation

Some silver stained black particles were observed in nuclei and classified. The positive staining rate was calculated[14]. A semi-quantitative evaluation system was used to determine the antigen expression in specimens[15]. Expression of p53 and c-erbB-2 was graded as the following scale: <10% "-",10-25% "+", 26-50% "++", 51-75% "+++", >75 "++++". Expression of PCNA was graded as the following scale: <25% "+", 26-50% "++", 51-75% "+++", >75% "++++".

Quantitative evaluation

The specimens were examined for multiple biomarkers with quantitative analysis using the computer processing image system (American Image Co. 8000 Type). Under the same power field of microscope, 150-200 cells were examined. Multiple biomarkers stained with silver or PCNA included DNA content, DNA index, nucleus area, Abs, granular shape factor, width and length of nuclei, the widest and longest diameter, and density of nucleus.

Statistical analysis

Student' s *t* test was used for analysis of variance. Discriminant analysis was done for multiple parameters and indices. The selected significant parameters were used to set up a diagnostic discrimination model. The SAS system for windows (version 6.12) was used for completing all the statistical analyses.

RESULTS

Multiple biomarkers (DNA contents, AgNOR, PCNA, p53, c-erbB-2) were quantitatively processed using the computer processing image system in 10 normal colonic mucosae, 37 colorectal adenomas and 55 colorectal carcinomas. The values of most parameters were increased in the order of normal mucosa, adenoma and adenocarcinoma. The tendency reflected the progression of colorectal malignancy. But the PCNA content was peaked in adenoma and decreased in carcinoma. The values were subjected to discrimination pattern method to evaluate the significance of parameters in multiple indices in order to set up the discrimination model. Then the model was used to recheck the samples. DNA index, shape factor, the widest diameter and density of nuclei were demonstrated to be the valuable parameters in feulgen-stained sections. The concordance rate was 86.02% for cancer group and 80.06% for adenoma group. The relatively valuable parameters in silver stained slides were average number, positive rate, density and aspect factor of particles. The concordance rate of the established model was 79.57% for cancer group and 78.65% for adenoma group. The valuable parameters in PCNA stained specimens were positive rate and density, and the concordance rate was 76.34% for cancer group and 73.55% for adenoma group. The valuable parameters in c-erbB-2 stained samples were Abs, positive rate and density, the concordance rate was 90.32% for cancer group and 90.14% for adenoma group. The valuable parameters in p53 stained positive samples were total positive area and density, and the concordance rate was 86.02% for cancer group and 83.55% for adenoma group. Discriminant analysis was performed for the above parameters. DNA index (X_1), positive rate (X_2), density (X_3) and aspect factor of AgNOR (X_4), density of c-erbB-2 (X_5), density of p53 (X_6), shape factor (X_7) and density of nucleus (X_8) were demonstrated to be the relatively valuable indices. Then a diagnostic discrimination model was established, and the model rechecked the samples, the concordance rates in cancer group and adenoma group were 96.36% and 89.19%, respectively. The models were as follows.

$Y(1)=-412.86-7.30X_1+34.83X_2+1.15X_3+2.54X_4+500.07X_5+17.34X_6+2.54X_7+409.68X_8$

$Y(2)=-480.40-7.04X_1+34.33X_2+0.98X_3+2.81X_4+560.55X_5+26.85X_6+2.47X_7+375.14X_8$

$Y(3)=-432.76-4.58X_1+41.97X_2+0.75X_3+2.63X_4+529.44X_5+52.13X_6+2.26X_7+321.11X_8$

DISCUSSION

Relationship among DNA content, shape parameters of nucleus and colorectal carcinoma

Most tumor cells had a certain amount of abnormal DNA; DNA content of tumor was closely related to biological behaviors[1,2]. Our study showed that the level of DNA content was increased in the order of normal mucosa, adenoma and adenocarcinoma with different significances. It has been confirmed that DNA content is a reliable and objective marker in early diagnosis of colorectal carcinoma and in distinguishing benign from malignant tumors.

In diagnosis of colorectal carcinoma with quantitative analysis as reported[1,2], the results varied with selected parameters. We believed that abnormalities of nuclei were the most important phenomena in hypertrophy of neoplastic cells in addition to abnormal structure of cells or tissues. It is well known that increased mitotic nuclei, multi-hierarchical structure, enlargement and pleomorphism of nuclei are expressed in most rapidly growing neoplasms. We suspected that increased nucleus area and much more irregular shape of nucleus were in accordance with the normal mucosa-adenoma-adenocarcinoma sequence. Parameters of nuclear shape and density of DNA reflected the malignancy of tumor significantly.

Although we considered that area of nucleus would play an important role in diagnosis of tumor with three-dimensional (3D) image processing, this study failed to show that areas of nuclei could reflect hyperplasic degree of tumor quantitatively. This might be due to the limitation of bi-dimension that could not reveal the whole nucleus.

Previous studies reported that Abs was an objective parameter to reflect the nucleus. But discriminant analysis showed that Abs was not relatively valuable. While Abs and density of nucleus had significant differences among the groups. We reason that sectional shape of nucleus is different from its real shape, so average area of Abs (density of nucleus) reflects of DNA content more objectively.

Relationship between AgNOR and colorectal carcinoma

Variation of the number of nucleolar organizer regions (NORs) could reveal the conditions of cellular activity[16-20]. It was suggested that AgNOR dot count of cells had a potential role in distinguishing benign from malignant tumors and in their early diagnosis[21]. Our results revealed that the count of AgNOR was increased in accordance with the normal mucosa-adenoma-adenocarcinoma sequence. Due to the strong correlation among type of particles, irregular factor and shape factor, irregular factor was rejected from the equations by discriminant analysis first. Type of particles used to be regarded as an important parameter[21], and turned out to have a limited value. The parameters, such as average number, positive rate, density and surface factor of particles were demonstrated to be valuable, and could reflect the characteristics of particles. The concordance rate was 79.57% for cancer group and 78.65% for adenoma group indicating this improved system is sensitive and very precise for quantifying the AgNOR dot count in cells and can provide a valuable objective measurement in differentiating benign from malignant tumors.

Relationship between PCNA and colorectal carcinoma

PCNA is a cell cycle related protein that is maximally elevated in late G1 and S-phase of proliferating cells and a key cycle regulator. It can be used as a marker of proliferation, and directly assessed using a thymidine analogue in suitably labeled pathological materials. Cellular proliferative activity has been accepted as a useful indicator of biologic aggressiveness in colorectal carcinoma[5-17]. PCNA immunohistochemistry could be used as a reliable marker of the proliferative compartment in both normal and neoplastic colonic mucosae[22]. The results revealed that all the parameters were significantly higher in adenoma and adenocarcinoma than in normal tissue, and the parameters in adenoma were higher than those in adenocarcinoma, indicating that multiplicative growth presented in G1 and S-phase when adenoma progressed to adenocarcinoma, and in other phase or in shock period while in adenocarcinoma. So the parameters of PCNA were descent in adenocarcinoma. Therefore we considered that the value of PCNA could distinguish benign from malignant lesions in the earlier stage of tumor.

Relationship among c-erbB-2, p53 and colorectal carcinoma

The c-erbB2 gene could be amplified in human adenocarcinomas, leading to elevated levels of expression of its encoded product, p185[11-23]. It has been shown that the accumulation of several alterations in p53 genes is most important for the conversion of adenoma to carcinoma. Critical genetic changes, including activation of oncogenes, mutation and deletion of tumor suppressor genes and disturbances in transcriptional regulatory sequences, might bring about aberrant expression of growth factors and their receptors in gastrointestinal carcinomas[10].

Mutations in the tumor suppressor gene p53 occur prevalently in a wide range of human tumors. Detection of a mutated p53 could provide useful information for the clinical management of colorectal neoplasms[24-29]. The p53 gene mutation and its subsequent over-expression in colorectal adenomas might therefore be a fundamental genetic event underlying the dysplasia and loss of proliferative control that are the characteristics of adenomas with a malignant potential[30]. Mutant p53 tumor suppressor gene and c-erbB-2 proto-oncogene were involved in human carcinogenesis, and detection of their protein product in human malignancies might influence the evolution of many neoplasms[31,32]. Therefore the aim of this study was to investigate the correlation of c-erbB-2, p53 with occurrence, progression of colorectal carcinoma and to determine the prognostic significance of oncogenes. The results showed that the values of c-erbB-2, p53 were increased in accordance with normal mucosa-adenoma-adenocarcinoma sequence (P<0.01), and the levels of c-erbB-2 were obviously increased in normal tissue and adenoma, suggesting that the occurrence of colorectal carcinoma was associated with activation of c-erbB-2, and c-erbB-2 could be considered as a more significant predictor of the occurrence of colorectal carcinoma. Meanwhile the value of total positive area and density of p53 were obviously increased in adenoma and carcinoma, indicating that p53 was a key oncogene in the progression of colorectal carcinoma. These results confirmed that p185 overexpression was associated with the early stages of colorectal cancer, whereas p53 was associated with more advanced stages[31].

Precancerous lesion of colorectal adenoma

The rate of carcinogenesis is associated with proliferation. To evaluate the proliferative degree of colorectal tumor with imaging analysis is helpful for the diagnosis of tumors. Our study of DNA contents, AgNOR, PCNA, p53, c-erbB-2 provided some useful prognostic information, their determination was useful for accurate evaluation of the prognosis.

The entity of carcinomatous change is a complicated process from quantitative to qualitative change. We studied the proliferative lesion with a mathematics model in order to provide the objective markers for accurate diagnosis. The result showed that DNA index, positive rates, density and aspect factor of AgNOR, densities of c-erbB-2, p53, shape factor and density of nucleus were relatively valuable. The result also showed that normal group was correctly diagnosed with the model (F>0.8). The 4 fault samples of papillary adenoma with grade 2-3 atypical hyperplasia by pathologic diagnosis were fault diagnosis of adenocarcinoma by the model (F<0.8). The 2 highly differentiated colorectal tumors were fault diagnosis of adenoma (F<0.8). Comprehensive evaluation showed the strong discriminating power of the model.

This study examined the value of PCNA. No parameter of PCNA was selected for the final model. As strong correlations were observed between PCNA and p53[33,34] we believed that the discriminating power of PCNA was hided by p53 due to the complex interface among the parameters. On the other hand, we doubted the value of PCNA in early cancer. However, some parameters of DNA, AgNOR, p53, cerbB-2 were selected for the model, and the diversity of tumor and occurrence and progression of colorectal tumor were related with multiple factors. Meanwhile, all the 4 indexes played a major role in the diagnosis of colorectal tumor, and inactivation of anti-oncogene and activation of oncogene were existed in the progression of colorectal malignancy.

Quantitative evaluation of some indices of colorectal tumor could provide reproducible data for its differential diagnosis. The discrimination model established can offer subjective parameters for distinguishing benign from malignant tumors.

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