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COMPARATIVE PATHOLOGICAL AND FCM STUDIES ON THE PRIMARY AND METASTATIC CANCERS IN STOMACH AND BREAST

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Pathological morphology, differentiation, cellular DNA content and cell proliferation index (PI) were comparatively studied in the primary and their corresponding metastatic lesions of 54 cases of gastric and breast cancers. The results showed that differentiation and types of metastatic cancers were in accordance with their corresponding primary cancers in more than half cases (18/34) of stomach cancers, differences could be found in the other cases (16/34), and among them, 10 were lower and 6 were higher differentially than their corresponding primary cancers. Similar results were found in breast cancer cases. Flow cytometry (FCM) analysis revealed that dramatic DNA Index (DI) differences between metastatic and their corresponding primary cancers existed in 6/34 of gastric and 4/18 of mammary cancers, among them 7 cases were higher than the primary while 3 were lower than the primary (DI) . Similar results could also be found in PI analysis . All these suggested that metastasis of gastric and breast cancer was a very complicated process and metastatic cancer did not necessarily always show lower differentiation, higher DNA contents and DNA aneuploidy as well as higher cell proliferative rate.

Key word: Gastric carcinoma, Breast carcinoma, Metastasis, Differentiation, FCM, DNA index, Proliferation index.

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In the past decades, pathological, flow cytometric and immunohistochemical changes have been widely studied in gastric and breast cancers. Yet, almost all of these studies were limited in the primary lesions in stomach and breast. To further explore the possible mechanism of cancer metastasis and set scientific bases for the clinical use of flow cytometry (FCM) in the diagnosis and treatment of malignant tumors, pathological morphology, differentiation, cellular DNA content and cell proliferation index (PI) were comparatively studied in the primary and their corresponding metastatic lesions of gastric and breast cancers.

MATERIALS AND METHODS

Cases

Thirty-four cases of gastric and 20 cases of breast carcinoma with lymphonode metastasis archived in the Department of Pathology, the Second Affiliated Hospital, Hebei Medical College were included in the series.

All the paraffin embedded tissue blocks of primary and their corresponding lymphonode metastases were used. Among the 34 cases of gastric cancers, 19 cases were male and 15 female. Their ages ranged from 23 to 72 years old. As for breast cancer cases, all of them were female and their ages ranged from 33 to 62 years old.

Pathological Morphology

All the sections of the cases in the series were reviewed by two experienced pathologists and definitely diagnosed as well differentiated adenocarcinoma, low differentiated adenocarcinoma, undifferentiated carcinoma, mucoadenocarcinoma and signet-ring cell carcinoma for gastric carcinoma and medullar carcinoma, sclerous carcinoma, ductal carcinoma etc. for breast carcinoma.

FCM Sample Preparation

Twenty-three cases of gastric carcinoma and 18 cases of breast carcinoma with typical pathological changes were chosen for FCM. Only these tissue blocks, of which the metastatic focus counting for over two thirds of a lymphonode in area on the section were used to get rid of false results.[1-4] Paraffin embedded tissue blocks (with an average of 2) of primary and metastatic tumors respectively were used. First, 5 μm section was cut, HE stained and the diagnoses checked, and then single nuclear suspension were prepared according to the method described before.[1,2]

Normal gastric mucosa from stomachs removed due to peptic ulcer and normal mammary gland tissues as well as normal lymphonodes were used as normal control (5 cases each).

FCM Analysis

Nuclei were stained with Ethethim Bromide solution and measured by FACS 420 flow cytometer (BD, USA) for DNA contents. Detected data were processed with HP-300 Consort 30 computer. Nuclear DNA contents and cell proliferating conditions were expressed by DNA Index (DI) and Proliferation Index (PI), their calculations are as follows:

$$DI = \frac{\text{cancer cell } G_0/G_1 \text{ peak mean channel}}{\text{normal control cell } G_0/G_1 \text{ peak mean channel}}$$

$$PI = \frac{S+G_2M}{G_0/G_1+S+G_2M} \times 100\%$$

Statistics

For the pathological results, Student's *t* test was used to check the changes of primary and their metastatic cancers based on the differentiation and pathological types.

As for the FCM results the significance of the changes on DI, PI between primary and metastatic lesions were also checked by *t* test.

RESULTS

Pathological Observation

Pathologically, for more than half of the cases (18/34), differentiation and types of the metastatic cancers were in accordance with their corresponding primary cancers in stomach. While in 47% (16/34) cases, there existed differences between metastatic and their corresponding primary cancers, some were higher and some lower differentially than the primary cancers. Among them, the differentiation of primary cancers were higher than their corresponding metastatic lymphonodes in 10 cases (Figure 1) and lower than the latter in 6 cases (Figure 2). There was no difference between these two groups (P>o.o5). The obvious differences in pathological morphology and differentiation were found between different metastatic lymphonodes in the same case.

The morphological changes in breast cancer were similar to that of gastric cancer, differences in pathological types and differentiation could be seen between metastatic cancers and their corresponding primary cancers in most of the cases. Among these cases, the most showed metastatic cancers with smaller cancer cells and increased stromal fiberous tissues (Figure 3).

On the other hand, metastatic cancers with abundant cancer cells and few stromal fiberous tissue, a medullary type appearance, could be found in sclerous type primary cancer in some cases.

Results of Cellular DNA Contents (DI)

There were some differences in DNA contents between metastatic and their corresponding primary cancers both in gastric carcinoma and breast carcinoma. Yet most of these changes were still in the range of 2 CV values. Dramatic DI differences were found between metasatic and their corresponding primary cancers in 6 of the 34 cases of gastric cancers. Among the 6 cases, 4 cases showed higher primary DI values (DI difference 0.23–0.56) and 2 showed relatively lower primary DI values (DI difference 0.24 and 0.38 respectively) (Figure 4). Among the 18 cases of breast cancers analyzed by FCM, dramatic DI value differences were found in 4 cases and 3 showed higher primary DI (DI difference 0.27–0.38) and 1 lower one (DI difference 0.14) (Figure 5). Statistically, there were no differences among these different groups ($P>0.05$). It was also found that there were some changes between different metastatic lesions of the same case, the DI changes can reach to 0.20.

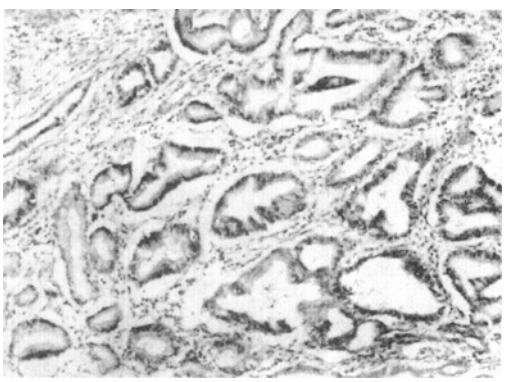

Fig. 1a. Primary carcinoma in stomach: well differentiated carcinoma.

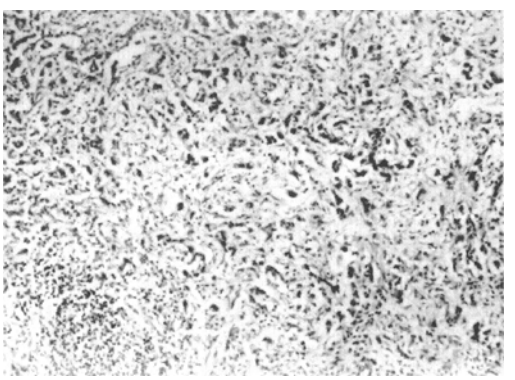

Fig. 1b. Lymphonode metastatic carcinoma in the same case: low differentiated carcinoma. HE 200×

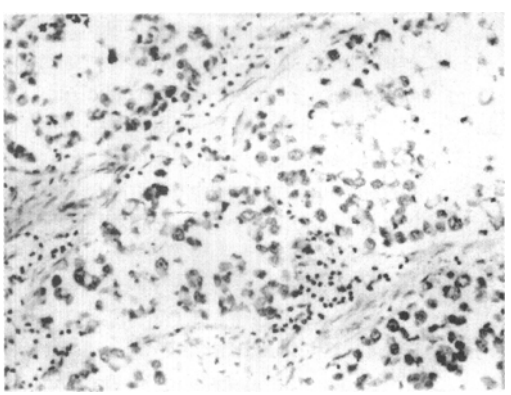

Fig. 2a. Primary carcinoma in stomach: signet-ring cell carcinoma.

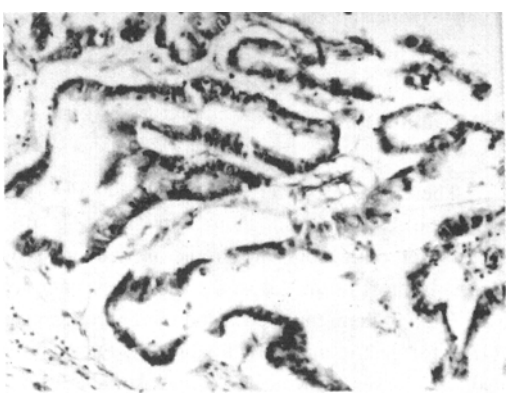

Fig. 2b. Lymphonode metastatic carcinoma in the same case: well differentiated carcinoma. HE 200×

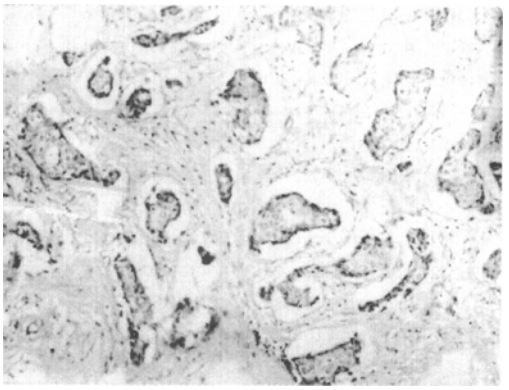

Fig. 3a. Primary carcinoma in breast, note the big cancer cells.

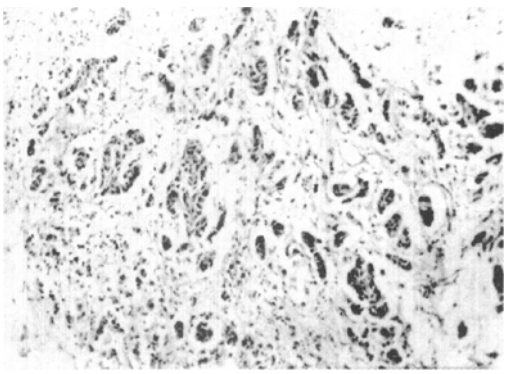

Fig. 3b. Lymphonode metastatic carcinoma in the same case, note the relatively smaller cancer cells with increased stromal fibrous tissue. HE 200 ×

Cell Proliferation

There were differences in cell PI between metastatic cancers and their corresponding primary cancers for both stomach carcinoma and breast carcinoma. In most of the cases, the PI of metastatic lesions were higher than their corresponding primary lesions (11/24 for gastric and 6/18 for breast). Yet there were also some cases showing lower metastatic cell PI (3/24 for gastric and 2/18 for breast). Statistical analysis showed that there were no significant correlation between PI of the metastatic and the primary (P>0.05). Difference of PI between different metastatic foci in the same cases could reach 16%.

No difference in DNA content among different sex and different ages could be found. There was no significant correlation between DI and PI in the cases.

DISCUSSION

Metastasis is the important malignant maker in biological behavior of neoplasm and the main causes of death for cancer patients. The lower the differentiation, the more frequent the metastasis. Occasionally, histomorphological differences could be found between metastatic cancer and their corresponding primary cancer in pathological practice. In the past a few years, as FCM technique gained continuous improvement and wider usages, DNA contents for various malignant tumors have been analyzed. Many authors[3–5] take DNA content abnormalities, such as DNA content increase, DNA aneuploidy etc. as the index for higher malignance in biological behavior and poorer prognosis for cancer patients. But up to now, most of the pathological and cell analysis work in cancer research were limited to the primary cancers. Few of them involved metastasis. Bjornhagen[6] et al. comparatively studied the nuclear DNA contents of primary and metastatic malignant melanomas with microspectrophotometer in 1991, and found DNA contents of metastatic lesions could be different from their primary counterpart. They attributed this phenomenon to melanoma's higher heterogenicity. The results in this study showed that although pathological morphology, differentiation, nuclear DNA content and cell proliferation condition of metastatic cancers were in accordance with their corresponding primary counterpart in more than half of the cases with gastric and breast carcinoma, significant differences in these parameters did appear in some cases. Pathologically, differentiation of the metastatic cancer could be higher than the primary cancer, and on the other hand, it could be lower than that of the latter ones. Nuclear DNA content of metastatic lesion might be higher and lower than that of their corresponding primary counterpart. Cell proliferation condition showed the similar change. There existed differences in

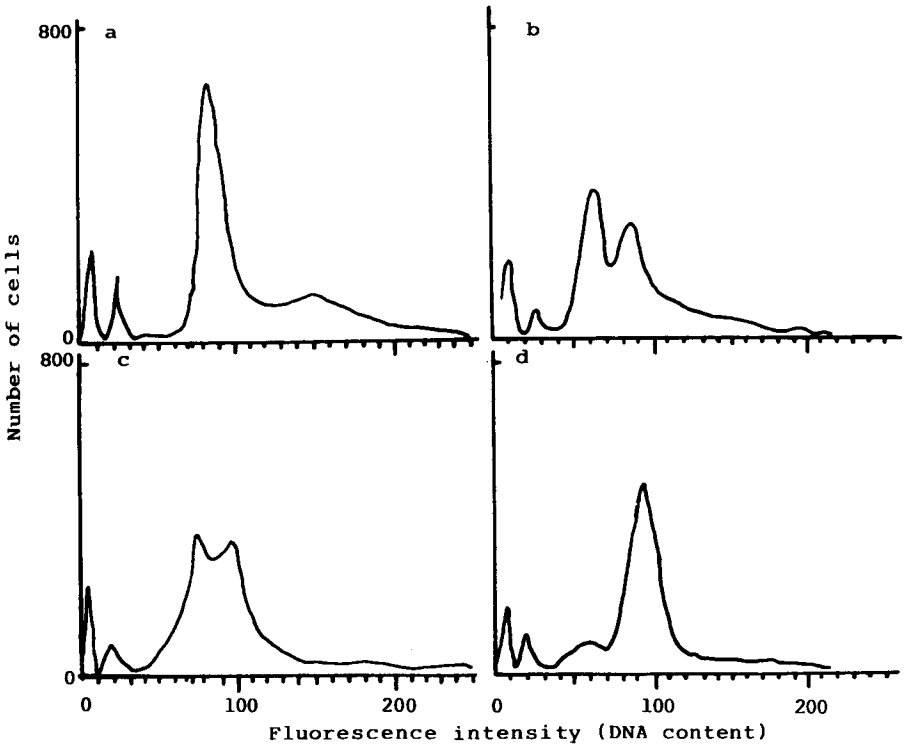

Fig. 4. DNA histogram in some gastric carcinoma cases:
a. Primary carcinoma, DI=1.51
b. Corresponding metastaic carcinoma in the same case as a, DI=1.51
c. Primary carcinoma, DI=1.32
d. Corresponding metastatic carcinoma in the same case as c, DI=1.70

different parts of primary cancer or different foci of metastatic cancer in the same case. All these suggested that metastasis of gastric and breast carcinoma was a very complicated process. There was no regular changes for metastatic cancers in pathological morphology, nuclear DNA content and cell proliferation. Metastatic cancer did not necessarily always show lower differentiation, higher DNA content or DNA aneuploidy and higher cell proliferating rate. As we all know that differences in pathological types, differentiation and DNA contents etc. may result in dramatic differences in response to the therapies. Oncologist take these parameters as the basis for the consideration of postoperative treatment. If clinicians do not consider the possible changes among primary and metastatic cancers and design their radio- or chemotherapy protocols only base on pathological and cell analytic results of some parts of primary cancers, satisfied clinical effects may not be resulted. Thus, we suggest that patho logists should take specimens from different parts of the primary cancer and metastatic cancers as well, and if there is any changes between different parts of the primary or between the primary and metastatic lesions, must describe the changes in report for the clinician's reference. The oncologists should always bear in mind the whole spectrum

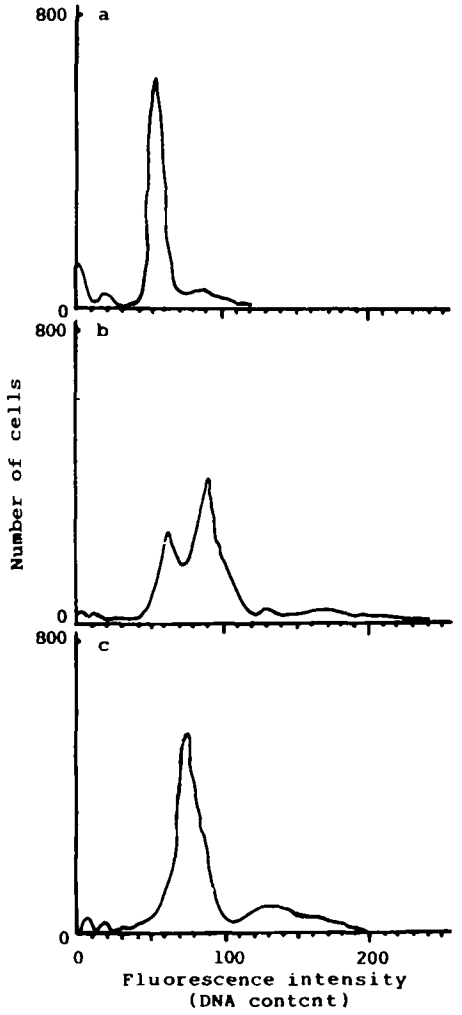

Fig. 5. DNA histogram in mammary carcinoma case:
a. Normal mammary tissue, DI=1.0
b. Primary carcinoma in breast, DI=1.55
c. Corresponding metastatic carcinoma in the same case, DI=1.30

of pathological and cell analytic changes in the primary and metastatic cancer to get better clinical results.

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