

Differentiation between Metastasis and Synchronous Double Cancers of the Esophagus

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ABSTRACT: Simultaneously two independent tumors were detected in the esophagus. Histological examination could not necessarily distinguish the two tumors from double cancers. It is emphasized that cellular DNA analysis by using FCM is of great benefit for this purpose.

It is difficult to determine clinically whether double cancers are simultaneously existing two cancers or cancerous lesion accompanying metastatic lesions. Until recently histologic finding was the only method to identify them. The criteria of identifying double cancers from primary cancer with metastasis are that different types of histology should be individually revealed and no histologic sequence between both lesions should be defined with or without submucosal lymphatic involvement.

It is well known that malignant tumors are characterized by abnormalities in cellular DNA content. However, it is no doubt that metastatic tumors arising from the primary one display a similar pattern of DNA, suggesting identical stem cells with an original tumor when surgeons encounter in independent cancerous lesions in the same surgical specimen, two separated cancerous lesions should be identified whether double primary cancers or metastasis.

Flow cytometry (FCM) provides a fast and precise means for determination of DNA-aneuploidy index.

we experienced with independent double cancerous lesions in the esophagus and these were regarded as primary cancer with skipping metastatic lesions with the help of analysis of DNA histogram.

PATIENTS

A 60-year-old man complained of dysphagia since May 1989 and this symptom was aggravated in October with body weight loss of 5kg. Precise examination revealed a diagnose of carcinoma of the esophagus. On his admission, he could not take solid food, and his nutritional condition was poor. Immediately after his admission, total parental nutrition was initiated.

Radiological X-ray film showed the serratus type in the upper portion of the thoracic esophagus with 4cm shadow of a defect, and the other deformity was observed 3cm distal to the proximal lesion (**Fig. 1**). Endoscopic examination revealed circular stenosis 16cm distal to an incisor line, and an endoscopic tube was unable to pass through the stenotic lesion (**Fig. 2**). Therefore, the distal lesion could not be defined through the endoscopic tube. CT scan showed no direct invasion to the aortic and bronchial

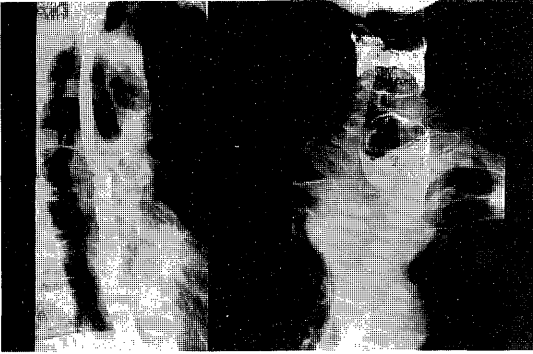


Fig. 1. Radiological X-Ray film of esophagogram, showing the abnormal findings of upper and middle portion of thoracic esophagus.

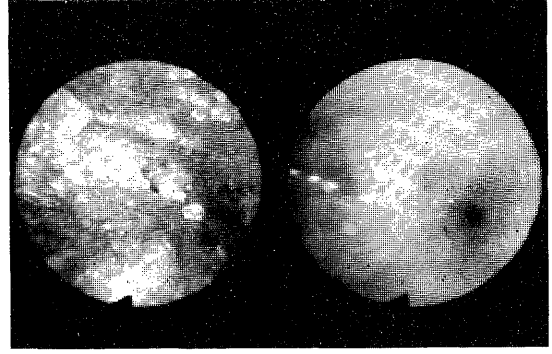


Fig. 2. Endoscopic finding, showing the circular stenosis which was unable to pass through forwards.

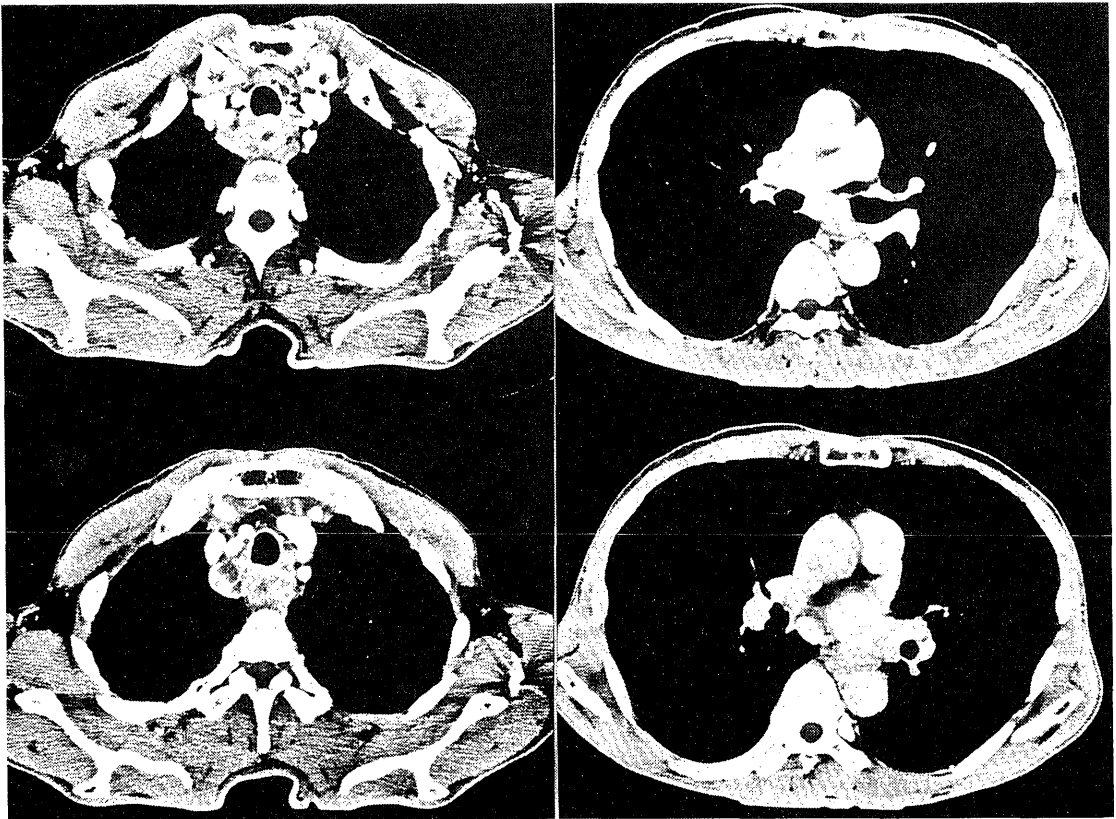


Fig. 3. CT scan, showing the tumor mass of the esophagus, identifying no direct involvement of the adjacent organ.

walls and also illustrated swelling in the subcarinal and paraesophageal nodes (**Fig. 3**). Right thoractomy was made to expose the esophagus. The tumor was situated in the middle portion of the esophagus with adventitial

invasion. Proximal lesion was resected with an additional resection. However, there was no direct involvement of the adjacent organs. The other lesion was not palpated through the esophageal wall. After subtotal esophagectomy,

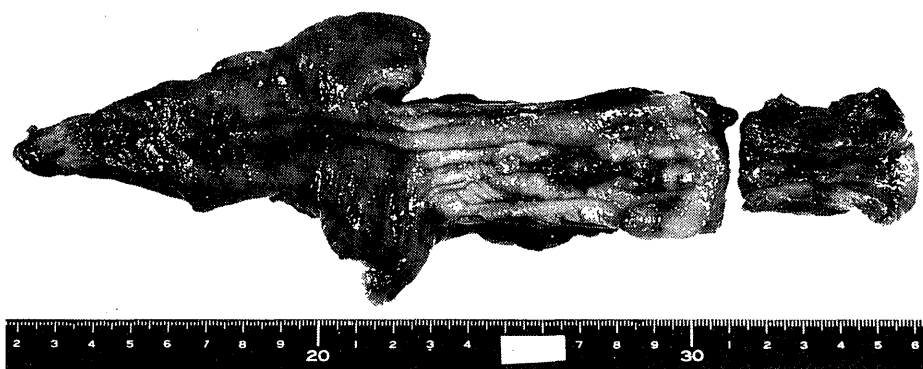


Fig. 4. Surgical specimen, showing independent two cancerous lesion, which include the proximal lesion in the additional resection specimen.

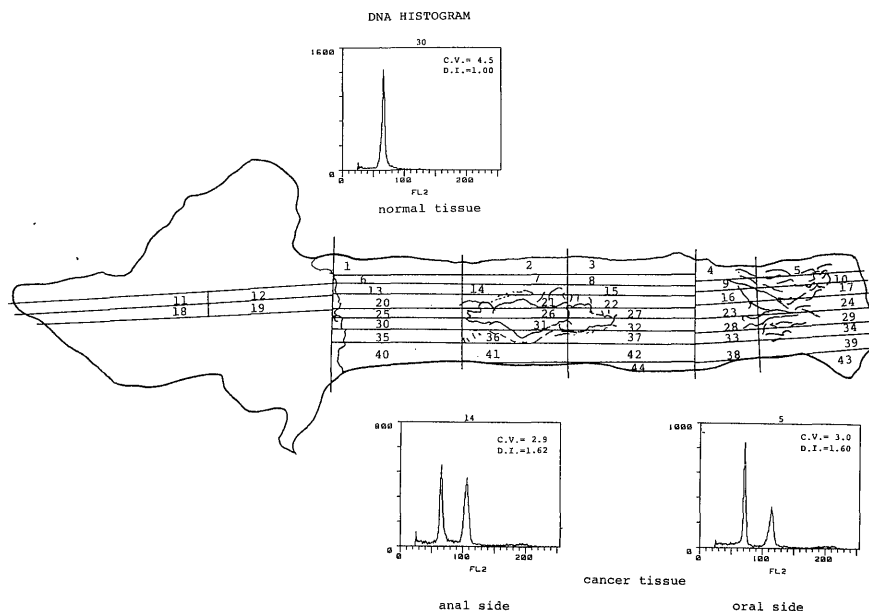


Fig. 5. Schema of the surgical specimen and DNA ploidy patterns in the corresponding sites.

reconstruction was performed with gastric tube via retrosternal route at the neck.

The surgical specimen showed independent, two ulcerative lesions with healthy mucosa in 3cm distance (Fig. 4, 5). These two ulcers were of irregular margins with interrupted mucosal folds. Histologic examination clarified squamous cell carcinoma with poor differentiation in the both lesions involving the adventitia. The

proximal lesion was characterized by the finding of that was protruded with lymph vascular invasion, which was compatible with a metastatic lesion.

However, it was not confirmable for the proximal lesion to be metastatic. Therefore, DNA analysis by FCM was applied for differentiation of double cancers.

Paraffin-embedded tissues were cut into 40µm

sections and dewaxed with xylene, rehydrated with ethanol and incubated overnight at 37°C in trypsin citrate buffer, before staining with trypsin, trypsin inhibitor (RNase) and propidium iodide, spermine tetrahydrochloride. Finally fluorescence measurement was made by using FACS IV.

DNA histogram showed a similar pattern in the both ulcerative lesions on the condition of 2.0 to 2.9 of CV. The DNA index indicated a range of 1.60 to 1.62 where DNA histogram in the separated healthy mucosa 2cm distal to the lesion showed a different pattern from those in the lesions which was regarded normal.

This was sufficient to infer that two independent lesions were arising from the same stem cells that one was primary and the other was metastatic.

DISCUSSION

FCM study is now prevalent as a clinical approach and aims at obtaining additional information in terms of tumor biology or prognosis. Furthermore, FCM provides a fast and precise determination in association with heterogeneity and different cellular clones of the tumors.

The two cancerous lesions existing in the same surgical specimen should be suspected whether there exist double cancers or metastasis.

The criteria to determine synchronous double cancers were cytomorphologically clarified by many researchers in various organ. The clear definitions of double cancers are that different histologic patterns should be observed and arising from a different tissue origin as reported by Billroth¹⁾ and Warren, Gate²⁾.

In general, it is kept constant between chromosom number and cellular DNA in living tissues and these cells are considered as being a stem cell associated with proliferation.

On the other hand, it is believed that the tumors pose inherent cellular DNA^{3~6)} in accordance with stem line theory⁷⁾.

In the case with identical DNA pattern on histogram, it indicates that the tumors originates from the same clonal origin each other.

Scott³⁾ reported that cellular DNA analysis by FCM was of great benefit to determine

double cancers or metastasis.

On the contrary, Peterson⁸⁾ pointed out that 31% of colon cancers showed a presence of mosaicism, as defined by the presence of a variety of cellular DNA content. Therefore, in analysis of cellular DNA the same pattern obtained on histogram imply a neoplasm arising from an identical stem cell. However, it is considered that multiclonarity may be underestimated if it is defined by the required presence of two aneuploid DNA stemlines because one of the tumor stemlines in a multiclonal tumor may be diploid and thus hidden within a peak of normal diploid cells. Furthermore, the presentation of multiclonality seems to reflect multifocal carcinogenesis, characteristic for certain tumor types (colon, small cell carcinoma of the lung) rather than changes during evolution of the tumor. Cellular DNA analysis by FCM is of clinical value to determine whether double cancers are presented or not in two independent tumors.

REFERENCES

- 1) Billroth T: In general surgery, pathology and therapeutics. Translated by Hackley CE: Appleton century Croft, New York 1889, p14.
- 2) Warren S, Gates D: Multiple primary malignant tumors. *Am. J. Cancer* 16:1358-1414, 1932.
- 3) Rognum TO, Thorud E, Eligok *et al*: Large bowel carcinomas with different ploidy related to secretory component. Ig A and CEA in epithelium and plasma. *Cancer* 56:1658-1666, 1985.
- 4) Peterson SE, Lorentsen H, Bichel P.: A mosaic subpopulation structure of human colorectal carcinomas demonstrated by flow cytometry. *Acta Pathol. Microbiol Scand.* 274:412-416, 1981.
- 5) Rognum TO, Thorud E, Elgio K *et al*: DNA flow cytometry (FCM) in carcinomas of the large bowel compared with the two functional cell markers secretory component (SC) and carcinoembryonic antigen (CEA) the histopathological tumor grade and the clinical stage, *Acta Pathol. Microbiol Scand (suppl)* 274:417-423, 1981.
- 6) Rognum TO, Brandzaeg P, Orysaether H *et al*: Immunohistochemical study of secretory component, secretory IgA and carcinoembryonic antigen in large bowel carcinomas. *Pathol. Res, Pract.* 170:126-145, 1980.

- 7) Sandberg AA: Tumors of the alimentary tract. In the chromosomes in Human Cancer and Leukemia Sandberg AA (ed) Elsevier, New York 1981. pp468-485.
- 8) Scott MB, Taylor MA, Wolff MM *et al*: Perianal metastasis from a sigmoid carcinomaobjective evidence of a clonal origin-Report of a case-Dis. *Colon rectum* 31:68-70, 1988,