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Metastatic Adenocarcinoma of Unknown Primary Site: Abnormalities of Cellular DNA Content and Survival

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Abstract—*Using a new flow cytometric technique we measured the cellular DNA content of tumour biopsies taken from 152 patients presenting with metastatic adenocarcinoma or undifferentiated carcinoma of unknown primary site. One hundred and six (70%) contained populations of cells with an abnormal cellular DNA content and the remainder were diploid. The incidence of aneuploidy was similar for the two sexes and bore no obvious relationship to the various patterns of metastatic involvement. Median survival of patients with diploid tumours was 4.2 months and for patients with aneuploid tumours, 4.8 months. Nine of the 46 patients with diploid tumours (i.e. 18%) survived for more than 2 yr compared to 10 of 106 (9%) of those with aneuploid tumours. These results indicate that the incidence of aneuploidy in this heterogeneous group of patients is similar to that reported for adenocarcinomas of known histogenesis, such as breast or colorectal cancer. In contrast to many of these tumour types, however, patients with metastatic adenocarcinomas of unknown primary which are diploid do not on the whole have a more favourable prognosis.*

INTRODUCTION

IN RECENT years it has become recognised that a significant percentage of patients with cancer present with metastases from an unknown primary site [1, 2]. Histologically the majority of these tumours are either adenocarcinomas of varying degrees of differentiation or undifferentiated carcinomas [3]. Several studies have now emphasised the low efficacy of radiological or endoscopic investigations to identify the primary tumour site, and it has been suggested that the search for a primary site should be limited to include only those tests which might diagnose tumour types for which specific and effective treatment is available [2, 4]. Examples of such tests would include mammography in females and measurement of serum prostatic acid phosphatase in males. For the majority of patients the primary site remains uncertain, even after detailed post-mortem examination, and treatment remains empirical. Several trials of chemotherapy have been published [1, 5, 6]. These have generally employed combinations of drugs which can be active against adenocarcinomas of known histogenesis, but the results have been very disappointing, with low objective response rates and median survivals of only a few months.

Despite the generally poor prognosis of patients with metastatic adenocarcinoma of unknown primary (ACUP), most large series contain a proportion of patients whose tumours run a relatively indolent course. It has been suggested that measurement of cellular DNA content might help to identify these patients [7]. Between 10 and 30% of human solid tumours have an apparently normal diploid DNA content, the remainder containing one or more aneuploid clones [8]. In most instances the ploidy of a tumour remains constant with respect to both time and metastatic site [9, 10], and there is increasing evidence that for many adenocarcinomas, such as breast, prostate and colorectal cancer, a diploid DNA content is associated with a markedly better prognosis [11–13]. We recently developed a flow cytometric method for measuring cellular DNA content which uses archival, paraffin-embedded tissue as a starting point [14].

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This method is particularly suited to the retrospective study of large series of patients whose clinical outcome is already known, because its resolving power and speed of sample handling are much greater than the older, static cytometry techniques.

Because we see large numbers of patients with ACUP and have a long-standing interest in this clinical problem, we decided to undertake an extensive clinico-pathological study of our patients encompassing pathological review with detailed immunocytochemical staining and flow cytometric measurement of cellular DNA content, to see if these tumours could be better characterised with respect to both possible histogenesis and clinical outcome. In this paper we describe the incidence of abnormalities of cellular DNA content and their influence on survival.

MATERIALS AND METHODS

Patients

Patients were included in this study if they presented with histologically confirmed metastatic adenocarcinoma or undifferentiated carcinoma with no primary site identified by history, physical examination or chest X-ray. They were divided into six broad clinical categories: disease dominated by lymphadenopathy, pulmonary changes, liver involvement, other abdominal presentations or involvement of bone or central nervous system. Serum αFP, βHCG and acid phosphatase were measured and a limited radiological or endoscopic search for the primary site conducted, appropriate to the dominant metastatic sites of disease. For the purposes of this study patients were considered to have had an 'unknown primary' even if these investigations, the clinical course or post-mortem examination subsequently identified the primary site. Treatment with cytotoxic drugs was in general only given to patients who had symptomatic disease. Combinations of drugs used included cyclophosphamide, methotrexate and 5-fluorouracil (CMF), adriamycin and mitomycin C (AM) [5] and *cis*-platinum, vinblastine and bleomycin (PVB).

Measurement of cellular DNA content

We used a flow cytometric method modified to allow the cellular DNA content of fixed, paraffin-embedded material to be measured. This method has been described in detail [14]. Briefly, 30-μm sections were cut from tissue blocks using a microtome, dewaxed in xylene and rehydrated by sequential immersion in 100, 95, 70 and 50% ethanol. After washing in water, nuclear suspensions were prepared by incubation in 0.5% pepsin

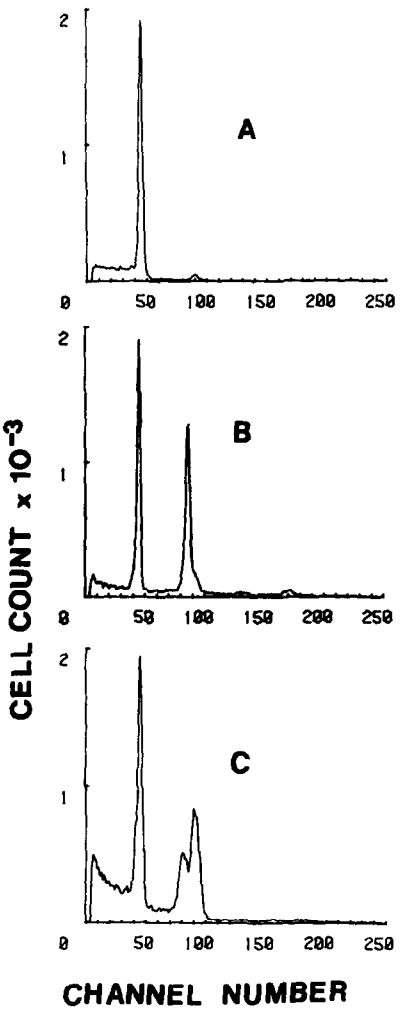

Fig. 1. Representative DNA histograms illustrating tumours with: (a) diploid, (b) single aneuploid and (c) multiple aneuploid cellular DNA content. Because virtually all human cancer biopsies contain normal diploid cells it is assumed that a solitary peak is diploid.

(Sigma, St. Louis, MO, product No. P7012, activity 2500-3200 units/mg protein), pH 1.5, for 30 min at 37°C. DNA was stained using 1 μg/ml DAPI (Boehringer Mannheim GmbH) and DNA content measured using a standard ICP 22 flow cytometer, the results being displayed as frequency-distribution histograms (Fig. 1).

RESULTS

Tissue for review was available from a total of 202 patients. However, in 36 cases we were unable to measure cellular DNA content because of the small size of the sample, the large majority of these being needle biopsies of bone, liver or lung metastases. In a further 14 samples apparently diploid DNA histograms were obtained which were of poor technical quality, i.e. they had a coefficient of variance greater than 6%. Because of the possibility that these wide peaks might be concealing a second, near-diploid population of aneuploid cells we omitted these patients from

detailed analysis. Technically satisfactory DNA histograms were obtained from 152 patients. Definite primary sites were eventually identified in 26 of these patients (either pre- or post-mortem), and these are shown in Table 1. Dominant sites of metastatic involvement and the results of cellular DNA measurements are shown in Table 2. A total of 46 (30%) tumours contained only a single G_1 peak and were considered to be diploid. The remainder were classified as aneuploid because of the presence of a second, or in nine instances multiple, aneuploid G_1 peaks. There were no significant differences in the percentage of diploid tumours between the different sites of dominant metastatic spread. Of 76 male patients 22 (29%) had diploid tumours, compared to 24 out of 76 (32%) females.

Survival curves, measured from the time of histological diagnosis, are shown in Fig. 2. Median survival was 4.2 months for patients with diploid tumours and 4.8 months for those with aneuploid tumours. Although a higher proportion of patients with diploid tumours survived for more than 2 yr (9/46 vs 10/106), comparison of the two curves using the log-rank test shows no statistically significant difference. Furthermore, replotting the survival curves on a semi-logarithmic scale does not suggest the possibility of a 'tail' of long-term survivors. Table 3 shows the median and range of survival of patients

Table 1. Identified primary sites

Lung	7
Renal	4
Stomach	3
Pancreas	2
Colon	2
Ovary	2
Breast	2
Melanoma	1
Gall bladder	1
Prostate	1
Nasopharynx	1
Total	26

Table 2. Incidence of aneuploidy according to dominant site of involvement

Site	Diploid	Aneuploid (%)	Total
Liver	14	19 (58)	33
Other abdominal	7	18 (72)	25
Lung	10	28 (74)	38
Bone	7	12 (63)	19
CNS	2	10 (83)	12
Lymph node	6	19 (76)	25
Total	46	106 (70)	152

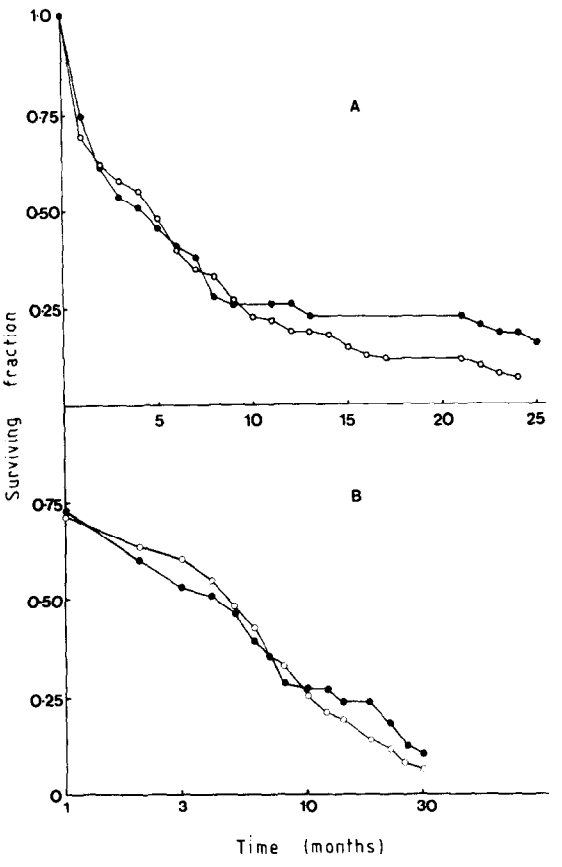

Fig. 2. (A) Survival curves, measured from the time of biopsy-proven diagnosis, comparing patients with diploid or aneuploid tumours. (B) Replotting the curves on a semi-logarithmic scale does not suggest the presence of a 'tail' of long-term survivors. (●——●) diploid, (○——○) aneuploid.

Table 3. Median and range of survival (months) by dominant site of involvement and ploidy

Site	Diploid	Aneuploid
Liver	2 (1-22)	4 (1-14)
Other abdominal	5 (1-60)	2 (1-37)
Lung	5 (1-13)	4 (1-28)
Bone	18 (3-44)	6 (1-22)
CNS	1 (1, 1)	4 (1-23)
Lymph node	16 (1-42)	16 (1-54)

according to dominant sites of disease and tumour ploidy. Again it can be seen that there is no obvious survival advantage for patients with diploid tumours in any of the clinical categories.

DISCUSSION

This study again confirms the poor overall prognosis for patients with metastatic ACUP, since half of them died within 5 months of diagnosis and only 20% survived for more than 1 yr. These figures are in close agreement with other published reports [1, 5, 6].

Recently there has been considerable interest in the clinical and biological significance of the cellular DNA content of human cancers.

Aneuploidy is now a well-recognised phenomenon, various series reporting an incidence of between 70 and 90% for solid tumours [8]. The majority of aneuploid tumours contain a single hyperdiploid population, whose DNA content tends to fall into either a near-diploid or a triploid-tetraploid mode. The significance of this non-random distribution of DNA content remains uncertain, although there is some evidence that proliferative activity might increase and prognosis worsen with increasing degrees of aneuploidy [15]. Between 10 and 30% of solid tumours have an apparently diploid DNA content. Although it is possible that some of these tumours have near-normal karyotypes, it seems likely that others have more marked abnormalities which have not been resolved due to the lack of sensitivity of the technique used. There is evidence that aneuploidy is a relatively stable marker, i.e. multiple tumour biopsies taken at different times from the same patient commonly show an identical cellular DNA content [9, 10].

The clinical relevance of aneuploidy has not yet been fully investigated. For many epithelial cancers it appears to carry an adverse prognosis, and this has been reported for adenocarcinomas of breast, prostate, lung, ovary and colon [11-13, 16, 17]. Possible explanations for this would include a tendency for aneuploid tumours to present at a more advanced stage, to be less well differentiated or more rapidly dividing, or to be less sensitive to drug treatment.

The incidence of aneuploidy in the present series was 70%, with 6% of biopsies showing the presence of more than one population of aneuploid cells. This is similar to that reported for the various primary adenocarcinomas from which we assume that our patients' metastatic tumours originated. In contrast to many types of adenocarcinoma of known primary site, however, there is no survival advantage for patients with diploid metastatic ACUP. This failure of cellular DNA content to influence survival in a substantial number of patients is of interest. Apart from uncertainties about the primary site, these tumours are characterised by the fact that they are by definition metastatic, and they show a general lack of responsiveness to cytotoxic drugs. It seems possible, therefore, that when these conditions apply, diploid tumours no longer have a better prognosis. In other words, the circumstances under which cellular DNA content influences survival could be related either to early clinical stage or to inherent drug sensitivity. Although this remains speculative, cellular DNA content is emerging as an important, objectively determined property of tumours. With the study of larger numbers of patients whose other prognostic features and survival are known, it should eventually be possible to define its clinical and biological significance with greater clarity.

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