Short Communication

Is DNA aneuploidy a good prognostic indicator in patients with advanced colorectal cancer?

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There is increasing evidence that abnormalities of cellular DNA within primary tumours are of prognostic significance. Deviation from the normal diploid DNA content of tumour cells has been associated with a poorer prognosis in carcinomas of the breast (Friedlander et al., 1984a), ovary (Friedlander et al., 1984b) and colon (Wolley et al., 1982). However, in the more advanced stages of malignant disease the association between DNA aneuploidy and prognosis does not appear to be so prominent (Hedley et al., 1985). With the current resurgence of interest in various forms of therapy for liver metastases from primary colorectal cancer e.g. hepatic resection, regional or systemic chemotherapy, hepatic arterial ligation and hepatic irradiation, it is of importance to assess the various prognostic factors that may influence the natural history. Several workers have identified a variety of indices which may be of use in assessing survival or for stratification within controlled clinical trials (Goslin et al., 1982; Lahr et al., 1983; Finan et al., 1985). In order to assess the value of ploidy in patients with advanced colorectal disease, we have studied a series of patients presenting with synchronous hepatic metastases from primary colorectal cancer whose survival has been documented and in whom the primary tumour was suitable for measurement of DNA content. Ploidy has then been compared with other well recognised prognostic variables.

From a retrospective study of 94 patients with colorectal cancer and synchronous hepatic metastases presenting to the two major teaching centres in Leeds over the period 1976–80, 46 cases, in whom the primary tumour was suitable for cellular DNA measurement, were selected. Clinical details of these patients are included in Table 1.

A standard proforma was completed for each patient recording personal details, symptoms and physical signs referable to both the primary lesion and distant metastases, the results of pre-operative haematological and biochemical investigation, operative findings and pathological examination of the resected primary lesion. Accurate survival figures were obtained from the hospital notes and the Yorkshire Cancer Registry.

Nuclear DNA measurements on the paraffin embedded primary tumour were performed using a modification of the method of Hedley et al. (1983), recently reported by Quirke et al. (1986). Briefly, a 30 μm section was cut from the paraffin embedded block, dewaxed, rehydrated and disaggregated using pepsin digestion. After centrifugation at 2,000 r.p.m. the pellet was washed and stained in a solution of 4′-6-diamidino-2-phenylindole-dihydrochloride (Boehringer Manheim, West Germany) in RPMI 1640 tissue culture medium. Following filtration samples were analysed on an EPICS V flow cytometer (Coulter Electronics, Hialeh, Florida, USA) using a coherent Innova –90 5w UV enhanced argon ion laser. Ten thousand nuclei were counted and DNA aneuploidy was defined as the presence of more than one G0/G1 peak. The DNA index was defined using standard criteria (Hiddemann et al., 1984).

The clinical information together with the ploidy value for the primary tumour was stored on computer for statistical analysis. Survival plots for different levels of individual clinical variables were obtained using the BMDP-PI1 programme based on the method of Kaplan & Meier (1958) and tests for statistical significance were computed using the Mantel-Cox 'logrank' analysis.

Table 1 Details of 46 patients presenting with synchronous hepatic metastases from colorectal cancer

| Site   | Number |
|--------|--------|
| R. Colon | 12     |
| L. Colon | 17     |
| Rectum  | 17     |

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Received 28 February 1986.
Figure 1 illustrates representative DNA histograms from a diploid tumour (top) and a tumour with a DNA aneuploid cell population. Of the 46 tumours, 27 (59%) were DNA aneuploid, the remaining 19 being diploid. There appeared to be no relationship between ploidy and the tumour site, grade and presence or absence of lymph node metastases. No significant difference between the Kaplan-Meier survival plots for DNA aneuploid and diploid tumours was found as illustrated in Figure 2. The clinical factors within this group of patients which did appear to have prognostic significance included percentage liver involvement by tumour, distribution of liver metastases, tumour grading and clinical enlargement of the liver. The median survival and levels of significance for these individual prognostic factors are summarized in Table II.

The prognosis for patients with synchronous hepatic metastases from primary carcinomas of the colon and rectum is poor; median survivals ranging from 6–10 months (Finan et al., 1985; Pestana et al., 1964; Jaffe et al., 1968). Many factors have been identified which significantly alter the prognosis (Goslin et al., 1982; Lahr et al., 1983; Finan et al., 1985). Although several of these relate to the extent of hepatic replacement by tumour or the distribution of metastases within the liver, other prognostic factors can be obtained from examination of the primary tumour, e.g., stage, grade and local fixity.

Three recent studies on colorectal cancer (Wolley et al., 1982; Armitage et al., 1985; Quirke et al., personal communication) have shown that the presence of DNA aneuploidy within the primary tumour is of poor prognostic significance. Wolley et al., studying 33 patients and following them for 3–5
years, noted that 12 of 13 cases in the non-diploid group had died (survival 4–34 months) as compared with only 6 of 20 in the diploid group. Similarly, Armitage et al. and Quirke et al. noted that 43% and 57% of patients with diploid tumours survived 5 years as compared with 19% and 34% of patients with DNA aneuploid tumours. However all of these studies were on patients with both local and advanced disease. In addition, it has been demonstrated recently that ploidy may be of some use in a variety of pre-malignant conditions of the colon e.g. colonic adenomas (Quirke et al., 1986; Van den Ingh et al., 1985) and cases of long-standing ulcerative colitis (Hammarberg et al., 1984). Doubt remains, however, as to the value of ploidy in patients with advanced colorectal malignant disease.

Our findings that 59% of tumours analysed were DNA aneuploid is in keeping with other series (Armitage et al., 1985; Quirke et al., personal communication). Like Armitage et al., we found no significant association between ploidy and either stage or grade of the primary tumour. However, others have noted quite a marked association between ploidy and staging (Banner et al., 1985). In their subset of 25 ‘Dukes’ D’ cases (Armitage et al., 1985) it is of interest to note that diploidy conferred no particular advantage in terms of 5 year survival. Using survival plots and log rank analysis, we too have been unable to show any significant difference in survival between DNA aneuploid and diploid tumours.

This finding mirrors the experience of workers studying ovarian carcinoma. Friedlander et al. (1984b) using multivariate analysis initially showed tumour ploidy to be an independent prognostic factor in patients with advanced ovarian cancer, diploid tumours surviving significantly longer than aneuploid tumours. However, more recently, they have noted no such difference when analysing patients with stage IV disease alone (ovarian metastases beyond the peritoneal cavity) (Hedley et al., 1985). Stuart-Harris et al. (1985) have not been able to demonstrate any survival advantage in patients demonstrating diploidy in locally recurrent or metastatic breast cancer although this finding may have been due to insufficient patient numbers or to the variety of treatments adopted.

As in previous series (Goslin et al., 1982; Lahr et al., 1983), we have been able to identify several factors which influence overall survival and if one expands this series then the number of significant factors increases (Finan et al., 1985). It may be that with a much larger series of patients a small difference might be noted between diploid and DNA aneuploid tumours. However, based on these studies it would appear unlikely that the routine measurement of ploidy in the primary tumour will have any useful role in assessing the prognosis of patients presenting with synchronous hepatic metastases from colorectal cancer. The relationship between DNA aneuploidy and response to treatment however requires further investigation in the light of recent reports that DNA aneuploid tumours are more susceptible to chemotherapy (Look et al., 1984; 3rd International Workshop on Chromosomes in Leukaemia, 1983; Morgan et al., personal communication) and radiotherapy (Wijkstrom et al., 1984).

This work was supported in part by a grant from the Yorkshire Cancer Research Campaign. We would like to thank Mrs. C. North and Mr. A. Roberts for technical assistance.

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Table II Significant variables affecting survival in patients presenting with synchronous hepatic metastases

| Percentage liver involvement | Median survival (months) | P value (Mantel-Cox) |
|-----------------------------|--------------------------|----------------------|
| <20%                        | 16                       | 0.002                |
| 20–80%                     | 6                        |                      |
| Clinically enlarged liver   |                          |                      |
| Yes                        | 4                        | 0.003                |
| No                         | 13                       |                      |
| Distribution of liver metastases |                  |                      |
| Single                     | 16                       | 0.05                 |
| Multiple                   | 9                        |                      |
| Tumour grading             |                          |                      |
| Well/Moderate              | 11                       | 0.05                 |
| Poor                       | 7                        |                      |
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