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Perspectives and Commentaries

Abnormal Cellular DNA Content as a Marker of Neoplasia*

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THE PROGNOSIS of patients with cancer is quite variable and only partially accounted for by the specific tissue diagnosis and the tumor burden. Many investigators have therefore sought to invoke tumor cellular characteristics to reflect the heterogeneity in the clinical course of comparably staged patients with identical morphologic diagnosis. The tumor cellular features of potential biologic and clinical importance in this regard relate to cytogenetics, proliferation, differentiation and, in the case of metastatic tumors requiring systemic anti-tumor therapy, to cellular pharmacology. While morphologic evaluation provides some insight into tumor proliferative and differentiative characteristics, this approach is not objective and quantitative enough to aid in cancer prognosis.

Recent technical advances have brought about the possibility of objective and quantitative cell analysis by flow cytometry [1–3]. One major requirement in these studies performed on single cells rather than tissue sections is the availability of an unequivocal neoplastic marker.

Abnormalities in cellular DNA content have long been recognized to be associated with malignant disease [4] and have recently been noted in flow cytometric investigations to be expressed with a high frequency of 80–90% in various human solid tumors and with a lesser frequency in hematologic malignancies (Table 1) [5–8]. These abnormalities in cellular DNA content have been demonstrated to correlate with cytologically recognized tumor cells [8, 9] and with cytogenetic aberrations in leukemia [8] and lung cancer [10]. There are only a few precancerous conditions that have recently been reported to express DNA-abnormal stemlines, although the transitions of atrophic gastritis [5, 11], ulcerative colitis [5, 12], benign monoclonal gammopathy [13], angioimmunoblastic lymphadenopathy [5] and pre-leukemia [8, 14] to overt neoplastic disease have not been observed in all cases reported. Except for tetraploid stemlines, normal tissue and reactive tissue alterations are typically not associated with DNA-aneuploidy [5].

The detection of DNA-aneuploid stemlines is greatly affected by the cytometric technique

Table 1. Aneuploidy in human malignancies

Diagnosis	No. of patients	% aneuploid
Leukemia	595	22
Lymphoma	360	53
Myeloma	177	76
Colon cancer	135	62
Breast cancer	385	79
Lung cancer	353	85
Prostate cancer	147	62
Bladder cancer	459	82
Testis cancer	74	93
Melanoma	643	76
Sarcoma	41	98
All solid tumors	3559	75
All malignancies	4691	67

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employed, the degree of DNA-abnormality and the proportion of DNA-aneuploid cells in relation to normal diploid cells engaged in DNA synthesis [15]. The detection rate of DNA-abnormal cells may be enhanced through the use of additional cellular properties that discriminate between host-reactive normal cells and the specific tumor population, e.g. cytoplasmic immunoglobulin and RNA in myeloma, specific cell surface characteristics in leukemia and lymphoma and other cellular expressions of tumor cell differentiation, including CEA, alpha-fetoprotein, enzyme expression, etc. These considerations, however, do not take into account the possibility that tumor stem cells may be more primitive and even of a different genomic makeup [16].

Hedley and colleagues have addressed the usefulness of flow cytometric detection of abnormal DNA content for the differential diagnosis of effusions [17]. While noting a lack of abnormal DNA stemlines in all 75 cytologically negative effusions from individuals with both non-malignant and neoplastic conditions, there was only one DNA-abnormal case among eight cytologically equivocal effusions, and 23 of 36 cytologically positive cases expressed a DNA-abnormal stemline (64%). This frequency of aneuploidy in malignant effusions predominantly secondary to solid tumors is entirely in keeping with the 75% incidence noted in our recent review of over 3500 cases of solid tumors [6] and with the 66% observed in Frankfurt's series of 656 tumors [7] if one considers, in addition to the 20–25% incidence of truly diploid tumors, an additional 10–15% near-diploid tumors. The detection of such near-diploid tumors will readily escape recognition when the proportion of tumor cells is relatively low, as is the case in malignant effusions (see also ref. [17]). Hence the incidence of DNA-derived aneuploidy in malignant effusions as reported by Hedley *et al.* could have been predicted unless there was a higher incidence of malignant effusions associated with a particular DNA-ploidy level. It remains to be determined whether the use of differentiation and/or cytokinetic markers in addition to ploidy may enhance the cytometric detection rate of diploid or near-diploid tumor cells. Alternative neoplastic markers have to be explored, and at least two such probes have already been used in conjunction with flow cytometry. The cytometric quantitation of the nucleolar antigen, which is apparently exclusively and universally expressed in a wide variety of human tumors [18], has been met with mixed success [6]. Similarly, the preferential association of double-stranded RNA with tumor compared to normal cells needs to be further investigated in clinically important differential-diagnostic problem areas such as effusions [19].

When present, however, a DNA-abnormal stemline even in pre-cancerous conditions is implicit of neoplasia and permits the assessment of tumor cell heterogeneity, including such important aspects as hormone receptor expression [20]. The observation of DNA-aneuploidy in pre-cancerous or overtly malignant conditions should not be equated with an aggressive clinical course demanding prompt therapeutic intervention. There remains considerable controversy with regard to the prognostic impact of the presence and degree of ploidy abnormalities [6, 21], which has to be viewed in the context of other already established prognostic factors and specific therapy employed. Thus, as of 1984, despite considerable progress in automated cytology, the expert pathologist still remains indispensable.

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