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Perspectives and Commentaries

The Heterogeneity of Human Cancers and its Influence on Metastases and Therapy

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(A COMMENT ON: Smith KA, Begg AC, Denekamp J. Differences in chemosensitivity between subcutaneous and pulmonary tumours. *Eur J Cancer Clin Oncol* 1985, **21**, 249-256.)

COMPLEX events dictate the effect of chemotherapeutic agents upon tumour cells at different sites in the body. The paper by Smith *et al.* concludes that evaluating drugs using subcutaneous tumours in animals may be a less than adequate model for drug testing. This observation adds to the body of clinical and experimental data that indicate a need to recognise the biological heterogeneity of human cancers.

EVIDENCE THAT HUMAN CANCERS ARE BIOLOGICALLY HETEROGENEOUS

Until the mid-1970s it was firmly believed that human cancer was clonal in nature, being the clinical expression of a proliferation of one cell type in the majority of instances. In favour of this was evidence from human leukaemia and the highly successful application of therapeutic strategy based upon *in vitro* clonal models of leukaemia. Against this clonal concept, however, was the weight of histopathological observations and, increasingly, clinical experience which indicated a different and differential therapeutic response amongst most other human cancers. What has happened in recent years to favour this concept of heterogeneity, and is the concept compatible with current ideas on how human malignant diseases progress?

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MECHANISMS OF HETEROGENEITY

In 1976 Nowell [1] hypothesised that cancer might develop biological heterogeneity in an evolutionary manner from a single clone origin. Inherent in the hypothesis is the idea that cancer cells are genetically unstable and that as the malignant cell population grows so mutational events accrue and genetic heterogeneity results. This heterogeneity manifests itself as phenotypic variation with alteration of cellular morphology, varying sensitivity to immune defences, varying growth rates, variable 'metastatic potential' and, most importantly, variable susceptibility to therapeutic intervention. It can be further postulated that epigenetic mechanisms might effect selection pressures upon mixed populations of cells, thereby encouraging the development of immune resistance to tumour cells, or the emergence of tumour cells which are resistant to the effects of chemotherapy following the destruction of sensitive populations. Aside from these observable mechanisms for influencing the genetic constitution of a mixed population of cells are yet other events which are more difficult to examine, such as the influence of growth factors [2] on primary and metastatic tumours, the latter frequently growing in a 'heterogeneous' stroma of different host tissues.

Inevitably, much of the research relevant to tumour heterogeneity has been on animal and experimentally induced tumours, the relevance of which may not be immediately apparent for human cancer [3]. However, experience of cancer medicine strongly supports the idea that clinical disease is characterised by heterogeneity.

HETEROGENEITY IN INDIVIDUAL HUMAN CANCERS

Breast cancer

This is probably the most carefully studied clinical model for metastatic heterogeneity in human cancer.

The presence and concentration of oestrogen-binding proteins (oestrogen receptors) have been studied by several investigators to examine the extent to which human breast cancer primary tumours and metastatic tumours are concordant for the expression of this variable phenotypic feature. Much ofthe data has necessarily required sequential study, although a few cases have been reported of simultaneous examination of two tumour sites. Results are somewhat conflicting. Earlier reports exemplified by Allegra *et al.* [4] indicate a high (80-85%) concordance for oestrogen receptor expression maintained over a considerable period in the absence of therapeutic intervention. However, more recently simultaneous and sequential studies indicate a much higher level of variation, with some originally ER-positive tumours becoming ER-negative and vice versa [5]. Likewise metastases biopsied simultaneously may be highly discordant for the expression of oestrogen receptor. More consistent, however, have been the reports which indicate that endocrine ablation or additive therapy converts oestrogen receptor-positive status to a receptor-negative status. These expressions of biological variation are supported by therapeutic experience, which suggests a discordance of response for tumour at different sites. It is commonly observed that node and soft tissue metastases respond consistently well to both cytotoxic and endocrine therapy. Concerning the influence of metastatic site, whereas endocrine therapy is consistently found to be less effective in bone and visceral disease, the reported efficacy of chemotherapy has been far less predictable. Thus response rates for similar chemotherapy regimes have been variously reported from 85 to 26% for bone metastases in some studies while remaining more consistent at around 60-70% when visceral sites and node disease are examined [6, 7]. Admittedly the technical problems of monitoring response of metastatic tumour in bone may confuse the picture, but quite how these data relate to the biological characteristics of breast cancer is unknown at present. It is certainly not clear, for instance, that oestrogen receptor expression can be related to sites of metastatic disease.

Small cell lung cancer (SCLC)

The evidence from morphology, nucleic acid analysis, analysis of cell surface antigens and biochemical markers have all been identified as indicators of the phenotypic variation in SCLC [8]. Work on *in vitro* models has identified an association between phenotype variation and drug sensitivity, and cell lines regularly show mosaicism with regard to the expression of chromosomal markers. There is accruing evidence that SCLC cells may be stimulating the growth, possibly through the production of peptide hormones, of other clones of SCLC cells within one tumour — in this sense there is an autocrine function within the SCLC population. Heterogeneity of DNA expression has likewise been convincingly demonstrated in human tissue by the studies of Vindeløv *et al.*, who conducted analyses of DNA content in metastases in patients on treatment [9]. Much of the work pointing to the heterogeneity of phenotype expression in terms of cell surface antigen has been done in cell lines. Few data exist on the examination of fresh biopsy specimens, but immunohistology on autopsy material shows marked variation in cell surface antigen expression as judged by intensity of staining using monoclonal antibodies. Evidence of variable growth potential exists from attempts to clone SCLC *in vitro* by biopsy from metastatic sites. There is an enormous variation in growth potential evaluated in this manner.

Human lymphoma

Human lymphomas have been particularly well characterised for cell surface antigen expression as models of tumour heterogeneity. Lymphoid cell surface antigens have been used to demonstrate the association between neoplasm and cell of origin, and also distribution of normal cellular counterparts in human tissues. These data have been summarised in several reviews, but of particular interest are the data of Nadler *et al.* [10], who have demonstrated that B cell differentiation antigens characterising varieties of malignant lymphomas may be important in determining the microenvironment to which B cells of different phenotypes may home in both healthy and diseased states. Thus the antigen characterised by the B2 marker shows a marked differential distribution in cells of the primary follicles and mantles of secondary follicles in human lymph node. Whether the anatomical distribution of lymphoid neoplasia is influenced by differentiation or other cell surface antigens is less certain. However, there are many examples of restricted metastic distribution in neoplasms of B cells and a particularly striking example is that of multiple myeloma. This tumour is characterised by restricted distribution of neoplastic cells to the bone marrow virtually to the exclusion of other lymphoid tissues. Although at post mortem

deposits may be found in some of the peripheral nodes, it is extremely rare for tumour to be demonstrable in the gastrointestinal tract despite the observation that the lamina propria of the gut is populated densely with plasma cells [11]. On the other hand, treatment itself may appear to induce anomalous distribution, suggesting a selection pressure of treatment on subclones of plasma cells which have a different homing propensity to subcutaneous tissues. Similar examples may be found in the low-grade lymphomas, including chronic lymphocytic leukaemia, where the phenomenon of 'dedifferentiation' is associated with a change in phenotype, a change in tissue distribution and an acceleration in the proliferation rate of tumour cells following prolonged treatment of an initially indolent tumour. Laboratory models of animal lymphosarcoma show an association between surface antigen expression and metastatic potential [12].

To assess the biological significance of tumour heterogeneity at different metastatic sites data obtained from human cancer studies are lacking, but the technology is now available to study these phenomena. For many years we have had the histological assessment of morphology which can point to extreme variation in tissue architecture between metastases and primary tumour, and between one metastasis and another. It is to be hoped that in the future more attention will be paid to the differential responses of cancers in various metastatic sites. We have recently reported much variation in metastatic response in human transitional cell bladder carcinoma [13]. With the evolution of techniques in immunology and molecular genetics a much higher degree of sophistication is becoming widely available for studying the biological features of human cancers [14]. Data from these approaches suggest that most clinical cancers consist of complex subpopulations of cells which may be variably influenced by therapeutic intervention. It seems likely that further improvement in the systemic management of these cancers will result from the frequent (and repetitive) sampling of human tumours — facilitated by techniques applicable, for example, to the study of cell aspirate preparations. In addition to consideration of factors such as drug access and uptake into metastases at different sites, therapeutic strategy should eventually take cognisance of the variation in the susceptibility of tumours that prove selectively capable of growth in different host tissues.

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