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Tumour Cell Heterogeneity and the Biology of Metastasis

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The metastatic spread of malignant tumours remains as one of the most intractable problems in clinical oncology. Experimental analysis of this phenomenon is fuelled by the hope that a more complete understanding of the process will give rise to insights allowing the eventual development of novel and successful therapeutic interventions. The purpose of this paper is to provide a brief overview of the pathogenesis of tumour dissemination and to discuss the implications arising from an appreciation of the biological principles revealed by studies with experimental tumour systems. It is hoped that this paper will provide an introduction to supplement the work described by Dr. Feldman in a subsequent chapter.

Metastasis is a process consisting of a series of linked, sequential steps. An inability to complete any one of these steps effectively abrogates the whole process. Thus, in order to establish a secondary focus, malignant tumours must invade locally and penetrate small blood vessels or lymphatics. This step is thought to be achieved by a combination of mechanisms including breakdown of tissue architecture by pressure atrophy, the release of proteolytic enzymes which digest the cohesive framework of the extracellular matrix and active movement of single cells or sheets of cells into such areas of tissue damage [1]. Penetration of vascular channels must be followed by release of single cells or small emboli into the circulation and the survival of these neoplastic cells in the face of turbulence and trauma or the effects of such specific and non-specific host immune effectors as natural killer (NK) cells, T lymphocytes, neutrophils and monocytes [2]. Having disseminated throughout the body metastatic cells must arrest and implant at distant sites, binding to areas of exposed basement membrane via specific attachment factors and cell surface receptors [3] or by direct penetration of endothelial cells [4]. Extravasation of cells into surrounding organ parenchyma is, presumably, accomplished by mechanisms much the same as those mediating initial invasion. Having reached the site of growth, tumour cells must establish and develop their own micro-environment; responding to local growth factors, releasing angiogenic factors to induce self-vascularisation and surviving host immune mechanisms to give rise to clinically obvious tumour deposits [5].

Given the highly complex nature of the process, it is perhaps not surprising that it is markedly inefficient [6]. Indeed, experimental analysis has shown that fewer than 0.1% of cells which gain access to the circulation may survive to form tumour deposits. This inefficiency posed the important question as to whether the survival observed was a consequence of random or selective events; that is do the eventual progenitors of secondary tumours result from the fortuitous survival of a few cells from the many shed into the blood or does it represent the selection of a pre-existent metastatic subpopulation from the parental tumour? Direct investigation of this possibility was reported by Fidler and Kripke [7] who used a modified Luria-Delbruck fluctuation analysis of clones, derived from single cells, to show that sub-popula-

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tions of metastatic cells pre-existed within the B16 melanoma. Subsequently several reports have described a similar degree of metastatic heterogeneity in a variety of rodent tumours [8–11].

A possible criticism of such demonstrations could be that they have all relied upon the use of transplantable tumours of rodent origin and the results obtained may not be applicable to human neoplasms. One obvious difficulty in any attempt to apply similar analytical procedures to human tumours lies in determining which animal can best be used to evaluate the degree of metastatic spread. The congenitally athymic nude mouse, which lacks significant numbers of T lymphocytes, would appear to be an ideal test animal in which to assess this parameter. Nude mice have been used frequently as recipients of human tumour xenografts, but while such implants commonly maintain their distinctive morphological and biochemical attributes they metastasize only rarely [12]. However, the situation with regard to the metastatic spread of established human tumour lines is much more equivocal and many lines have been shown to be capable of metastasizing readily in such recipients [13–15]. We have used this combination, the relevance of human material coupled with the ease of manipulation to be gained from established tissue culture lines, to determine whether human tumours manifest the degree of metastatic heterogeneity shown by rodent neoplasms.

The human melanoma line A375 was cloned by isolating discrete colonies growing in semisolid agar and individual lines were derived from these clones. The ability of the parental line and ten clonal lines to form lung tumour nodules in BALB/c nude mice was determined following i.v. injections. Four out of the ten clones examined differed significantly ($P<0.005$) from the parental line with regard to their ability to form pulmonary tumour foci [16] indicating that this human line was no less heterogeneous for metastatic capacity than its murine counterparts. Recovery of cells from these lung nodules to form metastasis-derived lines followed by re-examination of their metastatic capacity demonstrated that these selected lines consistently were more metastatic than the starting population both in the i.v. experimental metastasis assay and in the more stringent spontaneous metastasis assay from a s.c. site [16]. We have interpreted these results as showing that human tumour spread in the nude mouse results from the preferential selection of metastatic subpopulations and have confirmed the generality of this observation using the human prostatic carcinoma line PC3 [17] and a further melanoma line DX-3 [18].

The source of metastatic heterogeneity (or in fact heterogeneity for any other phenotype) is not known. Should tumours have a multi-cellular origin [19], then the diversity found simply may be the reflection of differences between the progeny of several transformed cells. However, since most tumours appear to be unicellular in origin [20, 21] other mechanisms have had to be invoked to explain such multiformity. Nowell [22] proposed that acquired genetic lability and variability in neoplastic cells, when coupled with the intense selection pressure of growth in a responding host, led to the rapid emergence of new sublines with increased survival ability which was manifested as enhanced malignancy. A corollary of such a mechanism might be that variants of increasing metastatic capacity would be accompanied by increases in genetic instability and supportive evidence for such a possibility is provided by the work of Cifone and Fidler [23]. These authors showed that rates of mutation to ouabain and 6-thioguanine resistance were increased in variants of high metastatic activity when compared with mutation rates shown by variants of low metastatic activity isolated from the same neoplasm [23].

Irrespective of the exact forces driving tumours toward a more aggressive behavioural pattern, the finding of metastatic heterogeneity within tumours, coupled with the ability to select out more metastatic variants, has profound implications for experimental analysis of metastasis. Determination of the cellular properties that are important for expression of the malignant phenotype can now be achieved by using cell variants or lines of different biological behaviour isolated from the same tumour. Such an approach obviates the need to include control cells, which frequently are of doubtful validity, derived from unrelated tumour or normal tissue. The demonstration

of a similar degree of metastatic heterogeneity in human tumour lines coupled with the ability to use the athymic nude mouse as a "selection vehicle" to pull out metastatic variants shows that similar investigations can now be conducted using human neoplasms.

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