Current Reviews

Invasion and metastasis

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Malignant tumours cause sickness and death largely because they invade and metastasize. Such spread is made possible by many cellular properties, including the ability of neoplastic cells to move and to release degradative enzymes. These properties enable tumour cells to break free of the primary tumour, penetrate blood or lymphatic vessels and, after being transported to distant sites, pass out of the vessels to establish new tumours. Not all cells in a tumour, however, are able to metastasize, so the process tends to select for greater malignancy in the secondary tumour. The heterogeneity of tumours probably accounts for the difficulty of providing effective treatment, in that the various subpopulations of cells arising from each tumour vary in their responses to chemotherapeutic agents. We do not yet understand the process sufficiently to treat cancer patients by interfering selectively with the metastatic mechanisms.

Les tumeurs malignes sont cause de maladie et de mortalité principalement à cause de leur caractère envahissant et métastatique. Une telle propagation est rendue possible par plusieurs propriétés cellulaires dont la capacité des cellules néoplasiques à se déplacer et à libérer des enzymes de dégradation. Ces propriétés prennent effet au fur et à mesure que les cellules tumorales se dégagent de la tumeur primaire, qu'elles entrent dans la circulation sanguine ou lymphatique et, qu'après avoir été transportées à distance, elles quittent les vaisseaux pour établir de nouvelles tumeurs. Toutefois, toutes les cellules d'une tumeur ne sont pas capables de métastaser, de sorte que le processus a tendance à sélectionner pour les tumeurs secondaires des cellules les plus malignes. L'hétérogénéité tumorale explique probablement la difficulté de trouver un traitement efficace; les diverses sous-populations de cellules retrouvées dans chaque tumeur répondent en effet de façon différente aux agents chimiothérapeutiques. Nous ne comprenons pas encore suffisamment ce processus pour traiter les patients cancéreux en intervenant sélectivement au niveau des mécanismes métastatiques.

A major proportion of cancer-related illness results from invasion and metastasis. The complex mechanisms responsible for the spread of cancer are governed by interactions between the tumour and the host tissues. Tumour cells may invade adjacent tissue and, if they become detached from the primary tumour, can enter the vascular pathways; then at some point they leave the blood vessels and proliferate in the extravascular tissues.

There are fashions in cancer research, and at present the processes of invasion and metastasis are being extensively investigated.[1-7] Most of our understanding has come from the study of nonhuman tumours and very often from experiments conducted in vitro. Hence, we must be cautious in applying this information to the human situation. This brief account is a synthesis for the general medical reader rather than for the specialist.

Neoplastic invasion

The relative importance of the many factors involved in invasion varies from one kind of neoplasm to another. Animal tumours, for instance, may grow as single cells, whereas human tumours, such as adenocarcinomas, are often composed of clustered, adherent cells and may well behave differently. Important factors contributing to the ability of the cancer cell to invade are the secretion of lytic substances, translocatory movement, decreases in cell adhesion, the build-up of hydrostatic pressure within a neoplasm,[8] the release of material from areas of necrosis[9] and increases in the rate of mitosis.

Role of lytic enzymes

The early breakdown of normal tissue structure, perhaps on a macromolecular scale, is a key phenomenon that allows cells to move. This breakdown may result from the release of proteolytic enzymes by either neoplastic or lymphoreticular cells within a neoplasm. Increased levels of cathepsin B or other proteinases that can digest the collagens, proteoglycans and pericellular pro-

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teins of connective tissue are sometimes present in the serum of patients with early neoplasms.[10] Cathepsin B is also present in considerable amounts in breast carcinomas.[11] In mice a highly metastatic variant of murine B16 melanoma contains more cathepsin B than one that is poorly metastatic.[12] Collagenases specific for type IV collagen (a major component of basement membrane) may be of particular significance in the transition from in situ to invasive carcinoma and in the penetration of blood vessels, for malignancy is correlated with the ability to digest type IV collagen in certain tumour lines.[13] Some tissues — cartilage, for example — have been shown in vitro to be protected from tumour penetration by the presence of anti-invasive factors, which are probably collagenase inhibitors.[14] Other enzymes, such as plasminogen activator, have likewise been associated with invasion.[15,16] While the ability of tumour cells to break down proteins has been relatively easy to assess, enzymes may also facilitate the breakdown of intercellular junctions, thereby allowing individual cells to escape from the primary tumour. Enzymes may also actually kill cells.

Tumour cell motility

Cell movement is probably very important in neoplastic invasion.[17] Most tumour cells have machinery for movement similar to that of normal motile cells such as leukocytes and smooth muscle cells. This motility generally depends on actin, myosin and regulatory proteins that are remarkably like those of striated and smooth muscle. Other cytoskeletal structures, such as microtubules and intermediate filaments, may also affect cell movement or shape. It is not certain whether movement is controlled in the same way in tumour cells as in normal cells. Manipulation of cytoskeletal components of experimental tumours alters the patterns of metastasis in vivo, and in vitro it changes a number of properties that may be involved in metastasis, including the rates of migration, the formation of tumour aggregates[18] and chemotactic responsiveness.[19]

Tumour cells, like many normal cells, show ameboid movement in tissue culture, but they often lack the "contact inhibition" characteristic of most normal cells.[20] None the less, a form of contact guidance may influence the direction of movement of malignant cells.[21] Adhesion, proliferation and differentiation are likewise affected by interactions between the tumour cell and the connective tissue matrix; even the matrix molecules secreted by the tumour cells vary with their malignant potential.[22]

The morphology of invasion

The early stages of invasion have been elucidated by ultrastructural studies of both experimental and natural carcinomas. The neoplastic cell protrudes fine cytoplasmic processes down through the basal lamina, which then thickens or reduplicates. The cell then penetrates and passes through the basal lamina.[23] This protrusion of cytoplasmic processes is characteristic of the leading edge of an invasive neoplasm and is clearly evident, for instance, in invasion of skeletal muscle.[24,25]

In-vitro models of invasion

Invasion has been studied extensively in vitro,[26] on both flat surfaces and in three-dimensional matrices. In some models tumour cells are cocultivated with "target" organs or tissues, but the mimicry of the natural situation is imperfect. However, there is some evidence that invasiveness in vitro may correlate with tumourigenicity in vivo and be a better index of malignant potential than other in-vitro measurements, such as morphology or loss of contact inhibition.[27]

Tumour cell metastasis

Via blood vessels

Before tumour cells can take the first step in distant metastasis and penetrate the circulation the tumour must be adequately vascularized. This involves the release by the tumour of angiogenesis factors — the chemical mediators that stimulate endothelial cell proliferation.[28] Our knowledge about the penetration of blood vessels is fragmentary.[29-31] We do not understand why a few invasive malignant tumours, such as basal cell carcinoma, fail to penetrate vessels or why some tumours tend to metastasize by one vascular pathway in preference to another. In any case, it seems that the cells enter the circulation by passing through an incomplete endothelial lining, by fusing with the endothelium or by extending fine cytoplasmic processes and inducing fibrin formation. It is fortunate that few tumour cells survive in the blood; in one experimental system 150 000 cells were found to have been released from a neoplasm in 24 hours.[32,33]

There is a tendency for metastasis to occur in the first organ encountered by the circulating cells, owing to a sieve effect, and the better the blood flow through an organ, the more likely it is that metastases will form there.[34] Tumour cells may be deformed and mechanically trapped in a small vessel. This is facilitated if the tumour cells aggregate with blood components. Several factors seem to control the "homing" capacity of tumour cells, including the surface characteristics of both tumour and endothelial cells, which may govern their mutual adherence.[35] The chemotactic responses of tumour cells can also influence their migration and the localization of metastases. The presence of numerous anionic sites on tumour cell membranes may be part of another mechanism leading to aggregation with other tumour cells or with host cells.[36]

Once tumour cells are caught in a narrow vessel, hydrolytic enzymes on their surfaces or released from them may damage endothelial and basement membranes, so that the cells lodge there. Endothelial swelling produced by unrelated factors, such as irradiation, may also, through resultant thrombosis, trap circulating tumour cells.

Tumour cells leave the circulation by means of mechanical or enzymatic disruption of the vessel wall[37] or pass through gaps, which may be normal or be due to shedding or endothelial retraction. The process of chemotaxis in tumour cells seems to be very similar to that in leukocytes. The chemotactic stimulus may make the tumour cell stick to

the endothelium and swell, perhaps (as in leukocytes) with concomitant discharge of lysosomal hydrolases, fluctuation of the membrane potential and cellular aggregation.[38-40]

Via lymphatics

Lymphatic metastasis is more characteristic of carcinomas than spread via blood vessels. Tumour cells probably penetrate the lymphatic capillaries by migrating through gaps between endothelial cells. Some of these gaps may normally be open, or the tumour cells may release signals that cause the gaps to open. Under some circumstances there may be massive necrosis of endothelial cells as the malignant cells break through. Thereafter the tumour cells drift singly or in clusters in the lymph and settle in the subcapsular sinus of a node.[41,42] At an early stage there is a reaction in the node involving the proliferation of thymus- and bone-marrow-derived lymphocytes (T and B cells)[43] and sometimes macrophages. With some human tumours a prominent T-cell reaction in the draining node indicates a good prognosis,[44] but usually the organism is unsuccessful in eliminating the tumour. Lymph nodes are relatively poor barriers to metastasis, delaying the passage of tumour cells for only a few days. Indeed, it is likely that they actually provide a favourable milieu for growth, since an experimental tumour may become established from a small number of tumour cells.[41]

To serosal cavities

The details of the penetration of serosal sacs are not known, but the fluid produced, in massive amounts, probably provides a good medium for the growth of tumour cells. Ascitic fluid and pleural effusions can also contain chemoattractants.[45,46]

Growth of the metastatic tumour

An important part of the establishment of a tumour in a secondary site is the acquisition of a blood supply, presumably through the release of angiogenesis factors.[28] The tumour may stay relatively small (a micrometastasis) or grow progressively. Regulation involves the interrelated functions of lymphocytes, macrophages and antitumour antibodies. It seems that immune mechanisms can either slow or accelerate tumour growth and therefore metastasis; manipulations intended to induce the former may actually be detrimental. There is evidence, too, that the primary tumour releases factors that suppress growth at the secondary site.[47] A metastatic tumour can invade locally as well as grow, thus serving as a source of additional metastases.

The heterogeneous nature of tumours

Many different cellular properties can contribute to the tumour cell's ability to invade or metastasize. Apparently not all cells in a primary tumour are capable of metastasizing. The process itself may act as a mechanism of selection for malignant cells, and there is evidence that cells in a metastasis are more malignant than those in the primary tumour. Within a single neoplasm, tumour cells do indeed vary in a wide range of other properties, from appearance to growth rate, karyotype, enzyme production and cell surface characteristics. The best evidence that these properties are important in relation to metastasis derives from experiments involving the multiple passage of tumour cell subpopulations, some of which are more metastatic than others.[48] In tumours of unicellular origin this heterogeneity may be due to the emergence of neogenetic variants, which are then subjected to selection pressures, while in tumours of multicellular origin the heterogeneity may reflect differences in the original subpopulations.[49] Clonal heterogeneity can explain many of the features of metastasis, but this does not mean that it necessarily *does* explain variations in the expression of such tumour markers as hormone receptors in breast cancer or pigment production in melanoma. Undoubtedly there are differences between the metastasis and the primary tumour, but these may be due to the random selection of metastasizing cells from genotypically different subpopulations, to transient characteristics of cells during metastasis or to modulation of tumour cells after they metastasize.[50]

The heterogeneity of tumours probably accounts for some of the difficulty encountered in selectively treating cancer metastasis. A single tumour may, for instance, give rise to subpopulations that differ in their sensitivity to chemotherapeutic agents. Eventually we may find that successful therapy depends on the development of treatments that act independently of tumour heterogeneity. One such approach, now only experimental, is to amplify the macrophage function in the host by using liposome-encapsulated immunomodulators.[51] We are a long way from being able to apply our growing knowledge about the metastatic process.

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