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Malignant and nonmalignant cells: Structural similarities and behavioural differences

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Invasiveness is the ability of cells to leave the boundaries of the tissue to which they belong, and to occupy neighbouring tissues. Invasiveness of tumours contributes largely to their malignancy both by local spread and by initiation of the formation of metastases. Because invasiveness constitutes the main difference between benign and malignant tissues, understanding of the mechanisms by which cells leave the tissue of origin and invade into the neighbouring tissues, may contribute to our knowledge of the biology of malignant cells. The role of in vitro culture in the study of some aspects of the invasiveness of malignant cells is reviewed here.

2 points are worth mentioning on beforehand. 1. There are theoretical (Nowell[1]) and experimental arguments (Liotta et al.[2], Fidler[3]) to accept that not all cells of a particular malignant tumour have or will acquire the ability to invade, so that in this respect the cell population constituting a malignant tumour is heterogeneous. This concept implies that by sampling populations of malignant cells one might overlook the activity of the invasive cells, which may represent a relatively small fraction. It may provide one possible explanation why quantitative evaluation of invasiveness is difficult (Easty and Easty[4], Hart and Fidler[5]). 2. As long as the mechanisms of invasion are unknown, invasion in vitro has to be judged on the basis of histological criteria bearing on the interaction between the tumour cells and the host tissue as it is done by clinical pathologists. Generally applicable cytological or ultrastructural criteria for distinction between invasive and non-invasive cells are not available. This implies that invasive cells have to be identified either directly whilst invading (cinemicrophotography) or indirectly through their position within the complex of tumour and neighbouring tissues. The drawback of the indirect method is that it infers kinetic features from static pictures.

Rationales for the in vitro study of invasion are isolation of the various factors that influence the invasiveness of malignant cells and direct observation of the cellular activities involved in invasion. Confrontation of biopsy specimens from animal and human tumours with a variety of embryonic and adult tissue fragments in three-dimensional culture, also called organotypical culture (Wolff et al.[6], Easty and Easty[7], Lumsden[8]), mimics the invasion of malignant cells in vivo. The argument is that the histological aspect of the frontier between tumour and host tissue is similar in three-dimensional culture and in vivo. Provided this argument is accepted, 2 conclusions can be drawn from these experiments. 1. Malignant cells conserve their invasive capacity when they are isolated from their natural host and brought into culture. 2. Invasion of malignant cells is not limited to their natural host but also occurs into tissues from different organs and from different species. The latter makes it unlikely that tumour cells become invasive because of alterations in the neighbouring tissues.

Using confrontation in three-dimensional culture, several authors (Easty and Easty[4], Schleich et al.[9], de Ridder et al.[10], Kuettner et al.[11]) have shown the invasiveness of cells from permanent lines established from animal and human tumours. These observations indicate that malignant cells do not loose their invasiveness during long-term culture. Furthermore, experiments by Barski and Wolff[12] and by Latner et al.[13] show that cultured cells become invasive when they undergo malignant alteration in vitro. Systematic analysis by independent investigators of in vitro invasiveness and ability to form invasive tumours in syngeneic animals has been done with 3 families of

cells: 1. MO cells derived from carcasses of fetal C3H mice confronted with embryonic chick skin before and after 'spontaneous', chemical and viral malignant alteration (Mareel et al.[14]). 2. ST/a cells derived from normal lungs of 3 to 5 months old ST/a Fib mice confronted with embryonic chick heart before and after 'spontaneous' malignant alteration (Kieler et al.[15]). 3. Brain cells from fetal BD IX rats transferred to monolayer culture shortly after exposure to ethylnitrosurea in vivo (Haugen and Laerum[16]) confronted with embryonic chick heart after various periods of culture (de Ridder and Laerum, unpublished data). For all cell types from the 3 families the ability to produce a tumour was predicted from their invasiveness in three-dimensional culture. These experiments show that, although invasiveness is not entirely specific of malignant cells - trophoblast cells and leukocytes are invasive (see review by Armstrong[17]) - invasiveness in vitro constitutes a criterion of the malignancy of cultured cells.

Three-dimensional culture appears to be a good model of invasion, that can be used for the study of its mechanisms (e.g. Mareel and De Brabander[18]), but with this model direct observation of the activities of the invasive cells is almost as difficult as it is in vivo (Ambrose and Easty[19]). Two-dimensional culture of cells on glass or plastic (also called monolayer or tissue culture) offers the opportunity for direct observation of locomotion, destruction (Mareel et al.[20]) and phagocytosis (Babai[21]) of host tissues, activities of malignant cells that are presumed to be involved in invasion.

Locomotion is performed by most cells when explanted on a suitable substrate, irrespective of their malignant or nonmalignant character, and the organelles supporting locomotion have been demonstrated in both types of cells. Microfilaments are present in the cytoplasm of malignant and nonmalignant cells (e.g. Haugen and Laerum[16]). Since the distribution of microfilaments may be relevant for the locomotory activity of the cell, examination of this distribution may offer an opportunity to recognize moving cells on static pictures from three-dimensional cultures or from invasive tumours in vivo (Gabbiani et al.[22]). De Mey et al.[23] have shown that malignant cells like nonmalignant ones have an intact cytoplasmic microtubular complex. Experiments with microtubule inhibitors indicate that this complex is necessary for directional locomotion of cells in tissue culture (De Brabander et al.[24]) and for invasion in three-dimensional culture (Mareel and De Brabander[18]).

As a whole, the observations on the locomotion of various cell types in tissue culture strongly indicate that malignant cells have an intact locomotory apparatus and in this respect do not differ from nonmalignant ones. The rules governing the locomotory behaviour of both types of cells when encountering other cells seem to be different (Abercrombie and Heaysman[25]).

Direct demonstration of the destructive activity of malignant cells in tissue culture appears to be difficult, and it has been argued that establishment of normal tissues in monolayer culture makes them resistant towards the cytolytic activity of malignant cells (Mareel et al.[26]). Promising results are obtained by Lysik et al.[27], who have demonstrated the cytotoxicity of various types of malignant cells for ^{51}Cr-labelled rat bone marrow cells in mixed suspension culture. The culture technique does not allow direct observation, either. The latter results and the data about the factors that influence the secretion of lytic enzymes in tissue culture (Kuettner et al.[28], Boggust and McGauley[29]) may lead to elaboration of culture conditions that do allow direct observation of the destructive activity of malignant cells. So far, our knowledge about the organelles involved in this activity does not allow to make comparisons between malignant and nonmalignant cells.

In tissue culture, malignant or nonmalignant cells, phagocytize cellular and other particulate material (Garfield et al.[30], Bosman et al.[31], Albrecht-Buehler[32]), and differences between both types of cells have not been found (Vasiliev et al.[33]). To our knowledge differences in the phagolysosomal structures have also not been described.

It appears to us that, in the present state, cultures of either malignant or nonmalignant cells do not reveal structural or functional characteristics that may explain the invasiveness of malignant cells. Confrontations of cells and tissues in culture allows demonstration of invasiveness. This indicates that the regulation of the cellular activities is different in various types of cells and may provide a key for understanding differences in behaviour between malignant and nonmalignant cells. Combinations of various culture methods and the further elaboration of individual techniques is promising for the study of invasive malignant cells, provided differences between cellular interactions in vitro and invasion in vivo are constantly kept in mind.

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Morphogenetic reactions of cultured cells

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Dissociated fibroblasts and epithelial cells placed in vitro on the appropriate substrate and surrounded by the appropriate fluid medium are able to perform many complex morphogenetic processes: these cells may spread on the substrate and further to move directionally on this substrate, epithelial cells may form coherent sheets, fibroblasts may orient themselves with regard to each other and to the structures of the substrate etc.[1-4]. Analysis of these complex morphological changes suggests that they can be regarded as combinations of a few types of cellular reactions, which may be called basic morphogenetic reactions[3]. In particular, pseudopodial attachment reactions (extension, attachment and contraction of pseudopods; figure) play the central role in all the changes of shape and in locomotion. Several other reactions inhibit the extension of pseudopods in certain parts of the cell surface.

Pseudopodial reactions

Extension of pseudopods. Suspended tissue cells (fibroblasts and epithelial cells) start to extend pseudopods (filopodia and lamellipodia) after contacting nonliving substrata of different types; pseudopods are usually extended from the areas of cell surface located near the site of cell-substrate contact. These observations suggest that extension may be a result of some signal change within the membrane produced by the contact. We do not know anything about the nature of the signal or of the membrane receptors involved in the reaction. Blood platelets extend pseudopods in response to contacting substances such as ADP, epinephrine, serotonin and others[5]. It is not clear whether any substances can induce the extension of pseudopods by suspended fibroblasts. Extension of pseudopods, almost by definition, should involve the transport of some intercellular material into a localized area of cell periphery but the nature of these transport processes remains unknown.

Attachment of pseudopods. Cell-cell contacts and focal cell-substrate attachment are usually formed only by the surface of extended pseudopods. Special adhesive properties of pseudopodial surfaces are best demonstrated by the experiments with cell sheets formed by various cultured epithelia. In these structures pseudopods are extended only at the free edges of marginal cells. Nonactive upper surfaces of the sheets were shown to be nonadhesive for homologous and heterologous tissue cells[6-8], for blood platelets, for solid particles of a varied nature[7,8]; they were also nonadhesive for liposomes made from the lipids which were in a crystalline state at 37 °C (distearoyl- and dipalmitoyllecitine)[10]. In contrast, pseudopodial surfaces at the free edges of the same sheets were adhesive for homologous cells, solid particles and solid liposomes. Formation of adhesive structures involves clustering of some membrane receptors within the plane of the membrane. To be kept in place these receptors have to be fixed by some mechanism; possibly, this mechanism involves anchoring of clustered receptors from the inside by some intracellular molecules, for instance, by α-actinin which is a characteristic component of at least some contact structures[11-13]. The adhesive properties of pseudopodial cytoplasm may be due to their special ability to build anchoring structures for clustered receptors; other explanations also remain possible at present.