Current concepts in metastasis

A K Nigam, M Pignatelli, P B Boulos

Metastasis remains an important clinical problem. In gastrointestinal cancers it is the single most important determinant of death. The incidence of metastatic disease from colorectal cancer at presentation is 25%. Even after curative surgical resection the five year survival rate is no more than 50% with an overall five year survival rate for colorectal cancer of 25–30%. The presence of liver metastases confers a median survival of only six months, figures that have essentially remained unchanged for over 40 years. At present our armamentarium against colorectal metastasis is weak. Surgery is limited to a fraction of cases and the alternative is systemic or regional chemotherapy. Results from such treatments are poor, only about 15–20% of tumours responding to systemic chemotherapy with marginal benefits in terms of survival. Such dismal figures lead to the conclusion that perhaps novel approaches are required in the prevention, early diagnosis, and treatment of liver metastasis. The questions that therefore arise are does modern science have anything to offer towards this problem and where is current research leading from a clinical perspective. A detailed description of the metastatic process is beyond the scope of this article. The role of oncogenes and tumour suppressor genes in the neoplastic process has been previously described and will not be referred to either. Here, we highlight two areas of current research in cell biology, namely, adhesion molecules and a group of matrix degrading enzymes, the metalloproteinases, and their roles in the progression and metastasis of tumours.

The understanding of tumour cell biology is a prerequisite for the development of new prognostic and therapeutic tools. Current theories embrace notions of imbalances and deregulation of host derived and tumour derived factors, which result in uncontrolled proliferation, invasion, and metastasis. The process of metastasis, however, is not random. It consists of a series of linked, sequential steps that must be completed if a metastasis is to develop. Tumour cells need to attach to host cellular and extracellular matrix determinants; this has to be followed by the enzymatic degradation of barriers such as the basement membrane to permit extravasation into the blood or lymphatic system. Locomotion within the system to the target organ followed by reattachment and the induction of independent growth of these tumour cells are further steps in the development of a metastasis. Moreover, it is now well recognised that tumours contain multiple cell populations exhibiting a wide range of biological heterogeneity in several parameters including their ability to invade and metastasise. As the vascular system is generally a hostile environment to tumour cells, metastases must arise from a cell subpopulation that is selected in terms of survival. Studies have shown that 24 hours after the intravenous inoculum of radio-labelled tumour cells, less than 0.1% survive to proliferate into metastases. The size of this subpopulation varies with time and between tumours. This biological variation represents a major obstacle to effective treatment.

Adhesion molecules

The concept that cellular adhesion is important in holding tumour cells together is not new. Coman, in 1944, showed that intercellular adhesion was decreased between tumour cells. Further research in this field, however, remained dormant until the comparatively recent identification and characterisation of families of cell surface receptors, named adhesion molecules. These mediate interactions between cells and between cells and the extracellular matrix (interstitial stroma and basement membrane). Cloning of the genes encoding for these molecules together with the development of specific monoclonal antibodies has facilitated the localisation of these molecules in vivo and the evaluation of their function in vitro. Adhesion molecules fall into one of four key groups: integrins, cadherins, selectins, and members of the immunoglobulin superfamily (Table 1). Integrins and cadherins are responsible for normal and transformed epithelial cell interactions, integrins being the prime mediators of interactions between cells and their surrounding extracellular matrix, whereas cadherins are responsible for intercellular interaction. Selectins play a part in endothelial cell adhesion, whereas members of the immunoglobulin superfamily share a diversity of expression on cells of the immune system.

<table>
<thead>
<tr>
<th>TABLE 1</th>
<th>Major families of adhesion molecules</th>
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<tbody>
<tr>
<td>Integrins</td>
<td>αβ heterodimers 1-4a and 88 units</td>
</tr>
<tr>
<td>Cadherins</td>
<td>E-epithelial, N-neural, P-placental, V and T-cadherins</td>
</tr>
<tr>
<td>Ig Superfamily</td>
<td>ICAM-1 and 2, V-CAM, L1, N-CAM, CEA, LFA-3 and others</td>
</tr>
<tr>
<td>Selectins (Lecitins)</td>
<td>3 identified, E, L and P-selectins</td>
</tr>
</tbody>
</table>

13-15 SHOWS Relevant references.
Current concepts in metastasis

neuronal tissue, as well as cells of epithelial origin such as colonocytes. A role for adhesion molecules has been implicated in biological processes (both physiological and pathological) such as wound healing, inflammation, coagulation, and embryogenesis. There is now ample experimental evidence for adhesion mechanisms, in particular these molecules, in the development of metastasis.

**INTEGRINS**

Integrins are transmembrane receptors found on the surface of cells and they mediate interactions with their ligands in the extracellular matrix. They are heterodimers composed of \( \alpha \) and \( \beta \) subunits, both of which are required for ligand binding. Several studies have shown that changes in integrin function at the time of intravenous injection of tumour cells into mice can influence the number of metastases formed. Human melanoma cells transfected with the cDNA for an integrin receptor that binds to collagen and laminin so that the integrin is overexpressed produce greater numbers of metastases in nude mice. Further, the injection of a tumour cell load with a synthetic peptide sequence recognised by many integrins in their ligands produces the opposite effect. The resultant blocking of the integrin receptors reduces the adhesiveness of the tumour cells and thus prevents anchorage and further growth. This is manifest by a reduction in the number of metastases found in distant organs. Proteins containing the same peptide sequence have also been shown to inhibit invasion across an artificial basement membrane in an in vitro system. Other studies have shown the effect of overexpression of \( \alpha V \beta 1 \) integrin (a fibronectin receptor) on the transformed phenotype. After transfection of a cDNA sequence for this integrin into a transformed cell line, features of normal growth control, reduced migratory phenotype, and the loss of tumorigenicity were seen in an experimental system. Thus, blocking and transfection studies support a role for integrins in metastasis.

Several human cancers have now been studied to evaluate cell adhesion molecule expression and function. The integrin repertoire on normal epithelium and malignancies has been determined on carcinomas of the colon, pancreas, stomach, kidney, breast, lung, prostate, and skin. Although most of these studies exhibit heterogeneous expression in the tissues reflecting the intrinsic variation between cells from the same tumour, in epithelial malignancies there seems to be a down regulation of expression of some of the integrins particularly in moderately and poorly differentiated carcinomas. This relationship suggests that tumour grade is consistent and seems to confirm the role of these molecules in the morphological differentiation of tumours.

**CADHERINS**

Cadherins are calcium dependent intercellular adhesion molecules. Recently, a strong link between metastasis and E-cadherin (epithelial specific) has been shown in colorectal tumours although other studies have not found this association. Gastric carcinomas show a down regulation of this molecule with increasing cellular dedifferentiation. This association with tumour grade is similar to that noted with the integrins and has been confirmed in several independent studies. There is indirect evidence to suggest that E-cadherin loss in vivo is possibly a later event in the genesis of these tumours when compared with integrin down regulation. This is of particular interest as another cell-adhesion molecule, namely N-CAM, a member of the immunoglobulin superfamily, has been shown to have large degrees of sequence homology with the DCC (deleted in colorectal cancer) gene product in colorectal cancer. This recessive gene is lost in about 70% of colorectal cancers and is also thought to be a late occurring event.

Further experimental studies have shown that blocking the function of E-cadherin by monoclonal antibodies renders cell lines invasive. Gene transfection experiments have clearly shown the importance of E-cadherin in maintaining intercellular adhesion and preventing invasion. Tumorigenicity when assessed by tumour harvest from xenografts is also reduced in colorectal cells transfected with the cDNA for E-cadherin. Despite the clear association between loss of cadherin expression and experimental invasion, however, these results have not always been confirmed by immunohistochemical studies in vivo. This is possibly because of biological response modifiers such as cytokines and growth factors that affect the function of cell adhesion molecules. A group of proteins termed the catenins present within the cadherin moiety have been shown to have a regulatory effect on cadherin function. Their absence does not seem to change the expression of the cadherin protein but prevents the function of the molecule.

Other factors that regulate such function are also being studied. One of the most interesting of these is the nm23 gene. Loss of this gene seems to be inversely correlated to metastasis, implying that one of its functions may be a metastasis suppressor. In breast and colorectal carcinomas this is a consistent finding. The nm23 gene product has been shown to have a high degree of sequence homology with a group of proteins, NDP kinases, that mediate signal transduction and morphogenesis. Both of these are functions of cell adhesion molecules and thus may be dependent on the nm23 gene. More recently another gene located on chromosome 16q has been cloned and characterised, which is thought to regulate cell adhesion. This regulator gene and its products are vital to the understanding of the control of adhesion molecules and their future clinical application.

**CEA**

CEA (carcinoembryonic antigen), whose role clinically is limited to being a marker of the
presence of metastasis after colorectal cancer resection, is another candidate for an adhesion molecule.\(^4\)\(^5\) CEA shows sequence homology with members of the immunoglobulin superfamily of adhesion molecules and has been shown to enhance the metastatic potential of a colorectal carcinoma cell line. The mechanism postulated for this finding is that CEA may be promoting attachment of tumour cells to the liver Kupffer cells.\(^7\) The function of CEA remains an enigma, however, and it possibly acts only at selective stages in the metastatic process. Preliminary data suggest that CEA modulates tumour adhesion function by down regulating integrin and cadherin molecule expression.\(^8\) This would be consistent with a role early in the metastatic cascade when tumour cell detachment is required.

**CD44**

Of the newer adhesion molecules described, CD44 seems to show much promise. The concept of organ homing and the selectivity of metastasis had been elegantly shown by Nicolson.\(^9\) He had shown that metastasis was not an adaptation phenomenon of disseminated tumour cells but an intrinsic property of those cells with selected sites for deposition.\(^0\) This is clearly seen by the fact that the liver receives only 30% of the cardiac output whereas the lungs receive 100% and yet, the liver is the commonest site for metastasis from cancers taken as a whole.\(^1\) This homing property of tumour cells possibly results from an adhesion molecule such as CD44. This is a cell surface molecule postulated to control lymphocyte circulation by facilitating entry into lymphoid tissue by modulating adhesion to specialised lymph node endothelium. Up to nine isoforms of the CD44 protein are known to exist. This is because different messenger RNA (mRNA) transcripts are created through a mechanism known as alternative splicing. These then translate into functionally diverse but structurally similar proteins.\(^2\) One particular variant of the gene, pMeta-1, was isolated from metastatic rat tumour cell lines. When the cDNA for this variant was transfected into non-metastatic tumour cells to overexpress pMeta-1, lymph node metastasis was induced when the transfected cells were injected into rat foot pads.\(^3\) A theoretical mode of action proposed for the CD44 variant is that it mimics circulating lymphocytes and acts as a molecular ‘disguise’ hence leading to metastasis, the so called ‘wolf in lamb’s clothing’. This finding has prompted several studies to consider whether human carcinomas also use this mechanism for lymph node deposition and avoid host defences. Colorectal neoplasms show a distinct pattern. All metastatic tumours express the variant, as do many of the primary tumours; few, however, are expressed in normal colonic mucosa.\(^4\)\(^5\) Whether CD44 turns out to be a true marker of metastasis remains to be determined, but a better functional understanding of this molecule may well lead to therapeutic applications.

**Matrix metalloproteinases**

The role of the extracellular matrix in tumour invasion and metastasis has been intensively studied in the past decade.\(^8\) Interactions between cells and the matrix occur at many stages in the metastatic cascade. The traversal of epithelial boundaries, a hallmark of invasive behaviour, may entail the enzymatic dissolution of the membranes that separate tissues. Research in this field has centred around a group of matrix degrading enzymes termed the matrix metalloproteinases (MMPs). They are broadly classified into collagenases, gelatinases, and stromelysins\(^5\) \(^6\) \(^7\) \(^8\) \(^9\) \(^0\) \(^1\) (Table II). Although many proteases can cleave extracellular matrix components, the MMPs are thought to be the main physiological mediators of matrix turnover. In disease states such as neoplasia a change in the normal balance between the enzymes and their naturally occurring inhibitors, tissue inhibitor of metalloproteinases (TIMPs 1–4), must occur.\(^5\) The expression of these enzymes is strictly regulated at the transcriptional level by growth factors,\(^5\) oncogenes, and various hormones. Post-translational modification is by \(\alpha\)2 macroglobulin, proteoglycans, and the TIMPs. A change in any of these factors may break the homeostatic paracrine loops resulting in tissue invasion and tumour dissemination. Several invasive human tumours show an increase in metalloproteinase expression detected by immunoreactivity at the protein level\(^5\) \(^6\) and in situ hybridisation at the mRNA level.

The cellular source of the enzymes in neoplasia still has to be identified.\(^5\) Recent evidence is suggestive of the host stroma rather than the tumour epithelial cells themselves as the producer of these enzymes. Poulsom et al have shown augmented signals for the mRNA of 72 kDa gelatinase and TIMP-2 in the stroma in an in situ hybridisation study of colorectal tumours.\(^5\) This finding has recently been confirmed by Pyke et al who also localised mRNA signals for the same MMP to the fibroblasts surrounding the invasive edge of colorectal tumours. No hybridisation signals were noted in the tumour cells themselves.\(^6\)

The protein itself has also been localised to the stroma (N C Gallegos et al, personal communication). Other in vitro studies have shown that manipulation of the TIMPs through genetic transfection experiments and antisense treatment can result in reduced invasive behaviour and metastasis.\(^5\)\(^6\) These initial findings have raised hopes for the future incor-

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<tr>
<td>Intimal collagenase</td>
<td>Gelatinase A (72 kDa)</td>
<td>Gelatin, elastin, collagen IV</td>
<td>Gelatin, elastin, collagen IV</td>
<td>Gelatin, elastin, collagen IV</td>
</tr>
<tr>
<td>Neutrophil collagenase</td>
<td>Gelatinase B (92 kDa)</td>
<td>Gelatin, elastin, collagen IV</td>
<td>Gelatin, elastin, collagen IV</td>
<td>Gelatin, elastin, collagen IV</td>
</tr>
<tr>
<td>Stromelysin 1</td>
<td></td>
<td>Proteoglycans, fbronectin, laminin</td>
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<td>Proteoglycans, fbronectin, laminin</td>
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<td>Stromelysin 2</td>
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<td>Stromelysin 3 (?</td>
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<tr>
<td>Matrixylsin (pump-1)</td>
<td></td>
<td>Proteoglycans, fbronectin, gelatins</td>
<td></td>
<td>Proteoglycans, fbronectin, gelatins</td>
</tr>
<tr>
<td>Metalloelastase</td>
<td></td>
<td>Elastin, fbronectin</td>
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poration of metalloproteinase inhibition on to the therapeutic menu.

The gradual accrual of knowledge of the cellular properties of tumours that determine the metastatic phenotype and the impact of molecular biology techniques entailed in their unravelling promises much for the future. Predictors of tumour behaviour may well facilitate recognition of tumours that are likely to metastasise and permit us a window in which treatment could be instituted. These same markers could form the basis of such treatment. Modulation of cellular function through genetic approaches or peptide treatments that block steps in the metastatic cascade can now be evaluated in animal models. The transition of such approaches from the laboratory to the clinical setting may not be far away.

The concept of neoplastic evolution being the ultimate expression of cellular anarchy is now no longer tenable. Metastasis is a segmented selective process whose control at the molecular level is hierarchical. The exploitation of this knowledge may provide the next real step forward in the creation of novel therapeutic strategies.

17. Albeda SM. Role of cell adhesion receptors and other cell adhesion molecules in tumour progression and metastasis. Lab Invest 1993; 68: 4-17.


