

Regulation of E-cadherin: does hypoxia initiate the metastatic cascade?

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Abstract

The ability of tumours to metastasise is regarded as one of the hallmarks of malignancy. The process through which tumours evolve to achieve this has been termed the metastatic cascade. This cascade has been the subject of much investigation over many years. One of the vital events identified by these investigations is the reduction of adhesion between tumour cells facilitating invasion of the surrounding tissues and vascular channels, ultimately leading to the development of a distant metastasis. E-cadherin and its associated catenin complex have been identified as key molecules in cell adhesion. This review looks at the structure and interaction of the E-cadherin–catenin complex and the factors that appear to regulate E-cadherin expression and thus cell adhesion. From the data gathered, it has become possible to propose the hypothesis that the development of tumour hypoxia is the initiating factor that sets the tumour on the road to metastasis.

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The ability of tumour cells to invade adjacent tissues and disseminate to distant organs has long been considered the biological hallmark of malignancy. This ability of malignant tumours to colonise and destroy distant organs was first recognised by Jean-Claude Recamier in 1829 and termed metastasis.¹ Since this first basic but profound observation, the study of the mechanisms and importance of metastasis has formed an important part of the investigation of neoplasia. The expansion of this body of knowledge has been particularly rapid over the past two decades, coinciding with the development of new techniques that have allowed crucial insights into the interplay of various factors at a molecular and genetic level. The resultant model shows metastasis to be a coordinated, multistep process encompassing the detachment of cells from the primary tumour to the development of a tumorigenic lesion in a distant site (fig 1).^{2–3}

The process of metastasis appears to be regulated by a variety of gene products.⁴ These include: (1) cell–cell and cell–extracellular matrix receptors^{5–6}; (2) proteolytic enzymes that facilitate the breakdown and invasion of the basement membrane, vascular channels, and organs^{7–9}; (3) motility factors that allow migration through tissues^{10–11}; (4) receptors mediating

organ specific invasion¹²; (5) growth factors necessary for the maintenance of the tumour microcolonies in the secondary organ¹³; and (6) angiogenic factors that result in neovascularisation of the metastasis, allowing the supply of nutrients, removal of metabolites, and haematogenous spread of metastatic cells.^{14–15} Consequently, it can be appreciated that the weakening of cell–cell adhesion mechanisms must be a basic prerequisite for tumour metastasis to occur. The weakening involves changes in homotypic cell–cell adhesion, heterotypic cell–cell adhesion, and interactions of cells with the extracellular matrix at the primary tumour site.¹⁶ In recent years, several families of biochemically and genetically distinct cell adhesion molecules have been described. These include the cadherins, integrins, adhesion molecules belonging to the immunoglobulin superfamily, selectins, and CD44.

The members of the cadherin family of cell–cell adhesion molecules are situated on the cell surface and have a wide distribution in normal tissues. Although the family as a whole shows a wide distribution, the individual members show pronounced tissue specificity. E-cadherin is one of the best characterised members of the family and is expressed by all normal epithelia. It has been the focus of much attention recently because of its apparent promise as a prognostic indicator, with loss or reduction of expression correlating with enhanced aggressiveness and dedifferentiation of many carcinomas.^{17–22} In this paper, the E-cadherin adhesion system and its relation to the metastatic potential of tumours will be reviewed.

Structure and function of the E-cadherin–catenin complex

To appreciate the role of the E-cadherin–catenin complex it is essential to be familiar with the interactions of these molecules and the mechanisms by which they exert their effects.

E-cadherin is a transmembrane protein with a molecular mass of 120 kDa. It is formed from a 135 kDa precursor, which undergoes cytoplasmic enzymatic trimming to form the mature molecule and is then routed mainly towards the basolateral surface of the epithelial cells, where it tends to localise to specialised junctions of the zonula adherens type (fig 2).^{23–24} The enzymatic trimming of the precursor at the extracellular N-terminal end of E-cadherin is essential for the mature molecule to exert its role in cell–cell adhesion.²⁵ The gene encoding human E-cadherin has recently been cloned and characterised.²⁶ It has been found to be situated on chromosome 16q22.1, within a large conserved linkage group that includes loci for haptoglobin, chymotrypsinogen B,

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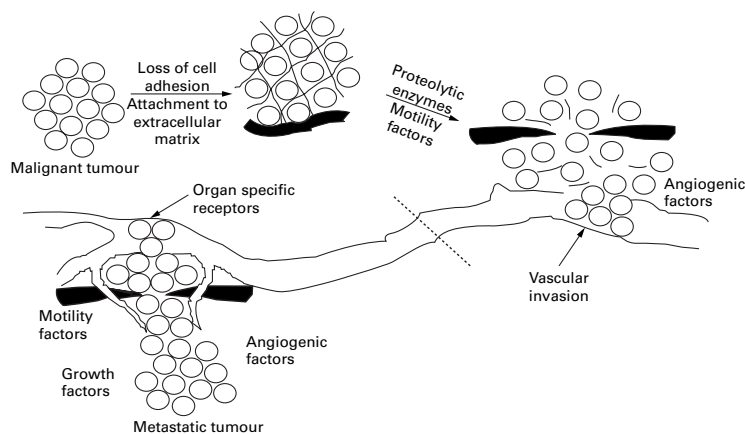


Figure 1 The multistep process of metastasis.

metallothionine-1, metallothionine-2, tyrosine aminotransferase, and lecithin cholesterolacyltransferase.^{27 28}

E-cadherin consists of an extracellular domain, which binds homotypically to E-cadherin molecules on adjacent cells, and a highly conserved intracellular domain, which binds non-covalently to the catenins. The homotypic binding is calcium dependent and is mediated by five homologous repeated domains that harbour two conserved regions representing the putative calcium binding sites.^{29 30} The extracellular domain also possesses a flexible hinge region.³¹ The cytoplasmic domain of E-cadherin contains a highly conserved region that is common to all members of the cadherin family.³² The presence of this region provides a target for immunological screening for the presence of cadherins. There is a catenin recognition site within the cytoplasmic domain that forms the link to the cytoskeleton through its interaction with the catenin complex.^{33 34} The two parts of the molecule are connected by a single, 32 amino acid, hydrophobic, membrane spanning domain.³¹

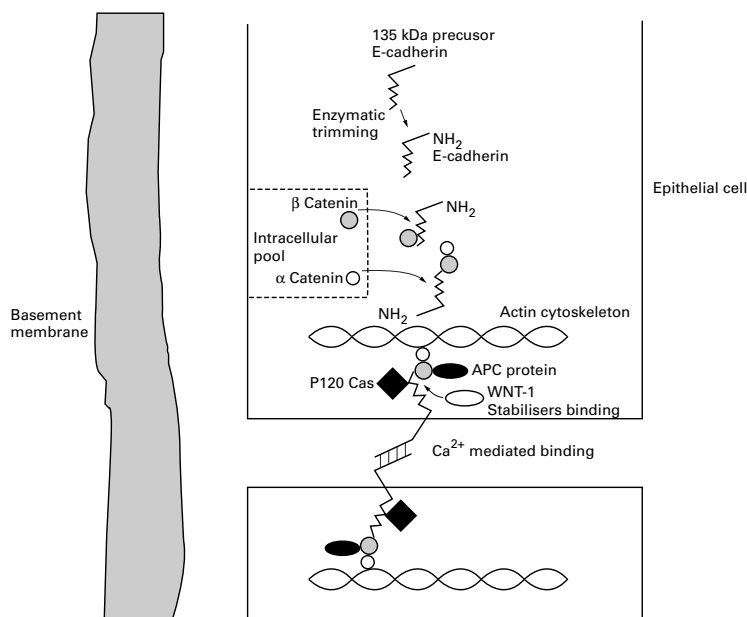


Figure 2 The formation of an E-cadherin complex using α catenin and β catenin in epithelial cells.

The binding of the transmembranous E-cadherin molecule and the actin cytoskeleton is essential for the formation of strong cell–cell adhesion mediated by the catenins, a series of associated cytoplasmic proteins that are classified according to their molecular weight. The catenin complex consists of α catenin (102 kDa), β catenin (92 kDa), and γ catenin/plakoglobin (83 kDa).^{35–37} The human genes have been assigned for all three catenins, with α catenin located on chromosome 5q31, β catenin on chromosome 3p21, and γ catenin/plakoglobin on chromosome 17q21.^{38–40} A fourth catenin-like molecule, p120cas, has recently been described and its gene localised to the long arm of chromosome 11q11, immediately adjacent to the centromere.^{41 42} The protein has been shown to be a tyrosine kinase substrate for epidermal growth factor (EGF) and platelet derived growth factor (PDGF) receptors.⁴³ Four isoforms of the p120cas molecule have been described.

The catenins bind to E-cadherin and each other in a specific manner. E-cadherin binds to either β catenin or γ catenin, whereas α catenin also binds β catenin or γ catenin but not E-cadherin.^{44–46} The existence in the same cell of two distinct E-cadherin–catenin complexes results from specific binding. One complex is composed of E-cadherin, α catenin, and β catenin, and the other of E-cadherin, α catenin, and γ catenin.^{46 47} The p120cas molecule appears to bind only to E-cadherin and does not associate directly with the other catenins or the 300 kDa adenomatous polyposis coli (APC) protein, which binds to β catenin.^{48–50} The wild-type (wt) and the mutated (mt) APC proteins both bind to β catenin.⁴⁸ Several other cytoplasmic molecules have been found to associate with the E-cadherin–catenin complex. Among these is the wnt-1 gene product, which has been found to initiate a mechanism by which the binding between E-cadherin and β catenin is stabilised, effectively promoting cell–cell adhesion.⁵¹ The c-erbB-2 gene product has also been found within the complex as a result of binding to β catenin or γ catenin.⁵²

The E-cadherin–catenin complex begins to form during the passage of E-cadherin to the cell membrane. The first catenin to interact with E-cadherin is β catenin.^{46 53} The initial interaction is followed by binding of α catenin to a short region close to the N-terminal of β catenin, which results in the formation of stable bonds between the complex and the actin cytoskeleton.⁵⁴ The binding domain responsible for the link to actin is located at the N-terminal and is also responsible for the linkage of spectrin to the complex.⁵⁵ The formation of the complex does not interfere with the catenins' ability to form complexes with other molecules, both cytoplasmic (such as APC) or at the cell membrane—for example, the EGF receptor (EGFR).^{45 46} Changes in the linkage to the cytoskeleton may be the mechanism by which EGF induces alterations in E-cadherin function.⁵⁶ The catenins forming the complex may also be exchanged for free catenins within the cytoplasm.⁴⁶ The final intercellular binding between E-cadherin molecules of adjacent cells

is calcium mediated and the resultant multimolecular structure has a “zipper” conformation.³⁰

Pathological mechanisms effecting cell adhesion mediated by the E-cadherin–catenin complex

The E-cadherin–catenin complex is dependent upon numerous interactions, which have been highlighted above. It should be obvious that cell adhesion is thus dependent not only on the structural and functional integrity of the E-cadherin molecule, but also that of the associated catenins and other molecules that mediate its binding to the cytoskeleton. Reduction in cell adhesion is of major importance in tumour metastasis and appears to be achieved by a variety of mechanisms affecting the E-cadherin–catenin complex. These include reduction or loss of E-cadherin expression, mutation of the genes of the constituent molecules, redistribution of E-cadherin to different sites within the cell, shedding of E-cadherin, and competition for binding sites by other proteins.⁵⁷

Reduction or loss of expression of E-cadherin has been documented in a large number of tumours from varying organs, including colon,^{58–59} stomach,^{20–60–61} pancreas,⁶² oesophagus,^{63–64} liver,⁶⁵ lung,^{64–66} bladder,^{67–69} prostate,^{70–72} breast,^{73–79} uterus,⁸⁰ ovary,⁸¹ thyroid,⁸² skin and oral carcinomas.^{83–87} The degree of tumour differentiation appears to be related to the proportion of E-cadherin expression, with poorly differentiated tumours more likely to show reduced E-cadherin expression, which might be a result of downregulation or defects in the catenins.^{63–88–90}

Mutation of the genes of the constituent molecules may result in structural or functional aberrations that result in reduction of cell adhesion. Mutations of the E-cadherin gene appear to be infrequent events. In frame skipping of exon 8 or 9 and deletion of exon 10 have been demonstrated in diffuse-type gastric cancer.⁹¹ Point mutations in exons 7 (invasive breast carcinoma),⁹² 12 and 13 (endometrial carcinoma),⁹³ and 16 (ovarian carcinoma)⁹³ have also been demonstrated, and these mostly affect the extracellular domain of E-cadherin. Deletions of the α catenin gene, resulting in a mutated α catenin that does not bind E-cadherin, have been identified in lung, colon, and prostate carcinoma cells.^{70–94–96} β catenin has been found to be deleted in a human gastric carcinoma cell line.^{88–90}

Redistribution of E-cadherin expression has been noted in some cancers, with the staining being variable or spotty in distribution, or located at abnormal sites along the membrane.^{64–66} Cytoplasmic (as opposed to membranous) expression has been noted in thyroid, breast, and some squamous carcinomas.^{78–82–97}

Shedding of E-cadherin from the cell surface, with resultant excretion of soluble E-cadherin in the urine has been reported.^{98–99} In these cases, the primary tumours have been noted to show reduced E-cadherin expression. Bladder cancers have also been shown to be associated with shedding of the molecule into the urine.¹⁰⁰

Competition for binding to E-cadherin by other molecules such as the APC protein may affect the normal E-cadherin–catenin interaction, thereby resulting in abnormal function.^{49–101–103}

Functional regulation of E-cadherin expression

The expression of E-cadherin may be downregulated as part of a physiological process. Embryonic morphogenesis is commonly associated with variations in E-cadherin expression occurring during specific events.^{104–105} An example is found during the development of the murine cochlea, where E-cadherin is downregulated on the lateral membranes of the reticular lamina, allowing the process of fluid space opening in the organ of Corti.¹⁰⁶ E-cadherin is downregulated during fusion of cytotrophoblast cells to syncytiotrophoblast.¹⁰⁷ Downregulation has been observed in liver undergoing regeneration after partial hepatectomy.¹⁰⁸

Downregulation occurring under specific circumstances suggests the existence of external control over the expression of E-cadherin, and thus cell adhesion. Treatment with epidermal growth factor appears to interfere with E-cadherin–catenin complex assembly and results in a more invasive phenotype *in vitro*.¹⁰⁹ The interference with complex assembly seems to be mediated by a mitogenic signal transmitted by the EGFR through its tyrosine kinase, resulting in tyrosine phosphorylation of β catenin and E-cadherin itself.¹¹⁰ Transforming growth factor α (TGF- α) has extensive homology with EGF, and produces most of the biological activities of EGF, as a result of binding with the EGFR. It has been shown recently that inhibition of E-cadherin, using a specific antibody, results in secretion of a urokinase-type plasminogen activator, which induces proteolysis of the extracellular matrix.¹¹¹ Thus, stimulation of the EGFR results in reduction of E-cadherin function, facilitating cell motility and proteolysis of the extracellular matrix, which would favour cell invasion. Regulation of cell adhesion by EGFR stimulation is also very important in wound healing, where EGF and TGF- α are produced in response to the cascade of active substances released by the injured tissue, allowing the proliferating epithelial cells to migrate, thereby facilitating closure of the breached epithelial layer. This appears to account for the downregulation of E-cadherin seen in epithelial cells adjacent to areas of ulceration in the gastrointestinal tract.¹¹²

Other motility factors that promote the proliferation and non-directional movement of discohesive cells have been isolated. These include autocrine motility factor (AMF),¹¹³ migration stimulation factor (MSF), scatter factor/hepatocyte growth factor (SF/HGF), and autotaxin.^{10–114} The SF/HGF receptor, c-Met, is a transmembrane tyrosine kinase and proto-oncogene.¹¹⁵ Binding of SF/HGF to c-Met appears to mediate mesenchymal–epithelial interactions that regulate cell growth, development, motility, and morphogenesis.^{116–117} Although the function and interac-

tion of these factors is beyond the confines of this review, it is clear that they form part of the cascade of active substances released during the process of tissue damage. The motility factors appear to act in concert with other factors, including EGF and TGF- α , to facilitate a reduction in cell adhesion and increased cell motility, in turn leading to tissue repair and healing. Adhesion molecules expressed by tumour cells could be regulated in a similar way, in response to tumour necrosis.

Several studies have reported that integrins are capable of signal transduction across the plasma membrane, resulting in local changes in cell adhesion and the cytoskeleton,¹¹⁸ giving rise to the question of whether E-cadherin itself is involved in signalling. EGF induced signal transduction and its effects have been mentioned previously. Recent studies have revealed an intersection between signalling (WNT-wingless pathway) and adhesion (cadherin-catenin complex).¹¹⁹ The Wnt-1 protein, which has been studied extensively in drosophila, has been found to bind to a seven transmembrane domain receptor called frizzled. The steps in the pathway downstream of membrane binding are still not fully understood; however, β catenin is known to be of major importance. The sharing of the cytosolic pool of β catenin means that the cadherin/WNT pathways are dependent upon each other. For instance, binding of Wnt-1 to the frizzled receptor results in accumulation of cytoplasmic β catenin.¹²⁰ The increased pool of β catenin will obviously have an effect on cell adhesion, with the β catenin in this cytosolic pool either linking with E-cadherin or acting in the WNT-wingless pathway. β Catenin may heterodimerise with leucocyte enhancer factor (LEF-1), allowing translocation to the nucleus, where LEF-1 induces DNA bending and gene transcription.^{121 122} The genes activated by the β catenin-LEF complex have not been defined.

The view that the E-cadherin-catenin complex might be involved in signalling has been strengthened by the demonstration that homophilic binding of E-cadherin to an adjacent cell can activate protein kinase C, leading to the assembly of tight junctions.¹²³ The assembly of tight junctions also involves the Rho subfamily of the Rho small G protein family.¹²⁴ Other members of the G protein family include the Rac and Cdc42 subfamilies, which are involved in regulation of E-cadherin mediated cell-cell adhesion through the action of a molecule known as IQGAP1.¹²⁵ In a recent study, Rho, Rac, and Cdc42 activation by tumour necrosis factor α (TNF- α) resulted in reorganisation of the actin cytoskeleton and the formation of intercellular gaps, indicating reduced function of tight junctions.¹²⁶ A similar effect on endometrial epithelial cells by TNF- α had been noted previously and attributed to disassembly of actin filaments.¹²⁷ The evidence above appears to indicate a role for TNF- α in the regulation of cell adhesion.

The role of the APC protein in the regulation of E-cadherin is not yet fully understood. The APC gene is located on chromosome 5q and is mutated in familial adenomatous polyposis and

in most sporadic colorectal carcinomas.^{128 129} As already mentioned, both wt and mt APC proteins bind to α catenin and β catenin. In addition, the wt APC protein, unlike mt APC, has the ability to promote microtubule assembly in vitro.¹³⁰ It has been hypothesised that, based on the selective localisation of the APC protein to the superficial differentiated crypt compartment in gut epithelium,¹³¹ the wt APC protein may regulate shedding of cells from the luminal surface through its interplay with the E-cadherin-catenin complex, resulting in downregulation of E-cadherin expression. Mutation of APC might result in interference of the normal mechanism, leading to accumulation of cells that contain potentially oncogenic mutations.¹³²

The role of *Helicobacter pylori* in the regulation of E-cadherin has been studied recently. It is widely accepted that *H pylori* is the main factor in the pathogenesis of peptic ulceration.¹³³ In addition, data have emerged recently that implicate this bacterium in the development of gastric cancer and lymphoma.¹³⁴⁻¹³⁶ The ulcerogenic potential of *H pylori* seems to result from the action of bacterial urease, which generates ammonia and protease that break down the protective mucous layer overlying the gastric epithelium. Recent studies have shown that *H pylori* infection is associated with downregulation of E-cadherin, probably by generating cell signalling events that counteract the normal function of protein kinase C.^{137 138} The resulting increase in permeability mediated by the reduction in cell adhesion might allow *H pylori* antigens to reach the gastric lamina propria and activate the mucosal immune system, with resultant tissue damage.

The ability of cell adhesion, represented in part by E-cadherin function, to be regulated by a variety of factors implies that the downregulation of E-cadherin seen in certain tumours may not just be a function of genetic mutations, resulting in expression of a dysfunctional, mutated protein, but may be regulated by factors within the microenvironment of the tumour. This modulation of E-cadherin expression has been noted in a recent study of 25 adenocarcinomas by Cowley and Smith.¹³⁹ They found higher levels of E-cadherin expression in the intravascular component of the tumours, compared with the adjacent, much larger extravascular component. It is tempting to speculate that the upregulation noted in the intravascular component may be the result of a relatively higher oxygen tension in this compartment. The effect of this upregulation would be an increase in tumour cell adhesion, facilitating the formation of a tumour embolus.

The interplay of various factors within the tumour microenvironment has been extensively studied recently, particularly in breast cancers. Reduction of E-cadherin expression in invasive duct carcinoma of the breast has been shown to correlate with the presence of lymph node metastasis, invasiveness, and EGFR expression.^{140 141} Poorly differentiated duct carcinoma in situ (DCIS) shows significantly less E-cadherin expression compared with well differentiated DCIS.¹⁴² The expression of peptide

growth factors TGF- α , EGF, and insulin-like growth factor I (IGF-I) has been found to be increased in a large proportion of breast carcinomas.¹⁴³⁻¹⁴⁵ Breast stromal cell cultures derived from human breast cancer lines are able to secrete an EGF-like substance, probably as a result of stimulation by the adjacent cancer cells.¹⁴⁶ These results indicate that the reaction to varying concentrations of stimulatory factors, such as EGF and TNF- α , within the various microenvironments of a tumour affect the degree of cell adhesion, allowing carcinoma in situ to transform into an invasive tumour.

Is there a role for hypoxia in the initiation of the metastatic cascade and downregulation of E-cadherin?

The conventional models of epithelial tumour metastasis start with a cell or population of cells that undergoes a series of mutational events resulting in a malignant population.¹⁴⁷ These mutations affect oncogenes and tumour suppressor genes, resulting in an uncontrolled proliferation of immortalised cells, which causes further genetic mutations that affect the differentiation of the cell, as well as the structural proteins dictating the interactions with adjacent cells.¹⁴⁸ The structural aberrations allow the tumour cells to detach from each other, break through the basement membrane, attach to and degrade extracellular matrix components, migrate through the tissue into a vascular channel, and eventually spread to a distant organ to establish a metastasis. With advances in the understanding of the interaction between epithelial cells, it is clear that to generate a clone of cells capable of metastasis, a vast amount of genetic damage is required at a multiplicity of sites.

Although most tumours appear to develop from a single cell, the mutations occurring within the resultant tumour population lead to the development of several phenotypes that differ with respect to their rate of growth, invasiveness, metastatic potential, karyotype, hormonal responsiveness, and resistance to anti-cancer treatment. This realisation that malignant tumours, although monoclonal in origin, are, at least by the time they manifest clinically, actually a heterogeneous population, has led to extensive study into the "metastatic phenotype". The factor that predisposes the original transformed cell to additional genetic damage is not known, but most researchers favour the notion that the original transformation event renders the cell's genome inherently unstable, making it susceptible to a high rate of spontaneous mutations.¹⁴⁹

The search for factors that affect the progression and behaviour of tumours has led to the investigation of the effect of the microenvironment on particular tumours. It is now known that the tumour microenvironment can affect the cellular heterogeneity of tumours and this realisation has spawned a number of studies.¹⁵⁰ Many of them have been conducted in vitro under well controlled conditions; however, the microenvironments produced have not been reproduced in vivo. There have been

some in vivo studies that have attempted to identify specific interactions. Drug resistant variants were found to be increased in murine carcinoma cells after exposure to activated macrophages,¹⁵¹ whereas progression of hyperplastic alveolar nodules to adenocarcinoma in mice was noted after natural killer cell infiltration of the nodules.¹⁵² Another microenvironment that occurs in malignant tumours and which is receiving attention is that of hypoxia. Oxygen deprivation seems to be present in almost all malignant tumours. With progressive and rapid growth of the tumour population, the blood supply is outstripped, resulting in cellular ischaemia and eventually the tumour necrosis that is invariably seen in malignant tumours. The effects of this ischaemia are listed below and provide compelling evidence that tumour hypoxia may well be the factor that initiates and promotes the metastatic cascade.

After the development of ischaemia within a tumour, the resultant necrosis leads to the release of inflammatory mediators such as cytokines, which recruit polymorphonuclear leucocytes, macrophages, and other cells that participate in the inflammatory process. Macrophages that are recruited to the site of tumour necrosis act in a similar way to those that are present at a site of non-neoplastic tissue damage. One of the factors released by the macrophages is nitric oxide synthetase (NOS), resulting in the formation of nitric oxide (NO), which acts as a free radical and is cytotoxic to tumour cells. It acts by oxidising sulphhydryl groups on proteins and reacting with superoxide anion to form nitrogen dioxide (a strong oxidant) and the highly reactive hydroxyl radical.¹⁵³ The demonstration of increased NOS activity within breast cancers of higher grade suggests that NO may provide a positive growth signal within the hypoxic tumour environment, resulting in increased growth rate, vascular density, and invasiveness.¹⁵⁴

The peptide growth factors, basic fibroblast growth factor (bFGF) and EGF, have been found to reduce NO mediated neuronal death in the hippocampus after exposure to an anoxic environment.¹⁵⁵ The reduction in neuronal death implies that these peptide growth factors might have a protective effect on tumour cells exposed to the cytotoxic effects of NO. As has been mentioned previously, EGF is found in breast carcinomas, where it appears to be synthesised by activated stromal cells within the tumours.¹⁴³⁻¹⁴⁶ In addition, experimental work on acute renal injury mediated by hypoxia has revealed that there is induction of mRNA for heparin binding EGF-like growth factor (HB-EGF).¹⁵⁶ A subsequent study by the same group confirmed that HB-EGF was produced in response to acute hypoxic renal injury.¹⁵⁷ Production of HB-EGF appears to be important in renal epithelial cell repair, proliferation, and regeneration. From these results, it is possible to suggest that the increase in peptide growth factors identified in carcinomas might be the result of the hypoxic injury suffered by the tumour. These growth factors might have a similar protective effect on the tumour cells as they appear to have on hypoxic neurons. This

protective effect would not only allow a larger percentage of the tumour cells to survive the period of hypoxia before the ingrowth of new vessels, stimulated by angiogenic factors, but would also have the effect of allowing the cells prolonged exposure to the mutagenic effects of the free radicals produced by NO. Thus, once the vascular supply has been re-established by the process of angiogenesis, these cells may have acquired enough genetic mutations over a relatively short period to establish a clone of cells possessing an aggressive malignant phenotype.

In several studies, the effect of a hypoxic environment on DNA synthesis and expression has been investigated. Although DNA synthesis appears to be inhibited by hypoxia, on reoxygenation, the previously arrested cells demonstrate large scale DNA replication.¹⁵⁸⁻¹⁶⁰ A similar effect has been observed with gene amplification.^{159 161} As a result, it appears that large parts of the genome, including any newly mutated parts (resulting from the damage precipitated as a result of the hypoxia) are amplified in a non-specific manner. In addition, a hypoxia induced increase in metastatic potential, which correlates with the generation of cells with over-replicated DNA, has been demonstrated in murine tumour cells.¹⁶² The cells that exhibited the highest experimental metastatic efficiency were those that were exposed to the most severe degrees of hypoxia because they were situated furthest away from the vasculature.¹⁶³ More recently, a number of different metastasis associated genes have been studied in various tumour cell lines to try and identify any correlation between increased expression of these genes and metastatic potential. No overall correlation between changes in the mRNA levels for cathepsin B, cathepsin L, nm23, tissue inhibitor of metalloproteinase 1 (TIMP-1), osteopontin, or vascular endothelial growth factor (VEGF) and metastatic ability could be demonstrated.¹⁶⁴ A previous study from the same group using similar cell lines had demonstrated increased cathepsin B and cathepsin L, and increased invasiveness after hypoxia and glucose starvation.¹⁶⁵ The variation in results obtained in these two studies appears to confirm the random nature of the genetic damage caused by hypoxia.

The random damage demonstrated above is by no means unique, with the literature being littered at the present with numerous studies indicating that a particular genetic mutation is associated with metastasis in a particular carcinoma. However, these findings are inconsistent between different research groups. An example of these differences is the investigation of the metastatic phenotype in squamous carcinomas of the head and neck. Bockmühl *et al* found that metastasising tumours frequently displayed deletions affecting chromosomes 7q, 10q, 11p, 15q, and 20p.¹⁶⁶ Over-representation of chromosomes 19q and 20q were also noted. In comparison, metastasising tumours studied by Carey *et al* showed patterns of loss affecting chromosomes 3p, 4p, 5q, 8p, 9p, 10p, 13q, 18q, and 21.¹⁶⁷ Patterns of gain were found in chro-

somes 1q, 3q, 5p, 7p, and 11q. These conflicting results occurring in a similar tumour population cannot be explained simply by the use of differing methodology and they indicate that the "metastatic phenotype" may not be a distinct pattern of chromosomal aberrations within a specific malignant phenotype, but may be unique to each tumour.

The release of peptide growth factors, such as EGF and TNF- α , in response to hypoxia has been mentioned above. The binding of these factors in normal epithelia to the EGFR has been described previously and results in down-regulation of E-cadherin and reduced cell adhesion. In the setting of a tumour, this interaction does not appear to be so simple. Reports from recent studies indicate that the binding of peptide growth factors to the EGFR is reduced in the setting of hypoxia.¹⁶⁸⁻¹⁷⁰ In one of the studies it was found that pretreatment with suramin, which binds to growth factor, resulted in increased tyrosine phosphorylation of the EGFR after exposure to high oxygen tensions.¹⁷⁰ This suggests that either there is an autocrine effect, or that other factors within the hypoxic environment are stimulating the EGFR, which results in reduced cell adhesion.

The overall effect of hypoxia on tumours appears to affect the prognosis adversely. Well documented examples of this include carcinomas of the head and neck and cervical carcinomas.^{171 172} The presence of hypoxia within these tumours has been associated with increased invasiveness and a propensity to metastasise. Soft tissue sarcomas with reduced oxygen levels have been shown to have a worse prognosis compared with those with higher oxygen tensions.¹⁷³ Other instances where tumours exposed to hypoxia assume a more aggressive phenotype are those tumours that have been subjected to subcurative radiotherapy.^{174 175} In this setting, hypoxia appears to be mediated by the vascular changes seen in response to radiotherapy.

From the foregoing evidence, it is hypothesised that the development of tumour hypoxia is the initiating factor that sets the tumour on the road to metastasis. To summarise, a malignant tumour that undergoes uncontrolled growth eventually outstrips its blood supply, resulting in hypoxia and starvation because of a lack of nutrients. The resultant necrosis releases active substances, including cytokines, peptide growth factors, and cytotoxic factors such as NO. The result of this is a population of cells exposed to sublethal ischaemia, which has the effect of reducing cell adhesion, increasing DNA mutations, and stimulating angiogenesis. With the ingrowth of new vessels and reoxygenation of the affected cells, the resultant clone assumes a more aggressive behaviour, as a result of the acquisition of a large number of genomic mutations imparting a "metastatic phenotype". The transformed, poorly adhered cells with reduced E-cadherin expression then have the ideal opportunity to invade adjacent tissue and the newly formed delicate vessels provided by the process of angiogenesis, using previously described mechanisms of the metastatic cascade. Therefore, although the devel-

opment of hypoxia within the tumour is essential for the initiation and promotion of the metastatic cascade, it is the resultant angiogenic response that allows the tumour to reach its full potential and metastasise.

Conclusion

The realisation that a reduction in cell-cell adhesion is essential for a malignant tumour to invade and metastasise has led to the identification of several different groups of cell adhesion molecules, of which the cadherins and catenins are important members. The finding that reduction in E-cadherin expression in malignant tumours was associated with a poor prognosis and metastasis led to much research, which has elucidated the structure of this molecule and its interactions and regulatory factors. The understanding of the processes involved in the control of E-cadherin expression, together with the explosion of research aimed at investigating the metastatic cascade, has allowed the formulation of the above hypothesis, which implicates tumour hypoxia as the principal initiator of the metastatic cascade. It is important to remember that the metastatic cascade is a result of complex interactions between numerous factors and that to regard a single factor as being the lynchpin on which all other steps are dependent would be naïve. Recent research into angiogenesis inhibitors is exciting and the results produced to date are encouraging. However, they should not be viewed in isolation but rather as part of a multidrug treatment combined with existing chemotherapeutic drugs. At present, work on targeting the cell adhesion system is in its infancy when compared with the progress made in the field of angiogenesis. One possible target area identified here is the peptide growth factors, molecules that exert a protective effect against the hypoxia present within tumours, which in turn seems to facilitate the generation of genomic mutations within tumour cells. Use of a peptide growth factor inhibitor in conjunction with an angiogenesis inhibitor might facilitate the development of lethal hypoxia within the target tumour, eliminating the development of a metastatic clone of cells.

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