

REVIEW

BIOLOGY OF METASTASIS : MOLECULAR ASPECTS OF METASTATIC CASCADE

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SUMMARY : *Cancer is a genetic disease in which not one, but several, mutations and other epigenetic alterations are required. So the disease is not cancer but the process of carcinogenesis, which often has an approximately 20 years latent period before invasion and metastasis occur. Recently, many investigators have identified some genes putatively involved in regulating metastasis, but the molecular basis of this process remains obscure. The present review summarises recent progress in understanding the molecular mechanism of metastatic cascade.*

Key Words : *Genes, Neoplasm, Metastasis.*

INTRODUCTION

Carcinogenesis is a multifactorial, multistage process of normal growth, differentiation, and development gone awry. In the simplest model for human carcinogenesis, that of retinoblastoma, the minimum number of stages required for the development in cancer are two. The human colorectal tumorigenesis is the best known model illustrated by Fearon and Vogelstein (1). However, leukemia is a poor model for the understanding of carcinoma (2).

The stages of carcinogenesis have been broadly defined as initiation, promotion, and progression (3, 4). Initiation is generally considered to result from an irreversible genetic alteration (5). Promotion may be defined as the clonal expansion of the initiated cells into a benign tumor or preneoplastic focus, resulting from reversible, epigenetic phenomena. Progression may be defined as the events necessary for the conversion

of the preneoplastic or benign population to the malignant state (3). The difference between benign and metastatic cancer is of utmost importance for prognosis and therapy. Experiments on the molecular mechanisms responsible have been less numerous than those focused on the earlier of neoplastic transformation, in part because of the complexity of tumor spread and in part because metastasis is often a late manifestation in tumor development (6, 7).

Biology of Tumor Metastasis

It can be considered that the process of metastasis is dependent on the outcome of a series of interactions between tumor cells and host cells or tissues, any of which could be affected by alterations in gene expression (8). In general, these sequential steps include transformation, growth of the primary neoplasm, angiogenesis, detachment and localised invasion, intravasation and extravasation of the circulatory system, survival

(host interactions) and finally, growth /angiogenesis to form a metastasis (4, 9).

Transformation is a precondition for metastatic spreading. Transformed cells become growth factor-independent, do not obey contact inhibition and lose features of differentiation, and they look phenotypically different as compared to their normal counterparts. Some of these variant cells die due to lethal mutations or host responses, or they fail to grow and become dormant, whereas other tumor cells become more competitive and malignant as they undergo phenotypic diversification (10). It is likely that most cancers are a clonal evolution driven by mutation. Generally, two mutations are more efficient than one : the first mutation would result in limited expansion of the progeny of a single cell. One of these cells would later acquire a second mutation, perhaps allowing growth of a small benign tumor. One cell within this benign tumor would then undergo a third mutation, and form a more advanced tumor composed of progeny cells with three mutations (11). In the colon, the gradual evolution of tumors (adenomas) is the first manifestation of neoplasia in colorectal epithelium (1).

ANGIOGENESIS

Solid tumors can not grow beyond microscopic sizes without forming new blood vessels. Well-developed vascularization is a prerequisite for both growth and metastasis. The chance of metastasis seems to increase with the size of the primary tumor so this angiogenesis may play an important part in determining cancer spread (7). New capillary blood vessels are needed if a tumor is to expand. New capillaries arise from pre-existing capillaries or venules, not from arteries, arterioles, or veins. Tumor angiogenesis factors secreted by neoplastic or normal cells promote the formation of a network of new blood vessels (12). For example, VEGF (vascular endothelial growth factor), first found to affect angiogenesis in malignant gliomas, also regulates angiogenesis in many different tumors (13). Both genetic, such as activation of the (ras) oncogene and inactivation of the (p53) tumor-suppressor gene, and environmental events (such as lack of oxygen) appear to affect the switching on of new blood-vessel growth (14). Malignant cells with mutations in the p53 gene can survive hypoxic conditions that kill less malignant cells (15), indicating that the supply of nutrients and oxygen to

a growing tumor can affect its growth, malignancy, and metastatic potential (16, 17).

The first step in the "metastatic cascade" is dissemination of tumor cells from the primary site and release of certain cells from their context during embryogenesis. For example, loss of function of the "cadherin" family has been correlated with the dissemination of both embryonic cells and metastasizing tumor cells (18).

INVASION

The main difference between locally growing and metastasizing cells is the ability of the latter to penetrate basal membranes, migrate through the interstitium and to invade lymphatic and blood vessels (18). After attachment of the cells to the basal membrane or to the extracellular matrix, tumor cells produce a number of lytic enzymes that degrade the basal membrane and extracellular components. Three different groups of molecules have been implicated as being involved in these processes. These are enzymes which disrupt the extracellular matrix (ECM) and create space for invasion, adhesion molecules which mediate affinity to specific ligands, and cell-surface and cytoskeletal proteins which mediate directional cell movement (18). The ECM is a barrier for diffusion and convection, and provides tissues with mechanical strength and elastic properties.

The ECM contains the mainly structural (such as collagens elastic) and the mainly adhesive (such as fibronectin and laminin) proteins that influence cell development, polarity, and behavior. The basal membrane (or basal lamina) plays an important part in controlling cell behavior. The basal membrane is also composed of collagen (mainly of type IV), proteoglycans, and glycoproteins (such as laminin and entactin) (12). Via the bridge between laminin and laminin receptors on the cell plasma membrane, malignant cells adhere to the basal membrane. Tumor cell binding to laminin increase collagen IV synthesis. Therefore, it can be expected that blocking the binding to laminin inhibits metastasis. Fibronectin apparently has the opposite effect, causing the cells to behave more normally. So metastatic cells have a reduced capacity to make and bind fibronectin (19).

CELL ADHESION MOLECULES (CAM)

Cell-cell and cell matrix adhesive interactions are involved in the regulation of many cellular

functions, including embryonic development, tumor cell growth and metastasis, programmed cell death (apoptosis), hemostasis, leucocyte homing and activation, bone resorption, and the response of cells to mechanical stress (18, 20). Adhesion to an extracellular matrix is also required for the progression of normal cells through the cell cycle, a phenomenon called anchorage dependence. Adhesion molecules include ligands and receptors.

The cadherins and also other cell adhesion molecules are proteins which can change the adhesive properties of tumor cells (18). These molecules may also function as tumor suppressors. For example, E cadherin, a Ca^{++} dependent epithelial cell-cell adhesion molecule, is regarded as an invasion-suppressor protein (21). The cytoplasmic domain of E cadherin is associated intracellularly with other proteins called "catenins". This association can be regulated by phosphorylation (18). In an attempt to clarify the mechanism responsible for inactivation of the E cadherin gene in carcinomas, it is suggested that the hypermethylation around the promoter may be a mechanism of E cadherin inactivation in human carcinomas (22).

It is well known that any significant change in expression or structure of one of the adherens junctions could lead to junctional disassembly and, consequently, to more mobile invasive carcinoma cells (21). Vinculin is an intracellular component of adhesion plaques, and plays an important role in the assembly and stabilisation of these junctions and the associated cytoskeleton (18). One of the genes encoding a member of the (CAM) family, DCC (deletion in colon carcinoma), is a recessive gene involved in colon tumor progression (1).

INTEGRINS

Metastatic spread is dependent on the surface properties of tumor cells. The other groups of surface molecules are the integrins and splice variants of CD44 glycoproteins. Integrins are a family of cell surface receptors that mediate cell-matrix and cell-cell adhesion. They are composed of alpha and beta transmembrane glycoprotein heterodimers which produce more than 20 different receptors and also function as signal transducers (23).

Divalent cations, such as Ca^{++} and Mg^{++} , are required for the association of these subunits and for ligand binding. Their binding to extracellular

matrix proteins often occurs via recognition of a specific tripeptide sequence, the arg-gly.asp (RDG) motif (23).

A number of observations indicate that integrins bind to the ECM which contains collagens, fibronectin, laminin and at the same time interact with cytoskeletal elements (such as talin, vinculin, actinin, tensin, paxillin and zyxin) connecting to the actin filaments (24). While changes in integrins promote the acquisition of an invasive phenotype by tumor cells, tumor invasion is also facilitated by the proteolytic degradation of ECM components induced by tumor cells (24). Integrins can also promote angiogenesis in tumors (25).

SURVIVAL/HOST INTERACTIONS

Because newly formed blood vessels in tumors are leaky, malignant cells can cross their walls fairly easily. Any branch of the vascular tree within a tumor mass is a potential site for the discharge of cells into the circulation (26). Most human cancers spread lymphogenically.

When the cells lose their habitual cohesiveness and enter the blood stream, they may interact with platelets and leucocytes. Platelets may protect tumor cells from the shear forces in the blood stream and immunologic surveillance. The aggregation of platelets and tumor cells arrest in the capillary bed of the target organ more easily than isolated tumor cells (12, 24).

Many tumors metastasize preferentially pass to certain target organs. For example, in general, melanomas tend to metastasize to the liver, and bone is the major metastatic site for neuroblastomas and carcinomas of the breast, thyroid, prostate, and kidney. Tumors arising from the breast, kidney, gastrointestinal tract, and lung also frequently are observed to metastasize to the brain (27). There are some factors involved in the organ selectivity of metastasis: the repertoire of adhesion molecules expressed by the metastatic cell and the availability of appropriate growth factors in the parenchyma of the target organ (24). When tumor cells express surface antigens which are recognised as abnormal by the host's immune surveillance, then it is possible for the tumor cells to be eliminated (18). Tumor cells may escape a major risk of being destroyed by immune cells, by expressing no such aberrant surface antigens, or losing the cell surface molecules, called "MHC proteins", that are needed

for recognition by some immune cells (18, 19).

Cancer Related-Genes and Controlling Metastatic Phenotype

Usually, the changes in cells that lead to neoplastic behaviour are genetic, that is, associated with structural changes (mutations) in the DNA sequence of critical genes. These mutations might lead to inappropriate activation or inactivation of important genes regulating cell proliferation and differentiation (28). Mutational events in cancer cells are not the only factors affecting cancer development. It is also possible that epigenetic changes, such as gene imprinting that requires DNA methylation, could result in a change in pattern of gene expression without there being a structural change in the underlying gene sequence (28, 29, 30).

Today, the main targets of modern molecular oncology are to identify at the level of the gene the changes that lead to cancer, determine how genetic damage takes place, and identify key steps where rational intervention might diminish mortality (31). Cancer-related genes can be classified into several groups: oncogenes, tumor suppressor genes, and metastasis suppressor genes. The oncogenes are initially identified through studies of oncogenic retroviruses (6). The precursors of oncogenes are called cellular protooncogenes. Protooncogenes are those having a counterpart within the genome of an oncogenic virus, usually a retrovirus. Viral oncogenes (v-onc) are dominant mutated forms of protooncogenes (32). Cellular oncogenes (c-onc) are defined as genes that have been shown to be capable of inducing the neoplastic transformation but that have no viral counterpart. The protooncogenes and oncogenes are involved in the growth control of normal cells and in the abnormal growth of neoplastic cells. The products of these genes which are important cellular factors include growth factors, growth factor receptors, protein kinases and nuclear proteins (31).

Tumor suppressor genes are normal cellular genes that act as negative regulators of tumor cell proliferation, and must be lost, inactivated or mutated for neoplastic transformation to occur (33). The best studied tumor suppressor gene is p53, which appears to be involved in the repair of damaged DNA. After DNA damage, its production is increased. Cells with normal p53 arrest in G1 before S phase, and then either repair the damage or

die (perhaps by apoptosis), thus halting propagation of the damaged genetic information. Cells with mutant or absent p53, on the other hand, continue to divide and can perpetuate genetic damage with the potential for cascading mutations ending in tumor development (31). As a result, loss of the normal p53 protein facilitates the emergence of malignant cells. However, it is suggested that p53 does not restrain cancer single-handedly. Rather, it seems to require an intimate partner- a protein encoded by another tumor suppressor gene, p33 (ING1) (34). So far, the cooperation between p53 and p33 (ING1) has been shown for only one gene (p21/WAF1) and, primarily, for one biological outcome-growth arrest (35). Another new putative suppressor gene, called p73, has recently been identified. Its protein resembles the p53 protein, and also seems to have similar activities (36). Classic tumor suppressor genes require two "hits" to be inactivated - a partial or complete deletion of the two gene copies. However it has been found that one p73 copy is already inactive in normal cells-the apparent result of a mysterious process called imprinting. Imprinting alters certain genes so that the copy inherited either from the mother or the father is specifically shut down (36).

METASTASIS SUPPRESSOR GENES

The close similarity of many malignant cell properties to those involved in normal cell proliferation, differentiation and development is not accidental. The genes that control or code many of these same cellular properties are probably the genes that have been misnamed "metastogenes" (10). Metastogenes are the genes which might function as metastasis suppressor genes identified in a variety of tumors including Wilms' tumor and retinoblastoma (3). Some attempts have been made to identify potential metastogenes. These approaches include somatic cell hybridisation, cytogenetic analysis, differential cDNA screening, transfection of known genes into recipient cells and transfection of genomic DNA from metastatic cells into nonmetastatic recipients (37).

In accordance with fusion studies, if metastasis is the result of the activation of metastogenes, hybrids from nonmetastatic and metastatic cells would be expected to be metastatic. Alternatively, if the loss of active suppressor genes induces metastasis then hybrids would be nonmetastatic (20).

TIMP (Tissue Inhibitor of Metalloproteases) :

In order to invade and metastasize, tumor cell must themselves produce, or induce surrounding stromal cells to produce various proteases such as collagenase, stromelylins, and gelatinases. Metalloproteases are a family of at least fifteen secreted and membrane-bound zinc-endopeptidases (37).

Metalloproteases are synthesized as enzymatically inactive proenzymes and their proteolytic activity is kept under tight control. Even an activated metalloprotease, however, will fail to cleave its target if a powerful tissue inhibitor of metalloprotease (TIMP) is present. TIMPs produced in normal tissues may have an important role in guarding against the excessive breakdown of the ECM. Tumor cells, too, can secrete TIMPs. The same malignant cell that produces a metalloprotease may therefore also produce an inhibitor of it (18). Enzyme function will occur only if the number of enzyme molecules is greater than the number of TIMPs. In other words, in this aspect of cancer cell invasion, the outcome depends on the balance of both positive and negative regulatory proteins (38). TIMPs are secreted glycoproteins which inhibit cellular invasion, and are therefore metastasis suppressor proteins (38).

Another potential metastasis suppressor protein (nm 23) was discovered by Patricia S. Steeg (1988) and her colleagues (39, 40). The biological function of the nm 23 protein is still poorly understood, although the presence of periodic leucine repeats suggest that it may function as a transcriptional factor : a protein that turns on gene activity (perhaps myc and others). Interestingly, it was found that the proteins coded by nm 23 genes function as nucleoside diphosphate (NDP) kinases, enzymes that synthesize GTP and other nucleoside triphosphates by transferring a phosphate from ATP to the corresponding diphosphate (41). The possible role of NDP kinase in cancer metastasis appears to relate to the involvement of these enzymes in microtubule assembly/disassembly and signal transduction through the "G proteins" as a consequence of the phosphorylation of 5'-diphosphate nucleotide- activities which could affect cellular adhesion and motility (7, 38).

The normal function of nm 23 will be revealed through studies of its fascinating conservation through millions of years of evolution. Human nm

23 is virtually identical to awd (abnormal wing disc), a fruit fly protein. In the fruit fly, awd is required for the correct formation of all the adult epithelial organs, such as the brain, the eyes, the wings, the legs, and the reproductive organs (42). Two distinct, independently regulated nm 23 genes (nm 23-H1 and nm 23 H2) have been identified in the human genome (39, 40). Perhaps only the nm 23-H1 serves as a metastasis suppressor, although the proteins encoded by the two genes are nearly 90% identical and similar functions would be expected (19). Expression of the nm 23 gene has been consistently down-regulated in tumor cells of high metastatic potential in many experimental models of tumor metastasis (37).

Ela :

The one of the most interesting oncogenes in terms of its effect on metastasis is the Ela gene (Adenom virus type 2). Ela protein may bind to and inactivate one or more cellular proteins that are an essential part of the metastatic cascade. Alternatively, E1a may bind to a DNA-bound protein and initiate transcriptional transactivation of one or more genes that possess the ability to suppress metastasis (37).

Oncogene Activities During Malignancy :

Many different oncogenes are implicated in the genesis of tumors. However, little is known so far about the genes which are activated at the latest stages of tumor progression. A protooncogene can be made oncogenic in many ways. The genetic alterations of protooncogenes include point mutations of a structural gene, translocations, amplifications, deletions and loss of heterozygosity, and insertion of a promoter or enhancer element (28). These mechanisms can lead to increased expression of the oncogenic product or may produce an altered gene product resulting in malignant transformation of the cell. Therefore, determining these alterations may be useful for diagnosis, prognosis, and determination of therapeutic regimes. For example, ras oncogenes have been implicated in the development of several tumors by either structural alterations or by elevated expression (2).

Ras genes:

p21 ras was first identified as a protooncogene that is mutated in a variety of human tumors. Now it is recognised as an important switch in a signalling

pathway that connects cell surface receptors to cellular targets (43). The three mammalian ras genes (K,H,and N-ras) encode 21 kDa, associated proteins that bind and hydrolyse GTP (44). p21 proteins in the GTP-bound state stimulate the PIP2 conversion to DAG and IP3 by phospholipase C. IP3 leads to increased levels of cytoplasmic Ca⁺⁺ levels ; DAG and Ca⁺⁺ then act to stimulate protein kinase C (45). The p21 ras oncogene product shows homology with the signal transducing G proteins, which suggests that H-ras may act as a coupling protein to relay extracellular messages to internal effectors that control a variety of cellular events, perhaps some of which are involved in the metastatic cascade (46).

Of particular importance for the possible role of ras in tumor progression are the following correlates of ras oncogene expression ; secretion of type-IV collagenase, and cathepsin L, increased cell motility, loss of intercellular communication and increased cellular growth autonomy allowing ectopic growth (11). Other oncogenes have been implicated in the metastatic process. The kinase-encoding oncogenes mos,raf,src,fes, and fms in addition to H-ras can induce the metastatic phenotype in some model systems (28). The fos oncogene acts to make cells malignant by turning on the gene encoding transin, an enzyme that dissolves the proteins found in the basement membrane and ECM (19).

CONCLUSIONS

The metastatic process is the end result of a complex sequence of events. Despite abundant literature about the properties of metastasizing tumor cells and molecules with the properties, as discussed in this review, the complexity of genetic or epigenetic changes seen in common cancers may at first seem daunting. However, it is likely that there may be a different critical event or events for the different common cancers. In the future, these events might be selected for screening programs for early detection of the tumor.

It is well known that a single gene can not directly manifest expression of the metastatic phenotype in normal cells unless it can induce pleiotropic effects. However, the possibility of a single gene suppressing the metastatic phenotype seems much greater. Therefore, determining the molecular structure of the communication pathways that regulate metastatic behaviour of

tumor cells may be useful for predicting or preventing future metastasis. Many studies have shown that there is a close association between expression of various members of the metalloproteases family by tumors and their proliferative and invasive behaviour and metastatic potential. Therefore, by preventing tumor cells from interacting with each other, and also with their microenvironment, tumor growth and metastasis can be suppressed.

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