

Review Article**Tumour Invasion and Metastasis: A review****Dr. Moni Thakur¹, Dr. Guttikonda Venkateswara Rao²**¹Senior Lecturer, Department of Oral and Maxillofacial Pathology, Mamata Dental College and Hospital, Khammam, Telangana, India.²Professor & Head, Department of Oral and Maxillofacial Pathology, Mamata Dental College and Hospital, Khammam, Telangana, India.***Corresponding Author:**

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Abstract: A key characteristic of the adaptive response of cells is that if the stimulus is removed any alteration in cell growth reverts to normal. In contrast to these reversible adaptive responses certain stimuli cause changes in genetic material that result in permanent alteration of the normal cellular growth pattern. Such altered cells, which are termed neoplastic, fail to respond normally to signals controlling the cell growth. They proliferate excessively in a poorly regulated manner, forming a lump or tissue mass called a neoplasm. A tumour is said to be benign when its microscopic and gross characteristics are considered relatively innocent, implying that it will remain localised, cannot spread to other sides. Malignant, as applied to a neoplasm, implies that the lesion can invade and destroy the adjacent structures and spread to distant sites (metastasize) to cause death. Mechanisms by which metastatic cells arise from primary tumours and why they metastasize to specific organs can be explained by cross talk between selected cancer cells (the 'seeds') and specific organ micro environments (the 'soil') implying that Stephen Paget's 1889 seed-soil hypothesis. Metastasis of a tumour cell depends on its interactions with the homeostatic factors that promote tumour cell growth, survival, angiogenesis, invasion and metastasis.

Keywords: Angiogenesis, Invasion, Metastasis, Oral cancer, Tumour.

INTRODUCTION:

A neoplasm as defined by Willis is "an abnormal mass of tissue, the growth of which exceeds and is uncoordinated with that of the normal tissues and persists in the same excessive manner after the cessation of the stimuli which evoked the change." Fundamental to the origin of all neoplasms is loss of responsiveness to normal growth controls. Neoplastic cells are said to be transformed because they continue to replicate, apparently oblivious to the regulatory influences that control normal cell growth. Neoplasms seem to behave as parasites and compete with normal cells and tissues for their metabolic needs [1].

Currently the term tumour is applied almost solely to neoplastic masses that may cause swellings on or within the body [1]. A tumour is said to be benign when its microscopic and gross characteristics are considered relatively innocent, implying that it will remain localised, cannot spread to other sides. Malignant tumours are collectively referred to as cancers, derived from the Latin word 'for crab-they adhere to any part they seize on in an obstinate manner, like a crab'. Malignant, as applied to a neoplasm, implies that the lesion can invade and destroy the

adjacent structures and spread to distant sites (metastasize) to cause death [2, 3, 1]. All tumours, benign and malignant have two basic components (a) the parenchyme, made up of transformed or neoplastic cells, and (b) The supporting, host derived, non neoplastic stroma, made up of connective tissue and blood vessels.

The parenchyme of the neoplasm determines its biologic behaviour, and it is this component from which the tumour derives its name. The stroma carries the blood supply and provides support for the growth of the parenchymal cells and is therefore crucial to the growth of the neoplasm [2, 1]. The most significant property of malignant neoplasm is that growth is not confined to the site of origin of the tumour, i.e., the primary tumour control of cell growth becomes so abnormal that cells can grow into adjacent local tissues, in a process termed invasion [4].

The most deadly aspect of cancer is its ability to spread, or metastasize. The cancer cells that travel through the body are capable of establishing new tumours in locations remote from the site of the original disease. To metastasize, a cancer cell must break away

from its tumour, invade either the circulatory or lymph system, which will carry it to a new location, and establish itself in the new site [5].

Mechanisms by which metastatic cells arise from primary tumours and why they metastasize to specific organs can be explained by cross talk between selected cancer cells (the 'seeds') and specific organ micro environments (the 'soil') implying that Stephen Paget's 1889 seed-soil hypothesis still holds true today. Metastasis of a tumour cell depends on its interactions with the homeostatic factors that promote tumour cell growth, survival, angiogenesis, invasion and metastasis. The tumour cells and their stroma co-evolve during tumorigenesis and progression. The precise nature of normal stromal cells and their effect on tumour initiation and progression are poorly understood. Invasion and metastasis are biologic hallmarks of disease. They are major causes for cancer related morbidity [6, 7, 8].

Characteristics of Tumour:

Majority of neoplasms can be categorized clinically and morphologically into benign and malignant on the basis of certain characteristics like (a) rate of growth, (b) Clinical and gross features, (c) Microscopic features, (d) Local invasion (direct spread) and (e) Metastasis (distant spread).

INVASION AND METASTASES:

Metastasis and invasiveness are the two most important features to distinguish malignant from benign tumours [3].

Invasion: Cancers grow by progressive infiltration, invasion, destruction and penetration of the surrounding tissue. Next to the development of metastasis, local invasiveness is the most reliable feature that distinguishes malignant from benign tumours [2].

Metastasis: the term metastasis connotes the development of secondary implants discontinuous with the primary tumour possibly in remote tissues [2, 9].

These cancer cells that travel through the body are capable of establishing new tumours in locations remote from the site of original disease. To metastasise a cancer cell must break away from its tumour, invade either the circulatory or lymph system which will carry it to a new location and establish itself in the new site. The body has many safeguards to prevent cells from doing this, yet many cancer cells have the ability to overcome these safeguards [5]. Malignant neoplasms disseminate by way of , (a) Seeding with in body cavities, (b) Lymphatic spread or (c) Hematogenous spread [2].

EVENTS IN METASTASIS:

1. Invasion of the extracellular matrix:
2. Vascular dissemination and homing of tumour cells

INVASION OF THE EXTRACELLULAR MATRIX:

- 1) **Detachment of the tumour cells** from each other by down regulation of E-Cadherins which increases the metastatic potential of cancer cells.
- 2) **Attachment to matrix components:** Receptor mediated attachment of tumour cells to laminin and fibronectin is important for invasion and metastasis. Normal epithelial cells have high affinity receptors for basement membrane. Cancer cells have more number of receptors. Tumour cells also have integrins that serve as receptors for components of extracellular matrix like fibronectin, collagen etc.
- 3) **Degradation of extracellular matrix:** occurs through the secretion of various proteases like serine, cysteine, and matrix metalloproteinases (MMP) which further promotes tumour growth, angiogenic and chemotactic activity.
- 4) **Migration of tumour cells:** is mediated by tumour cell derived cytokines, such as autocrine motility factors. Cleavage products of matrix components like collagen and laminin and some growth factors like insulin growth factor 1 and 2 have chemotactic activity for tumour cells (Fig.1) [2, 1].

VASCULAR DISSEMINATION AND HOMING OF TUMOUR CELLS:

Within the circulation the tumour cells have homotypic adhesion among other tumour cells and heterotypic adhesion with platelets. Formation of platelet tumour aggregates may enhance tumour cell survival. Extravasation of tumour cells at distant sites involves adhesion to endothelium, followed by egress through basement membrane which is mediated by proteolytic enzymes and adhesion molecules. (Fig.2)[2, 1].

PROTEASES IN INVASION AND METASTASIS:

There are several possible mechanisms by which these proteases can promote cancer cell invasion and intravasation. Individual proteases cleave cell adhesion molecules, such as epithelial (E)- cadherin, leading to the disruption of cell-cell junctions. The loosening of cell contacts facilitates cancer cell migration, either as individual cells or in groups, and protease degradation or turnover of proteins in the extracellular matrix, (ECM), and the basement membrane enables invasive cells to migrate into the surrounding tissue and vasculature [10].

E-Cadherin and oral cancer: E-cadherin plays an important role in maintaining the tight cell-to-cell contacts in normal oral epithelia. Decreased expression of E-cadherin during epithelial-mesenchymal transition leads to a decrease in cell-cell adhesion, and thereby contributes to cell dissociation and increased motility. Thus, E-cadherin helps to suppress tumour cell motility, invasion, and metastasis. Decreased or complete loss of

E-cadherin expression has been associated with lymph node metastasis and poor prognosis in OSCC [11].

Integrins and oral cancer: In OSCC, immunohistochemical evaluation of integrin $\alpha_6 \beta_4$ expression has shown this cell-surface protein to be associated with early recurrence and metastasis [47]. Expression of $\alpha_6 \beta_5$ is lower in OSCC than in normal epithelium. In contrast to $\alpha_6 \beta_5$ the $\alpha_6 \beta_6$ expression is up-regulated in oral dysplasia, where its expression is correlated with progression to malignant disease. It has been suggested that $\alpha_6 \beta_6$ may be useful in predicting malignant transformation [12].

MMP in oral cancer: Many studies have shown that gelatinases (MMP-2 and -9) are capable of degrading basement membrane and stromelysins (MMP-3, -10 and -11) MMP-3 at the invasive front and correlations between the above finding with regard to tumor size, thickness, and mode of invasion were also done. Overexpression of MMP-10 and -11 genes has been correlated to tumour differentiation and to local invasiveness. Collagenases (MMP-1 and -13) and membrane-bound MMP (MT1-MMP) are all expressed in oral cancer and may have roles in tumour progression. Tissue inhibitors of metalloproteinase (TIMP) can inhibit the action of MMPs, and a recent study showed that overexpression of TIMP is correlated with regional and distant metastasis, and poor prognosis [13, 12].

Metastatic Heterogeneity: Two approaches have been to show that cells within the parent neoplasm differ in metastatic capacity. In the first approach, metastatic cells are selected *in vivo*, such that tumour cells are implanted at a primary site or injected intravenously, metastases allowed to grow, individual lesions collected and expanded *in vitro*, and these cells used to repeat the process. In the second approach, cells are selected for an enhanced expression of a phenotype believed to be important to a step in the metastatic process and are then tested *in vivo* to determine whether their metastatic potential has changed [14].

PATHOGENESIS OF METASTASIS:

The process of cancer metastasis consists of sequential and interrelated steps each of which can be rate limiting because a failure at any step may halt the process. The steps or events required for metastasis are the same for all tumours, and consist the following: [54].

1. After the initial transforming event, the growth of neoplastic cells is progressive & frequently slow.
2. Vascularization is required for a tumour mass to exceed a 1- to 2-mm diameter, and the synthesis and secretion of angiogenesis factors has a critical role in establishing a vascular network within the surrounding host tissue ;

3. Local invasion of the host stroma by tumour cells can occur by multiple mechanisms, including, but not limited to, thin-walled venules and lymphatic channels, both of which offer little resistance to tumour cell invasion;
4. Detachment and embolization of tumour cell aggregates, which may be increased in size via interaction with hematopoietic cells within the circulation;
5. Circulation of these emboli within the vascular; both hematologic and lymphatic;
6. Survival of tumour cells that trafficked through the circulation and arrest in a capillary bed;
7. Extravasation of the tumour embolus, by mechanisms similar to those involved in the initial tissue invasion;
8. Proliferation of the tumour cells within the organ parenchyma resulting in a metastatic focus;
9. Establish vascularization, and defenses against host immune responses; and
10. Reinitiate these processes for the development of metastases from metastases (Fig.6) [14].

The Organ Microenvironment: Experimental data supporting Paget's "seed and soil" hypothesis have been derived from studies on the invasion and growth of B16 melanoma metastases in specific organs [14].

Seed and Soil Hypothesis: In 1889, Stefan Paget suggested that metastasis is not due to chance events, but rather that some tumour cells (the "seed") grew preferentially in the micro environment of selected organs (the "soil") and the metastasis resulted only when the appropriate seed was implanted in its suitable soil.

Concept of the "seed and soil" hypothesis consists of three principles [14, 15].

- The first principle is that primary neoplasms (and metastases) consist of both tumour cells and host cells. Host cells include, but are not limited to, epithelial cells, fibroblasts, endothelial cells, and infiltrating leukocytes. Moreover, neoplasms are biologically heterogeneous and contain genotypically and phenotypically diverse subpopulations of tumour cells, each of which has the potential to complete some of the steps in the metastatic process, but not all.
- The second principle is that a focused analysis into the metastatic process is needed to reveal the selection of cells that succeed in invasion, embolization, survival in the circulation, arrest in a distant capillary bed, and extravasation into and multiplication within organ parenchyma. These successful metastatic cells ("seed") have been likened to a decathlon champion who must be proficient in 10 events, rather than just a few.
- The third principle is that metastatic development occurs in specific organs, or microenvironments

(“soil”), that are biologically unique. Endothelial cells in the vasculature of different organs express different cell-surface receptors and growth factors that can support or inhibit the growth of metastatic cells. Thus, the outcome of metastasis depends on multiple interactions (“cross-talk”) of metastasizing cells with homeostatic mechanisms, such that the therapy of metastases can target not only the cancer cells, but also the homeostatic factors that promote tumour-cell growth, survival, angiogenesis, invasion, and metastasis[14,15].

MECHANISM AND BIOLOGY OF INVASION AND METASTASIS: The process of local invasion and distant spread (by lymphatic and haematogenous routes) involves passage through barriers before gaining access to the vascular lumen. This includes making the passage by the cancer cells by dissolution of extracellular matrix (ECM) at three levels - at the basement membrane of tumor itself, at the level of interstitial connective tissue, and at the basement membrane of microvasculature.

- 1. Aggressive clonal proliferation and angiogenesis.** The first step in the spread of cancer cells is the development of rapidly proliferating clone of cancer cells. This is explained on the basis of tumor heterogeneity, i.e. in the population of monoclonal tumor cells, a subpopulation or clone of tumor cells has the right biologic characteristics to complete the steps involved in the development of metastasis. Tumor angiogenesis plays a very significant role in metastasis since the new vessels formed as part are directly in contact with cancer cells.
- 2. Tumor cell loosening.** Normal cells remain glued to each other due to presence of cell adhesion molecules (CAMs), E (epithelial) - cadherin. In epithelial cancers there is either loss or inactivation of E-cadherin and also other CAMs of immunoglobulin superfamily, all of which result in loosening of cancer cells.
- 3. Tumor cell-ECM interaction.** Loosened cancer cells now are attached to ECM proteins, mainly laminin and fibronectin. This attachment is facilitated due to profoundness of receptors on the cancer cells for both these proteins. There is also loss of integrins, the transmembrane receptors, and further favouring invasion.
- 4. Degradation of ECM.** Tumor cells overexpress proteases and matrix-degrading enzymes, metalloproteinases that include collagenases and gelatinase, while the inhibitors of metalloproteinases are decreased. Another protease, cathepsin D, is also increased in certain cancers. These enzymes bring about dissolution of ECM—firstly basement membrane of tumor itself, then make way for tumor cells through the interstitial matrix, and finally dissolve the basement membrane of the vessel wall.

5. Entry of tumor cells into capillary lumen: The tumor cells after degrading the basement membrane are ready to migrate into lumen of capillaries or venules for which the following mechanisms play a role.

- a)** Autocrine motility factor (AMF) is a cytokine derived from tumor cells and stimulates receptor-mediated motility of tumor cells.
 - b)** Cleavage products of matrix components which are formed following degradation of ECM have properties of tumor cell chemotaxis, growth promotion and angiogenesis in the cancer. After the malignant cells have migrated through the breached basement membrane, these cells enter the lumen of lymphatic and capillary channels.
- 6. Thrombus formation:** The tumor cells protruding in the lumen of the capillary are now covered with constituents of the circulating blood and form the thrombus. Thrombus provides nourishment to the tumor cells and also protects them from the immune attack by the circulating host cells.
 - 7. Extravasation of tumor cells:** Tumor cells in the circulation (capillaries, venules, lymphatics) may mechanically block these vascular channels and attach to vascular endothelium. In this way, the sequence similar to local invasion is repeated and the basement membrane is exposed.
 - 8. Survival and growth of metastatic deposit:** The extravasated malignant cells on lodgment in the right environment grow further under the influence of growth factors produced by host tissues, tumor cells and by cleavage products of matrix components. These growth factors in particular include; PDGF, FGF, TGF- β and VEGF. The metastatic deposits grow further if the host immune defence mechanism fails to eliminate it. Metastatic deposits may further metastasise to the same organ or to other sites by forming emboli (Fig.7) [3].

ROUTES OF METASTASIS

Cancers may spread to distant sites through:

1. Local invasion
2. Lymphatic spread
3. Haematogenous spread
4. Others
 - a. Transcoelomic spread
 - b. Spread along epithelium-lined surfaces
 - c. Spread via cerebrospinal fluid
 - d. Implantation

1. Local invasion: Most common pattern of spread of malignant tumours is by direct growth into adjacent tissues. Tumours may also spread along natural tissue planes, e.g. along nerves [4].

2. Lymphatic spread: In general, carcinomas metastasise by lymphatic route while sarcomas favour haematogenous route.

Involvement of lymph nodes by malignant cells is by:

a) Lymphatic permeation: The walls of lymphatics are readily invaded by cancer cells and may form a continuous growth in the lymphatic channels.

b) Lymphatic emboli: The malignant cells may detach to form tumour emboli so as to be carried along the lymph to the next draining lymph node.

Generally, regional lymph nodes draining the tumour are invariably involved producing regional nodal metastasis. All regional nodal enlargements are not due to nodal metastasis because necrotic products of tumor and antigens may also incite regional lymphadenitis. Sometimes lymphatic metastases do not develop first in the lymph node nearest to the tumor because of venous-lymphatic anastomoses or due to obliteration of lymphatics by inflammation or radiation so, called skip metastasis. Due to obstruction of the lymphatics by tumor cells, the lymph flow is disturbed and tumor cells spread against the flow of lymph causing retrograde metastases at unusual sites [3, 4, 9].

3. Haematogenous spread: Blood-borne metastases are the common route for sarcomas but certain carcinomas also frequently metastasise by this mode. The common sites for blood-borne metastasis are the liver, lungs, brain, bones, kidney and adrenals, all of which provide 'good soil' for the growth of 'good seeds' (seed-soil theory).

Systemic veins drain blood into vena cavae from limbs, head and neck and pelvis. Therefore cancers of these sites more often metastasise to the lungs.

Portal veins drain blood from the bowel, spleen and pancreas into the liver. Thus tumours of these organs frequently have secondaries in the liver.

Arterial spread of tumours is less likely because they are thick-walled and contain elastic tissue which is resistant to invasion.

Retrograde spread by blood route may occur at unusual sites due to retrograde spread after venous obstruction, just as with lymphatic metastases [3,4,9].

4. Others: Some cancers spread by seeding at other surfaces, these routes of distant spread are:

a) Transcoelomic spread: Certain cancers invade through the serosal wall of the coelomic cavity so that tumor fragments or clusters of tumour cells break off to be carried in the coelomic fluid and are implanted elsewhere in the body cavity. Example, carcinoma of stomach seeding to both ovaries, carcinoma of breast seeding to pleura and pericardium.

b) Spread along epithelium-lined surfaces: It is unusual for a malignant tumour to spread along the epithelium-lined surfaces because intact epithelium and mucus coat are quite resistant to penetration by tumour cells. However, exceptionally a malignant tumor may spread through fallopian tube from the

endometrium to the ovaries and through the bronchus into alveoli.

c) Spread via cerebrospinal fluid: Malignant tumour of the ependyma and leptomeninges may spread by release of tumor fragments and tumor cells into the CSF and produce metastases at other sites in the central nervous system.

d) Implantation: Rarely a tumour may spread by implantation by surgeon's scalpel, needles, sutures or may be implanted by direct contact such as transfer of the lower lip to the opposing upper lip [3, 4, 9].

Tumour angiogenesis: Tumour associated angiogenic factors are produced by tumour cells themselves and by inflammatory cells (eg, macrophages) that infiltrate tumours. Of the dozen or so tumour associated angiogenic factors, the two most important are the basic fibroblast growth factor (bFGF) and vascular endothelial growth factor (VEGF). Others, such as TNF- α derived from macrophages, also contribute. Angiogenesis is not only essential for the growth of primary tumours, but also facilitates metastasis. Highly vascular tumours are more prone to metastasize because of ready access to the vasculature.¹

Tumour progression and heterogeneity: It is well established that over a period of time many tumours become more aggressive and acquire greater malignant potential. This phenomenon is referred to as tumour progression and must be clearly distinguished from an increase in tumour size. Careful clinical and experimental studies reveal that increasing malignancy (eg, accelerated growth, invasiveness and ability to form distant metastases) is often acquired in an incremental fashion. This biologic phenomenon is related to the sequential appearance of sub populations of cells that differ with respect to several phenotypic attributes, such as invasiveness, rate of growth, metastatic ability, karyotype, hormonal responsiveness, and susceptibility to anti neoplastic drugs. Thus, despite the fact that most malignant tumours are monoclonal in origin, by the time they become clinically evident; their constituent cells are extremely heterogeneous.

Most investigators believe that transformed cells are genetically unstable. Such instability may result, for example, from loss of p53, the so called "guardian of the genome". As mentioned earlier, inherited or acquired mutation in DNA repair genes may also contribute to genomic instability. These and other unidentified factors render tumour cells susceptible to high rate of random, spontaneous mutations during clonal expansion [1].

Molecular Genetics of Metastases: Are there oncogenes or tumour suppressor genes that elicit metastasis as their principal or sole contribution to tumorigenesis. This is more of an academic interest

because if altered forms of certain genes promote or suppress the metastatic phenotype, their detection in a primary tumour may have prognostic as well as therapeutic implications. At present no single “metastatic gene” has been found. Indeed, since metastatic cells must acquire multiple properties (eg, expression of adhesion receptors, production of collagenases and motility factors), no single genetic alteration is likely to render a cell metastasis prone.

Thus it might be expected that mutations in the E-Cadherin gene or genes that encode inhibitors of metalloproteinases would facilitate metastases by making the cells more invasive. Reduced expression of a gene called nm23 seems to be associated with metastatic potential in carcinomas of the breast, liver, ovary and stomach. The function of nm23 in the metastatic cascade is unknown [9, 1].

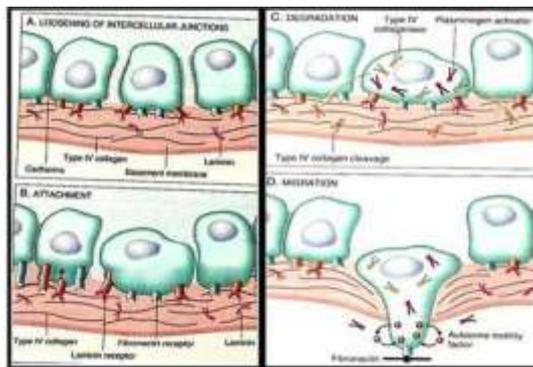


Fig-1: Invasion of extracellular matrix

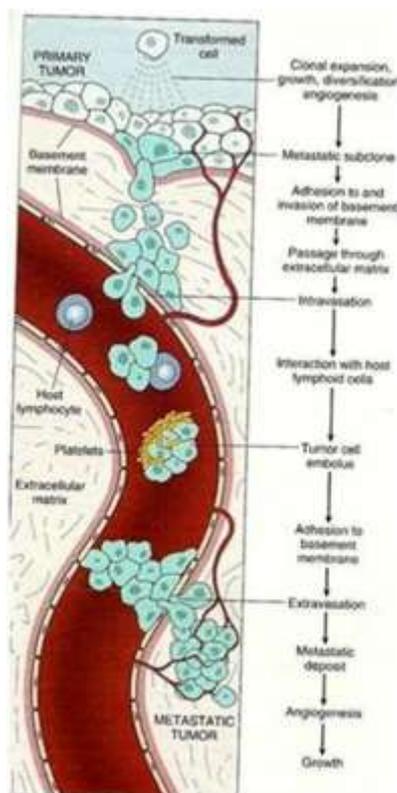


Fig-2: Metastatic cascade

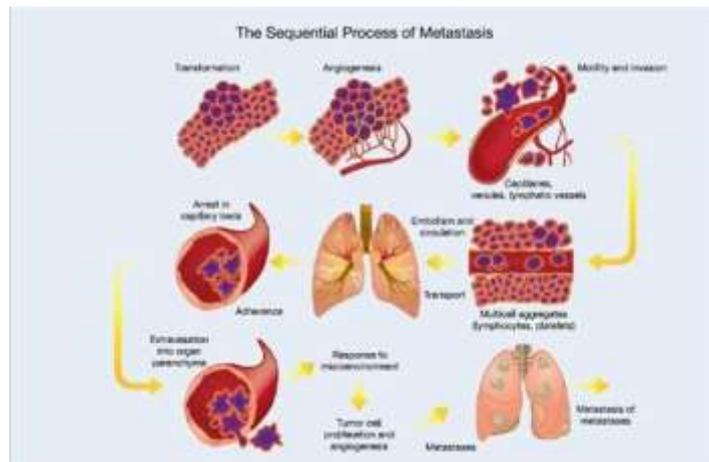


Fig-3: Sequential process of metastases.

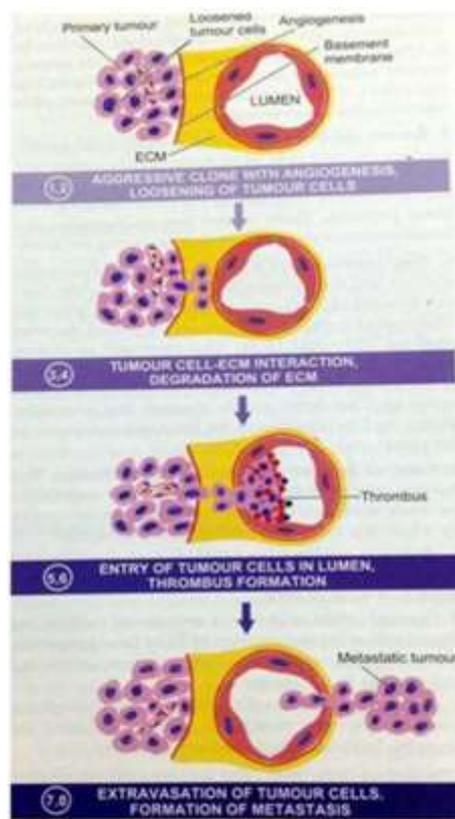


Fig-4: Mechanism and biology of invasion and metastasis

Summary and Conclusion:

The pathogenesis of metastasis depends on multiple interactions between metastatic cells and host homeostatic mechanisms. We posit that the interruption of these interactions will inhibit or help eradicate metastasis. Clinical efforts have focused on the inhibition or destruction of tumor cells; however, strategies to treat metastatic tumour cells and modulate the host microenvironment now offer new treatment approaches. The recent advances in our understanding of the metastatic process at the cellular and molecular level provide unprecedented potential for improvement and the development of effective adjuvant therapies.

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