Review Article

Metastasis: An Overview

M Keerthi[1]* and M Jaya Nagendra Krishna[2]

ABSTRACT

Metastasis means the spread of the malignant cells from the origin site to a distant area. It involves the process of separation / detachment, transportation of fragmented primary tumour cell and lodging in the new site, having a favourable environment. It does not result from random survival of cells released from the primary tumour, but from the selective growth of specialised subpopulations of highly metastatic cells endowed with specific properties that benefit them to complete each step of the metastatic process. To understand this complex network we need to explore the molecules involved in it. This article reviews the theories, mechanisms, genes and genetic alterations that are involved in the process of metastasis.

KEYWORDS: Metastasis, Separation, Detachment, Transportation, Genetic alterations.

INTRODUCTION

The study of cancer metastasis can be traced back to antiquity. Primary accounts by the Egyptians date back to 1500 BC and we owe much to the ancient Greeks in particular, who discoursed on the progressive nature of cancer, from its onset as localised growths to the development of pervasive disease[1]. Metastasis is the spread of a disease from one organ or part to another non-adjacent organ or part[1]. The word metastasis refers to "displacement" in Greek, from meta meaning "next", and stasis meaning "placement"[2]. A metastasis is defined as a growth, separate from the primary tumour that has arisen from detached, transported fragments of the primary tumour[3]. Metastases are the major cause of treatment failure in cancer patients. A total of 60% of patients with newly diagnosed solid tumours (excluding skin cancers other than melanoma) have clinically evident or microscopic metastases when the primary tumour is diagnosed. Dissemination of malignant cells throughout the body and their survival to form secondary growths constitute a complicated process dependent on both host and tumour properties[4].

MECHANISMS

BACKGROUND

Two historical arguments have been advanced to explain the mechanisms of metastasis. In 1889, surgeon Stephen Paget noticed that metastases in breast cancer were not random. He postulated the "seed-and-soil" hypothesis, meaning that a metastasis arose from the proliferation of a few tumour cells (the seeds) in the favourable milieu provided by certain organs (the soil)[5].

Forty years later, James Ewing postulated the "mechanical entrapment theory," hypothesising that invasion and metastases can be explained on purely anatomic grounds. The first organ encountered by the tumour cells would be the site of greatest tumour cell arrest and, consequently, the largest number of metastatic colonies[6].

After 60 years of observation and experimentation, it is now known that both Paget and Ewing were partially correct and that the two theories are not mutually exclusive. Some metastatic tumours colonise a wide

[1]Senior Lecturer, Department of Oral & Maxillo Faial Pathology, [2]Senior Lecturer, Department of Conservative & Endodontics, Kamineni Institute of Dental Sciences, Sreepuram, Narketpally, Andhra Pradesh, India
Corresponding author email id: *keerthikrishnamds@yahoo.com; [2]kittymjn@gmail.com

variety of tissues. For these indiscriminate tumours, the first site encountered will be the most common site of metastatic colony formation. Other tumours are far more selective; they bypass proximal organs and selectively colonise specific distal organs. Metastatic disease is a dynamic process, fuelled by the constant evolution of genetically variant tumours. For this reason, the clinical manifestation of metastasis can be diverse. In some instances, metastasis occurs relatively early, synchronous to the diagnosis of a primary tumour. Alternatively, metastases may grow asynchronously after remaining dormant for years. During this period of minimal residual disease, single cancer cells remain quiescent or a few undetectable micro metastatic lesions are maintained[7]. Metastases do not occur randomly but are the end result of a complicated series of tumour-host interactions[4]. Interruption of the metastatic cascade at any of these steps can prevent the production of clinically symptomatic metastases. Several discrete steps are discerniable in the biological cascade of metastasis: loss of cellular adhesion, increased motility and invasiveness, entry and survival in the circulation, exit into new tissue, and eventual colonisation of a distant site[8].

ALTERED CELLULAR ADHESIONS

Compared with normal epithelia, carcinoma cells almost invariably show diminished intercellular adhesiveness. In many instances, epithelial tumours lose E-cadherin-mediated adhesions as they progress towards malignancy. Integrins are also emerging as important mediators of the malignant phenotype during oncogenic transformation. In particular, the α6β4 integrin, which binds to the extracellular matrix protein laminin, forms signalling complexes with oncogenic receptor tyrosine kinases, such as Met, EGFR, and Her2. αVβ3 and α3β1 integrins have also been implicated in later stages of metastasis, specifically during adhesion of circulating tumour cells to the vasculature[9].

CELL MOTILITY

Metastasis fundamentally involves the movement of cells from one site to another. Studies done on the mouse model of melanoma have shown that an increase in copy number and overexpression of Nedd9, a Focal Adhesion Kinase (FAK) adaptor protein, fosters cell motility and invasion. Podoplanin, a mucinlike transmembrane glycoprotein that is expressed at the invasive front of many human malignancies, enhances cellular invasion[10].

RESISTANCE TO EXTRACELLULAR DEATH SIGNALS

Although evasion of apoptosis is a hallmark of tumour cells, it is possible that progression toward metastasis requires a further defence against microenvironmental death stimuli. Nutrient deprivation and hypoxia, alterations in extracellular adhesions, changes in cell shape during invasion, and exposure to novel stromal microenvironments can all trigger cell death. Ectopic overexpression of potent anti-apoptotic effectors, such as B- cell lymphoma 2 (BCL2), B- cell lymphoma extra large (BCL-XL), and X- linked inhibition of apoptosis protein (XIAP), in cancer cells can make them highly resistant to death stimuli, which has been shown to enhance the efficiency of metastasis in numerous experimental models[11]. Alternatively, loss of Caspase 8 expression, an apoptotic initiator Caspase activated downstream of unligated integrins, can also facilitate invasion and metastasis by making tumor cells more resistant

DISRUPTION OF THE BASEMENT MEMBRANE AND EXTRACELLULAR MATRIX

Basement membranes that underlie epithelial and endothelial cell layers are a dense meshwork composed of several glycoproteins and proteoglycans (such as type IV collagen, laminin and perlecan). For epithelial tumours in an incipient state, the basement membrane acts as a barrier to the invasion of transformed cells into the subjacent stroma. Tumour cells that are able to proteolytically disrupt the basement membrane can progress to overt malignancy and metastasis.

Cancerous cells use diverse mechanisms to unleash proteolytic activities on the basement membrane and interstitial extracellular matrices. In addition to

facilitating tumor invasion, extracellular proteases (MMP9 and MMP2) may generate a diverse array of bioactive cleaved peptides. These products can modulate migration, cancer-cell proliferation and survival, and tumour angiogenesis[4].

INVASION

During tumour invasion, malignant cells penetrate a variety of extracellular matrices, including basement membranes, interstitial stroma, cartilage, and bone. Penetration of the extracellularmatrix takes place at multiple steps in the metastatic cascade by cyclic repetition of three biochemical steps namely :

 1) Attachment 2) proteolysis; 3) locomotion.

 The first step is tumour cell attachment to the basement membrane or extracellular matrix by tumour cell surface receptors. The second step is local degradation of basement membrane or extracellular matrix by tumour-associated proteolytic enzymes. The third step is tumour cell locomotion into region modified by proteolysis. Direction of locomotion is influenced by tumour cell-derived (autocrine) motility factors, host-derived (paracrine) chemotactic factors, components of extracellular matrix and their proteolytic digestion products, and growth factors[5] (Fig 1).

ANGIOGENESIS

Tumour invasion is accompanied by angiogenesis, the growth of new host blood vessels into the tumor. This process is induced at a postcapilliry venule level by the release of tumoural and stromal angiogenesis factors. The rate of proliferation of vascular endothelial cells is 20-2000 times faster in host-induced tumour endothelium than in normal tissue endothelium[11]. Extravasated tumour cells proliferate as colonies but require a new vascular supply to grow larger than 0.5 mm in diameter. Angiogenesis is therefore necessary at the beginning and end of the metastatic cascade. Multiple host and tumour factors can alter the microenvironment required for survival and growth[12]. Tumour cells can synthesise and secrete their own functionally active growth factors (autocrine growth factors).

INTRAVASATION

Intravasation is the entry of tumour cells into the bloodstream. Newly formed tumour vessels are often defective, a property that may facilitate the entry of tumour cells into the circulation. At the invasion front, tumour cells also may invade pre-established host vessels[4]. Tumour cells are discharged into the vascular system in single - cell form and in clumps. From rapidly growing tumours, 1 cm in size, millions of tumour cells can be shed into the circulation every day. The blood compartment is a hostile environment for circulating tumour cells. Only a small percentage (<0.01 %) of circulating tumour cells initiate metastatic colonies[11].

ARREST

Circulating tumour cells use a variety of means to arrest in the vessels of the target organ, including mechanical wedging, entrapment with platelets and fibrin and attachment to target endothelium via specific tumour cell surface receptors[12].

EXTRAVASATION

The fate and time course of arrested tumour cells differ depending on where they are lodged; 90% of the tumour cells leave the circulation at the capillary or venule level. After adherence of the tumour cells, the endothelial cells retract, exposing the basement membrane. Degradation of the basementmembrane and extravasation are accomplished in 8-24hours. Altered host tissues can modify the arrest and extravasation of blood-borne malignant cells Metastases often form at sites of inquiry or inflammation, such as sites of trauma, surgical scars, percutaneous needle biopsies, subcutaneous injections of medications, and dental extractions[12].

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METASTATIC PATHWAYS

Once a metastasis has developed, it can itself progress, further amplifying the amount of tumor dissemination[4]. The paths of neoplastic spread in the body are tissue spaces, lymph vessels, blood vessels, coelomic cavities, cerebrospinal spaces and epithelial cavities.

TISSUE SPACES

Infiltration of tissue spaces represents the initial and fundamental pathway of the spread of most neoplasms. Many types of compact fibrous tissues in the body resist direct neoplastic invasion for long periods. These include the fibrous capsules of viscera (liver, spleen and kidneys), periosteum, pericranium, dura mater, ligaments, tendons, fasciae and cartilage. Cartilage contains antiinvasive factor, a low molecular-weight protein that inhibits proteolytic enzyme degradation and neovascularisation[14].

LYMPH VESSELS

Tumours (carcinomas) generally lack a lymphatic network. Therefore, communication of tumour cells with lymphatic channels occurs only at the tumour periphery and not within the tumour mass. In addition, tumour cells do not have to penetrate a basement membrane to enter the lymphatic system, as lymphatic vessels lack basement membranes. Most tumour cells reach the regional lymph nodes in the afferent lymph as emboli, in the form of either single cells or clumps. Within 10-60 mm after initial arrest in the sub capsular sinus of the lymph node, a significant fraction of the tumour cells detach and enter efferent lymphatic vessels[4]. As local lymph nodes are replaced by tumour, the afferent lymph will be directed into collateral vessels to fresh nodes. Along with increasing lymphatic obstruction, the lymph flow may be reversed, with retrograde spread of tumour to distant and sometimes anomalous locations. Sometimes anomalous locations Retrograde lymphatic embolism as seen in the carcinoma of the breast and in melanoma.

BLOOD VESSELS

Metastasis is more common in sarcomas because of their close proximity to blood vessels. Arteries are generally immune to tumour invasion. It has been shown that elastic fibres surrounding the vessels release antiproteolytic factors that inhibit proteases[15]. Venous invasion, exhibited in greater or lesser degree by virtually all malignant tumours, occurs mainly in the tumour vasculature at the level of the venous capsule of solid tumours. Most tumour emboli in the venous system are arrested in the first capillary bed encountered. Emboli liberated in systemic veins are arrested in the lungs, those liberated in the portal venous system are arrested in the liver and those liberated in the pulmonary veins are arrested in any peripheral tissue to which they are carried in the systemic arterial blood. Retrograde venous embolism can occur with occlusion of a main vein. Haemic dissemination from cancerous lymph nodes also occurs frequently. The tumour cells enter the bloodstream via three routes: direct invasion of venules or lymphaticovenous anastomoses within lymph nodes; direct invasion of the neighbouring veins; and through lymphatic tributaries of veins. Communications exist between lymphatic vessels and the portal veins, internal jugular vein, renal veins, inferior vena cava and azygous veins. Therefore, tumour spread via lymph vessels is important only as far as the regional lymph node from there, the venous system is the carrier[16].

COELOMIC CAVITIES

The coelomic cavities include the peritoneal, pleural and pericardial spaces. Transcoelomic dissemination of detached tumour cells is the most important mechanism of spread in the peritoneum. It occurs less frequently in the pleura and infrequently in the pericardium[15].

CEREBROSPINAL SPACES

Cerebrospinal fluid provides a metastatic pathway for cancer cells throughout the Central nervous system. Tumours may gain access to this space by invading the leptomeninges covering the brain and spinal cord or by invading the ependymal lining of the ventricles. Extracranial and extrathecal tumours can invade the

leptomeninges via the cranial dural venous sinuses and the spinevia perineural and perivascular lymphatic vessels traversing the intervertebral foramina[1].

EPITHELIAL CAVITIES AND SURFACES

Tumour extension within epithelial cavities and on epithelial surfaces can occur by direct extension, Implantation, or contact. Implantation metastases occur frequently on the epithelial surfaces. Implantation metastases within the epithelial cavities of the gastrointestinal and respiratory tracts are extremely unlikely because of the presence of digestive secretions, bacterial flora and ciliary action. Contact metastases on epithelial surfaces are rare. These include carcinomas metastasising from one lip to the other, from tongue to cheek, from eyelid to conjunctiva and from the skin of one thigh to the other[17].

THEORIES OF METASTASIS

CLONAL EVOLUTION THEORY

Nowell in 1976 proposed this theory. He stated that most neoplasms arise from a single cell of origin. Tumour progression results from acquired genetic variability within the original clone, allowing sequential selection of more aggressive sublines. Tumour cell populations are apparently more genetically unstable than normal cells, perhaps from activation of specific gene loci in the neoplasm, continued presence of carcinogen, or even nutritional deficiencies within the tumour. The acquired genetic instability and associated selection process, most readily recognised cytogenetically, results in advanced human malignancies being highly individual karyotypically and biologically.

This model is built on the theory of natural selection and posits of cancer cells ability to develop strategies to survive hostile environments (tissue barriers, immune response and programmed cell death) and use host resource (e.g. oxygen and nutrients) to grow and proliferate. Hence, each patient's cancer may require individual specific therapy, and even this may be thwarted by emergence of a genetically variant sub-line resistant to the treatment[18].

Vander Riet proposed the 'Molecular Progression Model' According to this model neoplasms arise clonally from transformed cells that have undergone specific genetic alterations in proto-oncogenes or tumour-suppressor genes. In head and neck carcinoma, first loss of chromosomal region 9p21 is the most common of all genetic changes and occurs early in the progression of these tumours. The main effect of this loss is the inactivation of the p16 gene, an inhibitor of cyclin-dependent kinase that is important in regulating the cell cycle.This early inactivation is consistent with the finding that keratinocytes in culture often lose p16 function and thus escape senescence. Approximately half of all head and neck cancers contain a mutation of the p53 gene located at 17p13.The loss of p53 function due to a mutation results in a progression from preinvasive to invasive lesions and increases the likelihood of further genetic progression. Amplification of the oncogene cyclin D1, which constitutively activates cell-cycle progression, is seen in about a third of all tumours and is usually associated with invasive disease. Tumour-suppressor genes have not been isolated or characterized for most of the regions that are commonly lost in tumours[19].

FIELD CANCERISATION

Patients with a head and neck squamous cell carcinoma often develop multiple (pre)malignant lesions. This finding led to the field cancerisation theory, which hypothesises that the entire epithelial surface of the upper aerodigestive tract has an increased risk for the development of (pre)malignant lesions because of multiple genetic abnormalities in the whole tissue region.

Slaughter in 1953 hypothesised that because of constant carcinogenic pressure the multiple genetic events occurred throughout the involved mucosa, allowing the development of multiple molecularly distinct lesion[20].

A COMPETING THEORY

This theory states that rather than molecularly distinct lesions that arise independently, a single group of molecularly similar transformed progenitor cells

migrates to distant sites, thus explaining the appearance of multiple primary lesions, second primary lesions and recurrent lesions[21].

CLONAL PROGRESSION MODEL

The evolution of metastatic competence is a generic predisposition that can be expressed at any time during the process of tumour development. This phenotype will predominate in the primary tumour through clonal expansion[21].

CLONAL DOMINANCE THEORY

A single clone initially present in the tumour cell inoculums, grows to dominate the primary tumour and this clone is metastatically competent [21].

METASTASIS SUPPRESSORS

Metastasis suppressor proteins regulate multiple steps in the metastatic cascade, including cancer cell invasion, survival in the vascular and lymphatic circulation and colonisation of distant organ sites.

NM23

NM23 is the first identified metastasis suppressor gene in this group. It is located on chromosome 17q21 and codes for an 18.5-kDa protein containing 166 amino acids, which functions as nucleoside diphosphate kinase and protein-histidine kinase. Clinically, NM23 has been shown to be down regulated in a variety of tumours including breast and prostate cancers. Ectopic expression of NM23 has also been shown to significantly reduce the in vitro and in vivo metastatic potential of highly metastatic carcinoma cell lines including breast, melanoma, colon, and oral squamous cells[22].Recently, Hartsough et al. reported that NM23 formed a complex with kinase suppressor of Ras1 and phosphorylated this protein at Ser-392 and Ser-434, which resulted in blockade of Ras/mitogen activated protein kinase pathway. Medroxyprogesterone acetate (MPA) and oestradiol were reported to suppress metastasis through up-regulation of the NM23 gene. Medroxyprogesterone is a progestin and commonly used as a component of hormonal contraceptives. Progesterone binds to the progesterone receptor, which is then transferred to the nucleus and acts as a transcription factor by binding to the progesterone response elements in the promoter region of the target genes. Progesterone receptor is known to directly regulate the expression of cyclin D1, beta-casein and p21WAF1 as well as MAPK[22,23].

KISS-1

KiSS-1 was originally identified as a metastasis suppressor gene using a combined strategy of **microcell-mediated chromosome transfer gene** and differential display. The introduction of an intact copy of whole human chromosome 6 into the C8161 human melanoma cell resulted in significant reduction of metastasis ability of this cell line without affecting tumorigenicity or local invasiveness in animals[24].

Metastin is a 54 amino acid peptide whose sequence is identical to a part of the KiSS-1 gene. Metastin was found to be able to suppress the degree of pulmonary metastasis, even when the peptide was administered to the mice that already had metastasis in the lung. Therefore, Metastin is considered to be a promising agent for the treatment of metastatic cancer patients[25].

MKK4

Yoshida et al. identified the MKK4/SEK1 (Mitogen-activated protein kinase kinase 4) gene in this 14p[38] chromosomal region as a candidate metastasis suppressor. Ectopic expression of MKK4 in highly metastatic prostate cancer cell line indeed significantly suppressed the macroscopic lung metastasis without affecting the primary tumour growth in animals[26]. It belongs to the MAPK family which plays central roles in cell proliferation, differentiation and apoptosis. It is known that MKK4 is activated in response to a variety of extracellular stimuli, including stress followed by activation of JNK(c-Jun N-terminal kinase) and/ or p38 MAPK pathways. It is plausible that, when a tumour cell reaches a distant organ site, the expression of MKK gene in cancer cell is suppressed in the stressful environment, and therefore, fails to establish colonisation[27].

E-CADHERIN

The E-Cadherin is a calcium-dependent adhesion molecule which constitutes the adherence junction in epithelial cells. Reduced level of E-cadherin is shown in a variety of human cancers at the advanced stages. It is believed that a low level of E-cadherin can give advantage to tumour cells on breaking the adhesion junction and detaching from adjacent cells so that these cells invade and metastasise to other distant organs[28]. Generally, E-cadherin plays an important role in epithelialmesenchymal transition (EMT) during which epithelial cells lose their cell cell junctions and acquire mesenchymal characteristics to endow the migratory ability to tumour cells. E- cadherin interacts with β-catenin to mediate actin binding. Therefore, loss of E-cadherin, in addition to reducing cell-cell adhesion, provides an oncogenic stimulus by freeing β-Catenin from the membrane, so that β-Catenin can travel to the nucleus to activate TCF-regulated genes such as c-Myc and Cyclin D1. Furthermore, E-cadherin has been recently found to be down-regulated by transcription factors Snail and Slug which are involved in the process of EMT, cell differentiation and apoptosis. Therefore, restoring the function of E-cadherin is considered to be a potential therapeutic option for metastatic disease[29].

NDRG1

N-myc downstream regulated gene 1 (NDRG1) was originally identified by differential displays as being significantly up-regulated by induction of *in vitro* differentiation of colon carcinoma cells. The protein encoded by the NDRG1 gene has a molecular weight of 43kDa and possesses three unique 10-amino acids tandem repeats at the C-terminal, among which seven or more phosphorylation sites were predicted and later
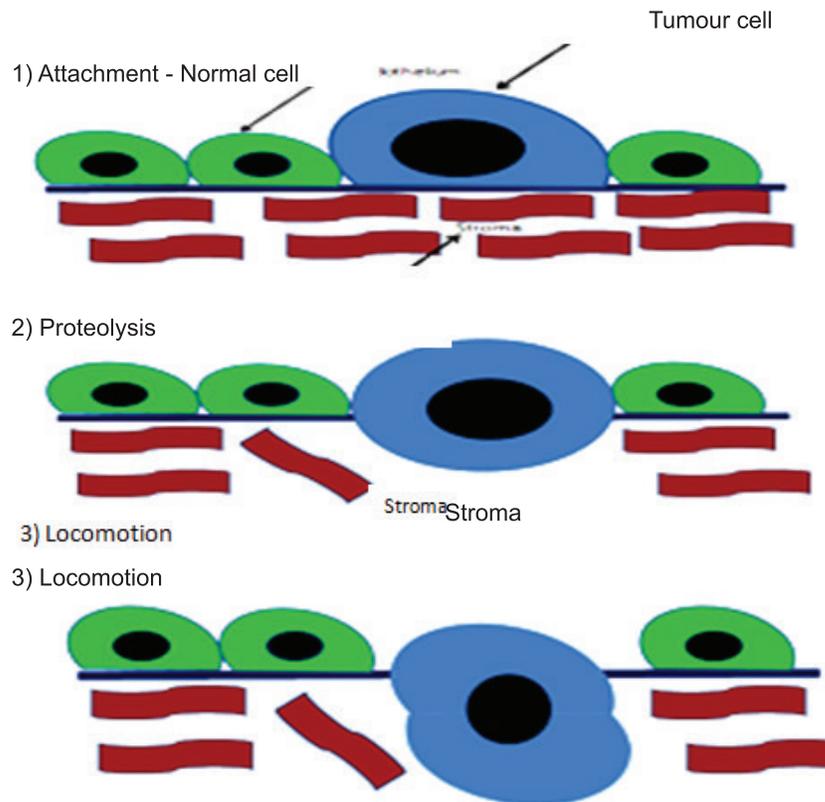
Figure 1: Steps in Metastasis

they were shown to be targets of protein kinase A in vitro[30]. Ectopic expression of the NDRG1 gene in a highly metastatic prostate cancer cell line significantly reduced the incidence of lung metastases, suggesting that NDRG1 was able to block the metastatic process without affecting the primary tumour growth. Similar metastasis suppressor effect of NDRG1 was also observed in the colon carcinoma cells by Guan et al. In addition, NDRG1 also significantly suppressed the invasive potential of prostate and breast cancer cells as tested by *in vitro* invasion chamber assay. Therefore, evidence from both clinical data and the results of *in vitro* as well as animal experiments overwhelmingly support the notion that NDRG1 is a metastasis suppressor gene and that the down-regulation of the gene results in acceleration of tumour metastasis. How NDRG1 suppresses the tumour metastasis is an intriguing question which is under active investigation[31]. Recently, iron chelators, desferrioxamine (DFO) and 311 were shown to be able to up-regulate the NDRG1 expression in human breast cancer cell line MCF7. In the previous years, dietary iron restriction has been shown to markedly decrease tumour growth in rodents, and iron chelators such as Triapine and desferrioxamine (DFO) were reported to be potentially useful for cancer therapy[32]. Dp44mT (di-2-pyridylketone-4, 4,-dimethyl-3-thiosemicarbazone) significantly augmented the expression of the NDRG1 gene in the tumour compared with that of the control group, suggesting a promising utility of this compound as an anti-cancer as well as anti-metastatic drug[33]. The goals of anti-metastatic therapy are three folds. First, we need to develop a specific drug that blocks secondary metastasis to treat patients who have already acquired metastatic disease but are still at an early stage. Second, a drug should also be developed to treat patients who underwent surgical resection of their primary tumours in order to prevent a possible recurrent disease. However, the ultimate goal is to develop a non-toxic agent that can be taken as diet for prevention of metastasis. In the past decade, the major effort of anti-cancer research has been focused on the development of drugs that can block the proliferation of tumour cells. They take advantage of the fact that tumour cells are more actively proliferating than other normal cells and therefore, can selectively kill the cancer cells. However, this selectivity has narrow margins and these agents inevitably cause severe side effects even when they are used in combination with lower the toxicity[34].

CONCLUSION

The molecular principles of cancer invasion and metastasis are highly complex and at the same time essential for a profound understanding of carcinogenesis. Moreover, the topic is immensely relevant since 90% of all cancer deaths can be attributed to metastases and not to the primary tumours. The currently applied therapy approaches mainly focus on rapidly proliferating cells. New insights into the molecular processes of invasion and metastasis, as well as the concept of cancer stem cells, may pave the way for new, highly specific and successful drugs. The precondition for this purpose is further research in the field for a better understanding of these processes.

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