Unveiling the Unpredicted and Uncontrolled Metastasis of Cancer Cells: A Minireview

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Abstract: Cancer as an historical disease is known to occur since several hundred years and still prevails till date as one of the most feared diseases. Its occurrence and/or progression are considered as the outcome of the series of accumulated ocogenic changes in the cell those transform it from benign to invasive or metastatic. It has been observed that most of the metastatic cancers are not curable and the available drugs focus on steadying the tumour growth to prevent further metastasis. Metastasis remains the cause of around 90% of the cancer deaths. Fundamental understanding of metastasis and recent advancements in metastasis driven cancer research may help to strengthen and bring in practice the new and advanced approaches for cancer treatment. In view of this, we review in detail the molecular mechanism of metastasis, problems in diagnosing and treating metastasis, and recent developments in cancer biology. Future efforts in order to unveil the unpredicted and uncontrolled metastasis of cancer cells have also been summarised.

Keywords: Cancer, Metastasis, Cancer stem cells, Cancer therapeutics, Cancer diagnosis.

1. INTRODUCTION

Cancers are remarkably diverse group of diseases that emerge through the augmentation of mutations in the functional biomolecules that control and limit the division, proliferation and migration of the cells. Cancer cells are competent of growing at will and may originate with different mechanisms to invade various organs. One of the dark sides of the cancer cells is their potential to be malignant, and the malignancy remains one of the root causes of many of the cancer. Most of the failures in cancer therapeutic regimes are typically attributed to two important aspects of malignant cell behaviour: a) ability of cancerous cells to spread (metastasis) by overcoming the natural defence systems (both innate and adaptive) and b) development of resistant against the targeted therapy. Some of the fundamental unanswered questions in tumor metastasis includes: a) How the cancerous cells overcome the natural defence system? and b) how the cancerous cells acquired drug resistance?

Metastasis is a process when a malignant cell withdraws its natural cellular adhesion mechanisms and split from its primary tumour [1]. Further, it penetrates the lymphatic system, and cultivate in the new location. Thus, lymph node infiltration appears to be a common feature of malignant cells, which in turn enhances the likelihood of metastasis. These cells demonstrate uncontrolled growth, local invasion and diverse colony formations (secondaries) in distant tissues. The fate of the metastatic cell depends on its angiogenic capabilities and its ability to bypass immune responses.

Cancer remains the leading cause of death throughout the world even after the considerable progress in understanding the cancer dynamics. For example, in the year 2016, approximately 1,685,210 new cancer subjects and 595,690 cancer mortalities are predicted in United States [2]. At present, most of the deaths are confined to the deadly attributes of metastatic cancers. It is estimated that 20-50% of the patients diagnosed with primary tumours develop metastasis [3]. It is important to note that most of the current cancer therapies have limited long term success rates and have severe side effects which ultimately lead to the deterioration of the overall health of the patient. Controlling the metastasizing tumours remains the most difficult task for both the researchers and the clinicians. Thus, discerning the molecular basis of metastasis is the crucial step towards defeating metastasis. Considering the facts in relation of metastasis and cancer, this article comprehencively reviews the importance of metastasis as one of the root causes of cancer.

2. CHARACTERISTICS OF CANCER METASTASIS

Following are the characteristics of metastasis cell and generalised molecular events known to trigger metastasis.

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a) To be metastatic, a cell must withdraw its natural adhesion characteristic, split from the primary tumour, and penetrates in the lymphatic/circulatory system to form the secondary tumour in the new site. In doing so, metastatic cells evades the natural immune responses by their inbuilt mechanisms.

b) A cell adhered to the other cell or to the extracellular matrix with the help of adhesion molecules (e.g. cadherins) [4]. This process involves the interactions of many cell surface proteins. In most of the metastatic cells, the adhesion molecules (e.g. E-cadherin) are found to be either missing or compromised. This facilitates the detachment of cancer cells from the primary tumour cells. Clinical data suggests that the desirable alterations in the E-cadherin activities in cancer cells can prevent metastasis. Cancer cells exhibit hyaluronic acid (HA) dependent cellular detachment from the primary tumours [5]. HA dependent detachment mechanism has been observed in most of the oral cancer patients due to the high amount of HA in the saliva. Binding of HA to the cell surface helps the cancer cells to move freely and escape the adhesion mechanism for cell growth. Cancer cells also demonstrate E-cyclin-dependent kinase 2 (E-CDK2) independent mechanisms for cell growth [6]. E-CDK2 is a regulatory protein. In the normal physiological conditions, if the adherent cell types do not exhibit cell to cell/cell to extracellular matrix interactions then the synthesis of E-CDK2 is shut down by the inhibitory substances in the nuclei leading to the suppression of the cellular growth and division. However, it is interesting to know that cancer cells can divide even without being adhered and E-CDK2 protein in the cancer cells remains active. The reason how cancer cells express E-CDK2 protein even after avoiding the adhesion mechanism is still unclear.

c) In order to enter the blood vessels, cancer cells secrets enzymes [metalloproteinases (MMPs)] that breaks the basement (epithelial) membrane and other extracellular matrices [7].

d) Malignant tumour cells, because of their thirst for nutrition, are also capable of developing new blood vessels (angiogenesis). This feature allows an opportunity to cancer cells to migrate at their will.

e) Once the cancer cells enter the bloodstream, body's immune cells kill most of the cancer cells. Host cells use all of its weapons (innate and adaptive immune systems) such as macrophages, T cells, antibodies, platelets, blood turbulence, natural killer cells etc., to kill the circulating cancer cells. Figure **1** entails the sequential events (cancer cell, benign, metastasis and infiltration of cancer cells in blood vessels/lymph nodes) occurring during the process of metastasis. Angiogenic factors promote the development of new blood/lymphatic vessels through angiogenesis/ lymph angiogenesis.

f) The frequency of survival of cancer cells is less than one out of the thousand cells. Blood flow is the important criteria to judge the migration of the cancer cells. Most frequently, cancer cells are trapped in the initial capillaries from the point of their entry. Often, the cells are trapped in lung capillaries as the deoxygenated blood from all the body organelles returns to the lungs. On the other hand, cancer cells residing in the intestines migrate to the liver due to the directional blood flow from intestine to the liver. Thus, liver and lungs are the two most preferred organelles for metastasis in the human cancers. Once in the new location, cancer cells again invade the blood vessels and continue its everlasting journey.

g) It is important to note that, only specific cells in primary tumours are capable of initiating metastasis. Most of the other circulating cancer cells die as they are not equipped for triggering the metastatic events. Metastatic cells are blessed with some of the very robust characteristics such as their ability to bypass natural defence mechanism, deformability, proliferation without being anchored, secretion of MMPs, uncontrolled mitosis, non-differentiation etc.

h) Sometimes, even after reaching the new destination, metastatic cells remain unable to recognize and respond to the signal transductions of a destined body organelle. In this condition, metastatic cells eventually die. It has been reported that less than 0.1% malignant cells were found to be metastatic when a mice was injected with B16 melanoma [8]. Fractions of survival rates of cancerous cells give us an idea about the metastatic potency of a few cancer

cells out of the whole primary tumour cell population.

i) It has also been demonstrated that certain tumours cells, even after reaching to the various parts of the body, metastasize in a particular body organelle. These cells start to adhere and reproduce after reaching the designated organ. Ivan Stamenkovic (Harvard Medical School, USA) reported that the metastatic spread of certain tumour cells can be diverted by supplementing the body organelle (to be metastasized) with certain adhesion molecules [9]. Ivan Stamenkovic was able to divert metastasis to liver when the necessary adhesion molecules were inserted into a mouse's liver. There are many other reports which demonstrate that the site of metastasis is highly dependent on compatible signal transductions between the tumour and host cells.

j) Most of the malignant tumours metastasized via lymphatic system (Figure **1**). This justifies the significant role of thoracic lymph nodes in pre-neoplastic developments. Tumour staging helps clinicians to determine the spread of metastasis. The criteria may differ in different cancers. For example, in breast cancer staging (I to IV) depends upon various criteria's including the size of tumour, invasiveness, metastatic spread (including lymph node metastasis) in and out of the breast tissues etc [10]. Metastatic cells can also be categorized based on TNM (tumour node metastasis) scores.

k) Bone metastasis is largely dependent on the equilibrium between the osteolytic and osteoblastic factors [11]. Deregulation in various osteoblastic factors (adrenomedullin, PDGF, VEGF, BMPs, IGFs, TGF-beta, FGFs and ET-1) leads to the formation of osteosarcomas.

l) In liver, metastasis occurs mainly due to the toxic metabolites generated during the process of detoxification (especially in phase I) and the migration of metastatic cells from the intestine.

There are several other important factors which decide the fate of cell migration and metastasis. Chemokines are known inflammatory cytokines which can stimulate the migration of different subsets tumour cells. For example, macrophage inflammatory protein-2 (MIP-2) and stromal cell-derived factor-1 (SDF-1) are the members of CXC chemokine superfamily and have an important role in the directional metastasis in colorectal cancer [12-13]. Metastatic cells often recruit healthy cells to help them travel along the different body organelles. Recently, researchers at university college London (UCL, UK) reported that neural crest cells (alike cancer cells) dramatically transforms themselves to track placode cells (alike healthy cells) [14]. Tracking efficiency of neural crest cells depends upon the small chemical molecules secreted by the placode cells. These findings open a new paradigm wherein future anticancer therapies can be designed by modulating the interactions between the cancer and the healthy cells to suppress metastasis and secondary tumour formations.

3. MOLECULAR MECHANISM OF METASTASIS

Since there are no definite causes of developing cancer, it's hard to find out a specific reason why a particular individual develops cancer while other individuals do not. Even if the causes of developing cancers are not specific, the risk of developing cancer in individuals can be predicted depending upon their exposure to the known cancer causing agents. Approximately 90% of the cancers may be developed due to improper nutrition, excessive alcohol consumptions, UV radiations, tobacco products and exposure to carcinogens [15]. Cancer may be developed over a period of time (e.g. years) due to the complicated undesirable alterations at genetic and epigenetic levels. After the onset, malignant cells can multiply rapidly leading to the development of metastatic tumours. Metastatic cells are mostly erratic. Characteristics of metastatic cells are greatly influenced by host factors and often differ from the primary malignant cells. Several reports suggest that neoplastic lymph nodes, especially mammary lymph nodes, are the consequence of metastasis and not the primary site of cancer. Common complexities and side effects of metastatic cancer involves the sleep disorders, brittle bones, weight loss, problems initiating urination, physical and psychological disorders, fractures and fatigue.

Different models which propose different mechanisms of tumour dissemination and colonization are briefed below.

3.1. Progression Model

Progression model (Figure **2a**), originally proposed by Nowell [16], suggest that only few cells of

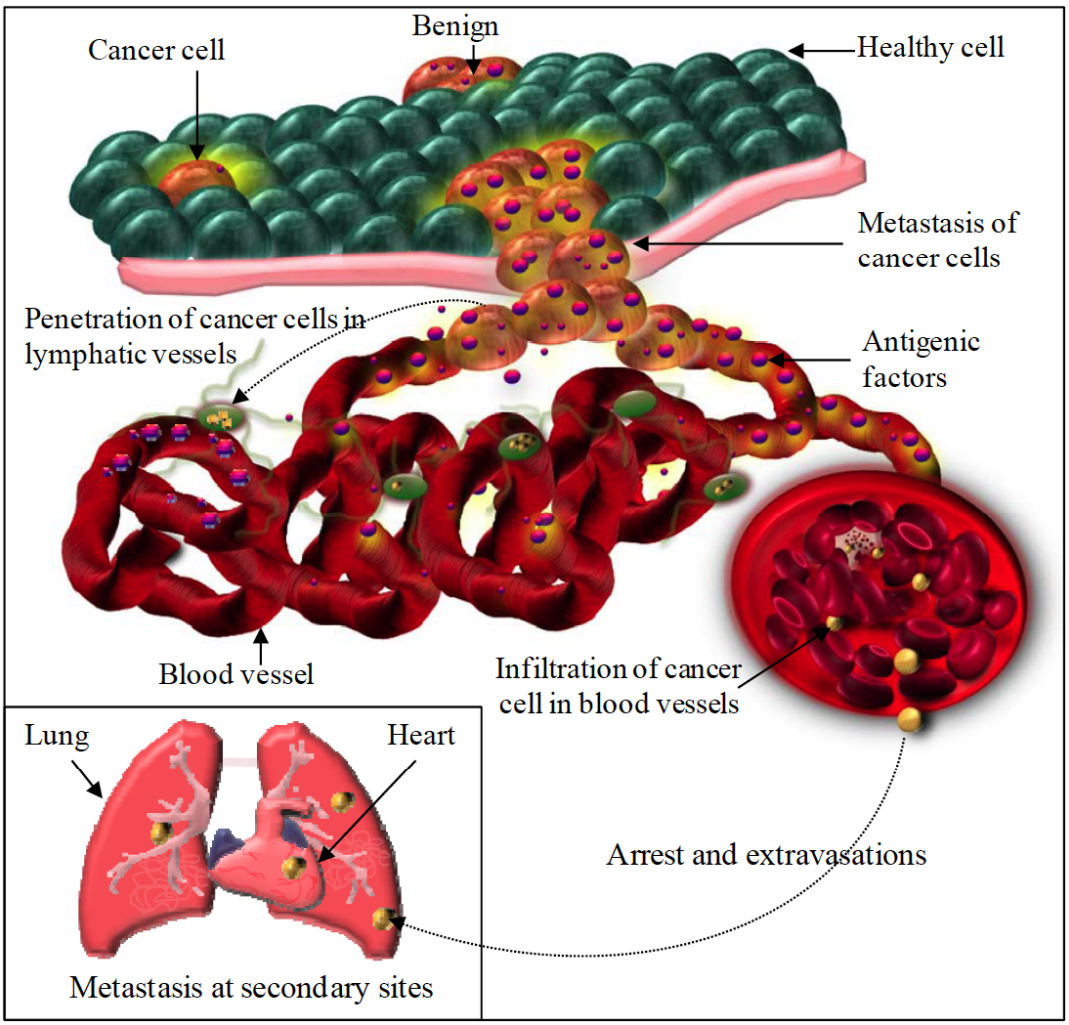

Figure 1: Sequential events in cell metastasis.

subpopulations of primary tumour or disseminated cells acquire full metastatic potential after sequential somatic mutational events. Studies on clonal subpopulations, metastatic suppressors [17]. support the progression model of metastasis. However, this model has failed to explain cancer metastasis in patients with unknown primary which constitutes almost 5% of total solid tumours. It is observed that subclonal populations with high metastatic potential lose their potential after several generations of cell division. These finding raise a question on the progression hypothesis that metastatic potentials are acquired due to mutational events in somatic cells as most of the somatic mutations are stably inherited in further generations.

3.2. Transient Compartment Model

Dynamic heterogeneity model [18], later extended by Weiss [19], explains the lack of metastatic potential of secondary tumours compared to primary tumours due to positioned and/or random epigenetic events (Figure **2b**). In 1990, it was demonstrated that the predictions of somatic genetic mutations made in the progression theory were deviating from the fact that secondary tumours gradually lose their metastatic potential [19]. Thus, Weiss suggested that all viable tumour cells acquire metastatic potential but a few cells retain the metastatic potential and colonize in the secondary sites due to positioned and/or random epigenetic events. Studies on methylation inhibitors [20, 21, 22, 23, and 24] support this model. However, studies on global demethylation [25] suggested that the alterations in the metastatic potential could be due to chromosomal aberrations/mutations and not due to epigenetic alterations. Secondly, if entire cells of the tumours could acquire metastatic potential, as suggested in transient compartment model, then significant fractions of secondary tumours would have appeared, even after the transient epigenetic alterations, due to heterogeneous nature of neoplastic cells of primary tumour.

3.3. Genetic Predisposition Model

The genetic predisposition model (Figure **2c**) considers that the genomic diversity as a result of inherited polymorphism in addition to metastasis-promoting somatic events that plays crucial role in tumour colonization. These findings were established with the identification of the gene *Sipa*1 which is related to polymorphic metastasis efficiency [26]. RNA interference studies have revealed that the minor reduction in *Sipa*1 levels had significantly lowered the lung colonising ability of mammary tumour cell lines. These and other studies demonstrate that SIPA1 with additional polymorphism plays a vital role in development and establishment of metastasis susceptibility [26].

3.4. Seed and Soil Model

Paget proposed the "Seed and Soil" model which reveals that the metastasis site has the "factors" which probably contribute to cancer cell proliferation (Figure **2d**). This theory was focused on process of cell immigration. This model is based upon three principles. One, a cancerous tissue has heterogeneous subpopulation which varies with the angiogenic, invasive and metastasis abilities. Second, intact metastasis is limited to the cells after surviving the long journey to the distant organ. Finally, the potential of the cells to metastasise depends upon their ability to utilize the factor/soil in their niche.

Recently, Bragado *et al*. (2013) validated "Seed and Soil" model of metastasis (Figure **2d**). Authors have observed that bone marrow can be the "restrictive soil" where the non-proliferative disseminated tumour cells (DTCs) were found to persist in comparison to the lung which showed growing metastasis [27]. Specific transforming growth factor-β2 (TGF- β 2) activates the MAPK, and through cascades of signalling events cyclin-dependent kinase 4 (CDK4) is down regulated in turn restricting the metastasis potential of the cells. On the other hand; in lung, low TGF- β 2 causes systemic inhibition of TGF- β -R1or p38α/ β activities that leads to activate dormant DTCs resulting in multiorgan metastasis. Therefore, these findings validate the "seed and soil" model and known to regulate metastasis in bone marrow (restrictive soil) and lung (permissive soil) (Figure **2d**).

3.5. Fusion Model

Fusion model of metastasis (Figure **2e**) suggest that epithelial cells turn into metastatic tumour cells and develop lymphoid characteristics mainly through nuclear transduction which is either by fusion with cells of myeloid origin or by uptake of circulating tumour DNA [28,29]. Further, it has been proposed by Aichel and Mekler (2000) that the development of more metastatic phenotype is preceded by the accumulation of leukocytic characteristics by epithelial cells [30]. In turn, it helps them to detach, spread and colonize distant sites [31,32 and 33].Various studies demonstrates the *in vivo* and *in vitro* fusion of tumour and host cells [34, 35, 36, 37 and 38]. However, metastatic progression occurring due to cell fusion in cancer cases is still a debatable knowledge. Some studies have revealed that the subclones generated by fusion of cells vary in their metastatic potential. Therefore, the substantiation over changes in the metastatic ability of the hybrids acquired due to fusion remains in question, especially when the experiments with the cell lines originally derived from metastatic tumours were carried out. Therefore, a firm and conclusive proof regarding the role of cellular fusion in development of metastatic capacity in hybrids is still expected.

3.6. Gene Transfer Model

Gene transfer model for metastasis acquisition is based upon horizontal gene transfer (Figure **2e**). With advancements in microscopy and histological technology, the hypothesis that the malignant cells escape from the primary tumour was confirmed. Once the presence of circulating tumour DNA was detected in animal tumour models, the idea of inducing metastatic capacity by horizontal gene transfer was re-borne. In addition, this concept was seconded with genometastasis hypothesis which explains that in some cases the horizontal gene transfer was observed in experimental system [39]. This model suggests that the metastases arise from *in vivo* uptake of circulating DNA by the stem cells and not from the circulating cells. No explanation to organ specific tropism of metastasis is the shortcoming of this model. In addition, uptake of sufficient quantity of DNA is required for the cell to exhibit metastatic properties at the secondary sites.

3.7. Early Oncogenesis Model

Early oncogenesis model (Figure **2g**) is based upon quantification of gene expression pattern by microarrays and identification of gene signature profiles that distinguish metastatic and non-metastatic tumours [40, 41]. Microarray gene expression data suggests that the major population of the cells of the primary

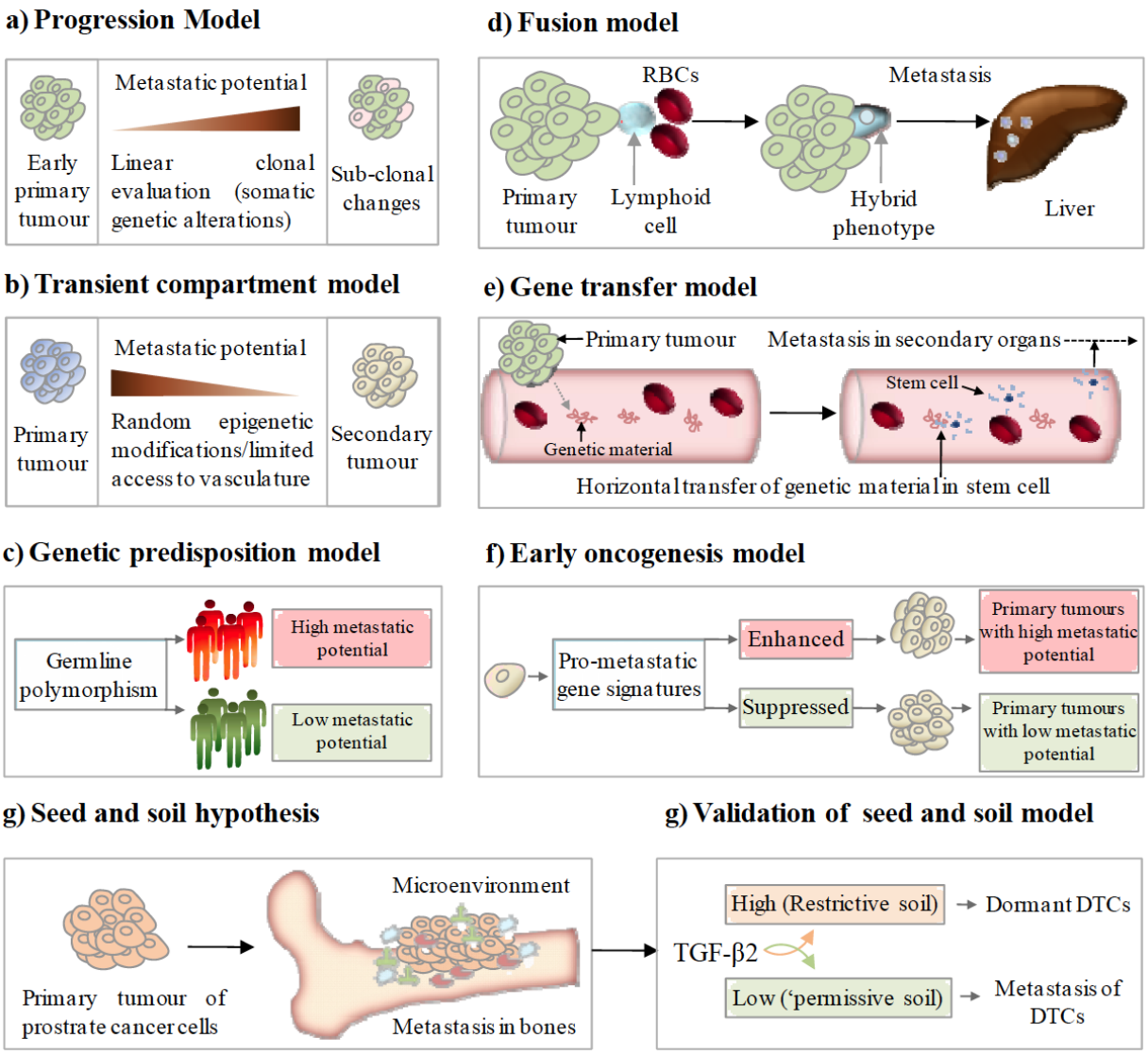

Figure 2: Different models of metastasis.

tumour must possess an intrinsic metastatic capacity. Like earlier models, the shortcomings in this model have also been noted. It states that if metastatic characteristic is detected earlier in epithelial cells, then the efficiency of colonization of distant organs would be much higher than observed in clinical practice, which is not observed. Additionally, microarray results do not rule out the possibility that the rare cellular subpopulations exist within primary tumours, and it only considers gene expressions induced by somatic oncogenic events.

4. PROBLEMS IN DIAGNOSING AND TREATING METASTASIS

Majority of the metastatic cancers are not curable and thus clinicians mostly focus on steadying the tumour growth to prevent further metastasis. But, it is difficult to envisage and the response of the patient along with its durability. Many of the existing tolls including radiation therapy, surgery, chemotherapy etc., have been moderately effective in treating metastatic tumours [42]. Controlling the brain metastasis have been especially challenging due to the restricted permeability of blood brain barrier, which is designated as the preferred site of tumour cells. To circumvent this issue, many chemotherapeutic drugs were developed that can pass through the blood brain barrier (e.g. doxorubicin). To understand the mechanical insights of the metastasis, number of *in vivo* models (e.g. mouse) were used to induce metastasis in different parts of the body including lungs, lymph nodes etc. Interestingly, a few mouse models showed metastasis in brain, liver and bones which are the common sites of metastasis in human. This could be attributed to the dissimilarities in the

lymphatic systems and the hormonal dependencies of tumour cells in human and *in vivo* models [43]. These dissimilarities have also been observed within the *in vivo* models (e.g., mouse and rat etc). Surprisingly, no significant association between the number and size of lymph nodes could be established in the animal models (dog and cat) closely related to mammals. Further, variability's were found in the hemal/ hemolymph nodes and hemolymph glands which were specifically found in some mammals but not in humans. To overcome this problem, direct injection techniques were developed to implant cancer cells in the designated organelles (e.g. Rag-2 knockout immune-deficient mice for retinoblastoma) [44].

Today, cutting-edge technologies demand an amalgamation of multi-dimensional (3D and above) and combinatorial modalities for cancer staging. The state of art of cancer diagnostic is based on the simultaneous analysis of multidimensional imaging, combined with molecular screening. This approach ensures the possibility to correlate the image with gene expression or metabolite changes. The current diagnostic modalities including CT, PET, single photon emission computed tomography (SPECT) which have their own inherent lacunas. Whole body imaging appears to be key approach in elucidating the metastatic spread including the primary lesions. At present, PET allows the whole body imaging and facilitates the monitoring of metastatic spread in 3D format. However, it cannot be used in differentiating cancer subtypes and tumour staging due to its inability to use multiple tracer molecules (e.g. fluorodeoxyglucose etc.) [45]. On the other hand, SPECT/ SPECT-CT offers the multi-tracer analysis but with more physical limitations. With the recent advances, magnetic resonance imaging (MRI) is becoming an alternative option for whole body imaging, complementary to PET and CT [46].

Recently, circulating tumour cells (CTCs) in the peripheral blood and dormant disseminated tumour cells (DTCs) in the bone marrow have gain considerable importance due to their candidature as potential prognostic cancer markers [47]. The biggest hurdle in dealing with the CTCs/ DTCs is their availability in the specimen. CTCs are present at astonishingly low frequencies in blood (≥5 CTC/7.5 ml blood in breast cancer patients) [48]. Thus, different methods have been designed to enhance the detection sensitivity using immune-magnetic separations, density gradient separations, filtrations etc. Immuno-magnetic separation techniques in combination with the epithelial markers have shown promising results in the detection of CTCs in metastatic breast cancer patients. The clinical relevance and prognostic significance of CTCs over the traditional prognostic markers has been rapidly evolving. The presence of CK19 mRNA-positive CTCs implies poor prognostic results especially in ER negative, HER2 positive and triple negative subtypes of breast cancer [49]. Thus, it can be assumed that the prognostic attributes of the CTCs are independent of the genetic features of the primary, secondary or recurrent tumours. The presence of HER2 positive CTCs in the HER2 negative primary tumours demonstrates the heterogeneity in breast tumours. This fact raises the questions regarding current therapeutic modalities and the involvement of cancer stem cells in breast cancer development.

Sometimes, small benign/primary tumours could not be efficiently detected due to the lack of efficient modalities to monitor thoracic lymph node (tLN) lesions [50]. This could explicate the growing incidences of metastasizing tumours in neck, head, genitourinary, breast and gastro-intestinal melanomas. High prevalence of human mammary tumours could be attributed to the possible crosstalks between tLN and mammary lymph node (MLN). Still the lymph node anatomy in human and in other mammals is not properly understood. The interplay between the parathymic lymph nodes (PTNs) and MLN could enlighten our knowledge about the directional metastasis from the peritoneal cavity to MLN and PTNs.

5. RECENT DEVELOPMENTS

Recent studies reveal the importance of cancer stem cells in ameliorating the diagnosis and treatment of cancer. Cancer stem cells are now recognized to play a very important role in triggering the primary tumour formations and metastasis. Traditional contingent models of oncogenesis were based on the ability of any cells in the body organs to initiate cancer (malignant tumours) after certain undesirable alterations in their genetic makeup. On the other hand, recent hypothesis suggest the origin of malignant tumours through co-ordinated events of cancer stem cells/cancer progenitor cells. Studies on *in vitro* and mouse xenograft models revealed that a single subtype of cancer may have different origins. This makes the clinical predictions more complicated. For example, in human breast cancer, ER positive cells generated from ER negative and aldehyde dehydrogenase 1 (ALDH1) positive stem cells have the unsatisfactory clinical

outcome compared to other ER positive breast cancers [51]. Thus, it is highly important to accomplish molecular characterization of different subtypes of cancers before deciding the therapeutic regime.

Some specialized cells in our body can also adopt the characteristics similar to the cancer stem cells and can participate in the tumour progression and metastasis. For example, mammary epithelial cells can mimic the self-rejuvenating properties of stem cells through epithelial mesenchymal transformations (EMTs) [52]. These cells are capable of CD44/CD24 alterations and mammosphere, myoepithelial, luminal, and bi-potential cell formations. It is interesting to note that both healthy and cancerous stem cell/like cells expressed EMT specific markers. These studies give a new dimension to our understanding of cancer development and metastasis.

Patients with advance non-resectable liver metastasis are the common victims of the relapse due to surgical incisions. In this regards, modern technologies like hepatic artery infusion (HAI) chemotherapy, radio-frequency ablation and cryoablation have significantly helped to improve the clinical outcomes with low side effects.

With the continuing efforts in understanding the lymph node micro-metastasis, molecular diagnostic platforms for sentinel lymph node (SLN) analysis have been developed in the recent years. These include the Gene Search BLN assay (Veridex, LLC, and Warren, NJ) and RD-100i analyser (Sysmex, Japan) [53-54]. Gene Search BLN assay employs reverse transcription-polymerase chain reaction (RT-PCR) to determine CK19 and mammaglobin transcripts in the specimen. CK19 and mammaglobin have been identified as the biomarkers for detecting lymph node metastasis. RD-100i analyser involves direct one step PCR based amplification of CK19 to predict the lymph node metastasis. Combining the traditional histopathological methods with the molecular diagnostic platforms could enable the extensive examination of lymph node micro-metastasis which alternatively would have remained hidden in the paraffin sections. Having said that, the current molecular diagnostic platforms for lymph node micro-metastasis needs further improvements. Current limitations to this approach are the appearance of false positive due to specimen contamination during surgical procedures and the inconsistency of targeted markers due to heterogeneity in lymph node tumours.

Recent studies have also revealed the importance of exosome signatures in determining the fate of forthcoming metastasis. Exosomes signatures differ with the cancer types and they can be employed as the potential prognostic markers. Available reports suggest 2-20 fold up-regulations in the protein content of exosomes in metastatic melanoma cells compared to the control [55]. Thus, exosomes profiling could be a new way of predicting the fate of metastasis in cancer patients.

Researchers have found out that erbB2 (HER2) up-regulation is associated with all the crucial defects occurred during the expression of metastasis initiation, progression and virulence genes. Furthermore, several erbB2 downstream signals [VEGF, mammalian target of rapamycin (mTOR), protein Kinase B (PKB or Akt), signal transducer and activator of transcription 3 (STAT3), proto-oncogene tyrosine-protein kinase (Src), etc.] were thoroughly scrutinized, which could be designated as potential therapeutic targets against breast cancer metastasis [56-58].

Recent advances in immunotherapy have helped the researchers to either stimulate or suppress the activities of specific molecules of immune system to stop cancer progression. For example, Food and Drug Administration (FDA) approved of immune checkpoint inhibitors (ipilimumab, Yervoy®, etc) have shown promising results against advanced melanoma.

6. FUTURE PERSPECTIVES

Continuous efforts need to be made in understanding the mechanistic insights of abovementioned recent developments in unveiling the unpredicted and uncontrolled metastasis of cancer cells. Pragmatic developments in this regard can be made by refining the existing therapeutic modalities by optimization of drug repositioning process and tailoring of therapeutic protocols. In addition, studies can be carried out for discovering novel modalities for early detection of micro-metastases, especially in PLNs, MLNs, tLN and thymic lymph nodes (TLNs). The correlation between internal MLNs and TLNs, with special reference to PTNs, has to be extensively studied in human and other *in vivo* cell models. Combinatorial cancer therapeutics has to be applied to diagnose and treat micro-metastatic tumours through micro-surgery, HAI, radio-frequency ablation, cryoablation in combination with chemo and radiation therapies. In addition, novel methods should be

designed for detecting micro-metastasis in lymph nodes.

Molecular pathogenesis of lung cancer is still not properly understood. Lung cancer patients often experience bone metastasis at an advance stage of cancer. In this regards, animal models [e.g., natural killer (NK) cell depleted severe combined immune deficiency (SCID) models] mimicking both the lung cancer and bone metastasis could be useful to elucidate the molecular mechanism and biomarkers associated with the pathogenesis. Recent literature reports several anti-osteoclast agents (bisphosphonates, reveromycin A etc.) against bone metastasis. For improving the clinical outcomes, combinatorial approach (multi-modality therapy) may be needed to stabilize/cure lung cancer bone metastasis.

Another critical area that needs to be thoroughly investigated is the alterations/mutations in the p53, p63 and TAp73 family of tumour suppressor genes/transcriptional targets and their other regulatory networks [including the members of RNA-induced silencing complex (RISC)]. p53, p63 and TAp73 are regarded as the guardians of the cell as they are involved in regulating most of the crucial processes in cell including cell cycle, proliferation, invasion, metastasis etc [59]. However, in most of the cancers, the efficiency of these guardians in regulating these crucial cellular processes is largely hampered by the alterations/mutations within the tumour suppressors or their transcriptional targets, including oncogenic and tumour suppressor microRNAs. For example, p53 is known to be mutated in 50% of the human cancers [60].

With the developments in the molecular techniques, researchers now started to understand these critical alterations in tumour suppressor genes and their transcriptional targets (both genetic and epigenetic). Some of the representative examples of tumour suppressor genes, their transcriptional targets, alterations in transcriptional targets and cancer types are given in Table **1**. Most of the conventional anticancer strategies were mainly focused on targeting the undesirable modifications in tumour suppressor genes and their transcriptional target, and not on their other regulatory networks. Interestingly, it has been found that the guardians of the genome (p53, p73, and p63) do not have the sole powers to control the undesirable cellular processes such as cancer development, progression, metastasis etc.

Their efficiencies are highly dependent on the tumour suppressor microRNAs (miR-26, 29, 34, 15/16a, 30, 145, 146a and let-7), tumour suppressor genes [Phosphatase and tensin homolog (PETN), retinoblastoma protein (RBs), multiple tumour suppressor-1 (MTS-1)] and metastasis repressors [Raf kinase inhibitory protein-1 (Raf-1), cyclin G2 (CycG2), Basic-helix-loop-helix (bHLH) transcription factor (DEC2)] [61-62]. Most of the times, combined regulatory cascades (p53/ TA-p73/p63 activated and c-

Table 1: Alterations in Transcriptional Targets of Tumour Suppressor Genes

Tumour suppressor genes	Transcriptional targets	Type of alterations in transcriptional targets	Type of cancer
p73/FoxO3a	tuberous sclerosis 1(TSC1)	Mutation	Head and neck cancers [66]; Bladder cancer [67], etc.
p63	acute myeloid leukemia-2 [(AML)-2 or Runx-3]	Promoter methylations	Esophageal squamous cell carcinoma [68]; Gastric cancer [69], etc.
p53/p63/p73	miR-29, miR-34, miR-101, miR-143, miR-145 and miR-200	Down-regulation	Bladder cancer [70, 71], etc.
p73/p63	fasciculation and elongation protein zeta 1 (LZTS-1/FEZ1)	Down-regulation	Ovarian cancer [72]; Lung cancer [73], etc.
p53 (possibly, p73/p63)	PTEN	Down-regulation/mutations	Prostate cancer [74]; Breast cancer [75]; Glioblastomas [76]; Endometrial neoplasms [77]; Haematological malignancies [78], etc.
E2F-1/p53/p73/p63	miR-let-7	Down-regulation	Breast cancer [79]; Colon cancer; Hepatic cancer [80]; Lymphomas; Uterine leiomyoma [81]; Bladder cancer, etc.
p53	cyclin-dependent kinase-1 (CDK-1)	UP-regulation	Esophageal adenocarcinoma [82]; Breast cancer; Glioblastoma [83]; Astrocytoma, etc.

myc suppressed) takes charge of the situation to control the undesirable modifications. This is achieved by suppression of c-myc via Tripartite motif-containing protein 32 (TRIM32)/ PETN/ F-box/WD repeat-containing protein-7 (FBXW7)/ miR-145/ miR-34/let-7, regulated by p53/ TA-p73/p63 [63]. This in turn up regulates tumour suppressor miRNAs expression. Thus, targeted knockout of c-myc (in c-myc over-expressing cancers) with the help of tumour suppressor miRNAs could be one of the promising approaches in triggering the combined regulatory cascades in cancer cells. For instance let-7 family is a heterochronic controller and regulate correct timing of cellular development and differentiation. It is straightforward that their altered expression could be a cause of de-differentiation of cells which is a typical feature of cancerous cells. Recently, researchers had demonstrated the importance of non-coding small RNAs in controlling various undesirable alterations in the cell and assumed that these RNAs could be regarded as the guardians of the genome [64-65]. These important facts could help us in changing our traditional approach to new therapies (e.g. RNA interference, immunotherapy, etc.) of designing anticancer targets. Future investigations should be made in elucidating the crosstalks between the guardian and other regulatory networks for designing better therapeutic anticancer targets.

7. CONCLUSIONS

Recent developments in electronic technologies have led to the development of advanced imaging tools. In recent times, non-invasive imaging technologies are of great importance due to their significant potential and scope in detecting micro-metastasis. Further improvements in the current diagnostic platforms including the multi-dimensional confirmations (3D and above), sensitivity, accuracy, reliability, advance graphics, quantitative algorithms and compatibility with other modalities could help in improving the diagnostic outcomes. There are important undiscovered domains which needs to be investigated to unveil the unpredicted and uncontrolled metastasis of cancer cells. These includes, understanding the metamorphosis of primary tumour cells to metastatic cells, large scale trials and validation of newly identified diagnostic/prognostic markers within different subsets of populations, effect of cancer stem cells markers on cancer initiation and progression, validation of new modalities (e.g., HAI chemotherapy) using large randomized trials, crosstalk's between PTNs, MLNs and TLNs, enrichment methods for CTC/DTC and micro-metastasis detection, exosome profiling, consistency of molecular diagnostic markers while elucidating the mechanistic insights of metastasis, etc. With regards to the use of *in vivo* cell models, studies on compatibilities and dissimilarities between humans and *in vivo* cell models has to be kept on highest priorities. Therefore, we could only hear better answers from the mouse if we ask right questions.

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