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Computational systems biology in cancer brain metastasis

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1. ABSTRACT

Brain metastases occur in 20-40% of patients with advanced malignancies. A better understanding of the mechanism of this disease will help us to identify novel therapeutic strategies. In this review, we will discuss the systems biology approaches used in this area, including bioinformatics and mathematical modeling. Bioinformatics has been used for identifying the molecular mechanisms driving brain metastasis and mathematical modeling methods for analyzing dynamics of a system and predicting optimal therapeutic strategies. We will illustrate the strategies, procedures, and computational techniques used for studying systems biology in cancer brain metastases. We will give examples on how to use a systems biology approach to analyze a complex disease. Some of the approaches used to identify relevant networks, pathways, and possibly biomarkers in metastasis will be reviewed into details. Finally, certain challenges and possible future directions in this area will also be discussed.

2. INTRODUCTION

Systems biology is computational and mathematical modeling of a complex biological system (1), which requires an integration of experimental and computational research (2). Computational systems biology, through pragmatic modeling and theoretical exploration, provides a powerful foundation for addressing critical scientific questions fundamental to our understanding of life and leads to practical innovations in medicine, drug discovery and engineering.

Traditional systems biology approaches used for studying biology rely mainly on linear verbal logic and illustrative descriptions without mathematical explanations (3). These approaches are only satisfactory for addressing mechanisms that are involved in a small number of elements or short chains of causality. Therefore, these approaches are unable to capture and unravel the elaborate webs of molecular interactions. Most diseases, including cancer, involve a large number and variety of elements that interact via complex networks and, consequently, display highly nonlinear dynamics. Therefore, simply knocking out one target molecule in a biochemical pathway is not sufficient for treating a disease like cancer, because the cells often find alternative molecular routes to escape from the blockage of one pathway. This is one key reason why current drug

design strategies often fail. It is increasingly believed that a systems perspective, rather than the current gene-centric view, could solve these problems and open up entirely new options for cancer treatment.

The systems approach used for biological studies combines empirical, mathematical, and computational techniques to gain an understanding of complex biological and physiological process. For example, hundreds of proteins may participate in the signaling network to ensure proper functioning of a cell. If such a network is disturbed or altered, a cancer phenotype could be induced. Systems biology helps to shed light on these complex processes by generating detailed route maps of various cellular networks and developing sophisticated mathematical, statistical, and computational methods and tools to analyze these networks. Understanding the complex systems involved in cancer development will make it possible to develop smarter therapeutic strategies. For example, two or three key intersections in a biochemical network can be disrupted at the same time. The new applications could lead to significant advances in the treatment of cancer and transform the traditional reductionism-based methods into unbiased systems-level approaches for drug discovery.

The birth and development of systems biology have been driven by the innovation of high-throughput techniques applied to life science research. Over the past few years, high-throughput techniques, such as next generation sequencing, RNA-seq, chip-on-chip, chip-seq, microarrays, and others, have been developed for genome analysis, gene expression profiling, protein-DNA interaction, transcription factor binding. These technologies have triggered a dramatic change in the style of biological studies from a “one gene model” (i.e. focusing on the identification of individual genes and proteins and pinpointing their roles in the cell) to a “multiple gene model” (i.e. based on the belief that molecules never act alone and biological entities are *systems,* collections of interacting parts). These technologies have generated many “large-scale biology projects”. As these technologies become more affordable and accessible, the implementation of large-scale biological projects is more popular. These projects have generated a large amount of data and the only effective way to analyze such data is through mathematical representation and computation. Systems biology can be used to deal with these challenges by integrating many types of -omic data and developing effective computational tools to decipher the complex systems.

Metastasis is a complex process and remains the main cause for cancer-related deaths in the United States. The progression of a primary tumor to metastastic disease is a multiple step process and involves detachment of primary tumor, local invasion, intravasation, transport, extravasation, and colonization at the secondary site, especially in the brain which is the most complex biological system in human. Because the metastatic cascade involves many complex steps, it is generally considered to be an inefficient process. When metastasis does occur, it is almost always fatal to the patients. For these reasons, increased understanding of each step in the metastatic cascade will be important for the development of better therapeutic interventions. Research in the field of metastasis has been ongoing for decades and various mechanisms of metastasis have been suggested, all of which have added another layer of complexity to the metastatic cascade. Recent technological advances such as high throughput genomic, proteomic, and metabolomics analyses have provided better platforms for studying this complex disease at a system level. The incorporation of multiple systems or data types will promote new biomarker discoveries for metastatic diseases and demonstrate more suitable and individualized targeted therapy.

A recent review paper pointed out the systems biology approach can be used to understand and study the mechanisms of cancer metastasis (4). Technological advances for high-throughput screening of cells such as expression profiling, next generation sequencing, as well as global network analyses have further advanced the studies of these mechanisms. Combined with new insights into the various mechanisms of metastasis, a systems biology approach has shown to be useful in identifying metastasis-specific gene signatures as well as predicting disease outcome, leading to identification of biomarkers for metastatic diseases.

The related biological mechanisms of cancer brain metastasis have been intensively reviewed in the literature previously regarding different types of cancers such as melanoma (5) and lung cancer (6, 7). In this review, we will focus on illustrating strategies, procedures, and computational techniques for the study of systems biology in cancer brain metastases (Figure 1). We will give examples on how to use a systems biology approach for analyzing this complex disease. We will highlight some of the approaches used for identifying relevant networks, pathways, and possibly biomarkers in brain metastasis. Finally, certain challenges and possible future directions in this area will also be discussed.

3. PROCESS OF CANCER BRAIN METASTASIS

Cancer metastasis to the distant organs involves various steps. Chaffer *et al* has described six distinct steps that are crucial for cancer cells to metastasize (8). The initial step in the acquisition of metastatic phenotype involves invasion of the primary tumor to the surrounding stroma. Cancer cells gain invasive property by the virtue of the phenomenon known as Epithelial to Mesenchymal

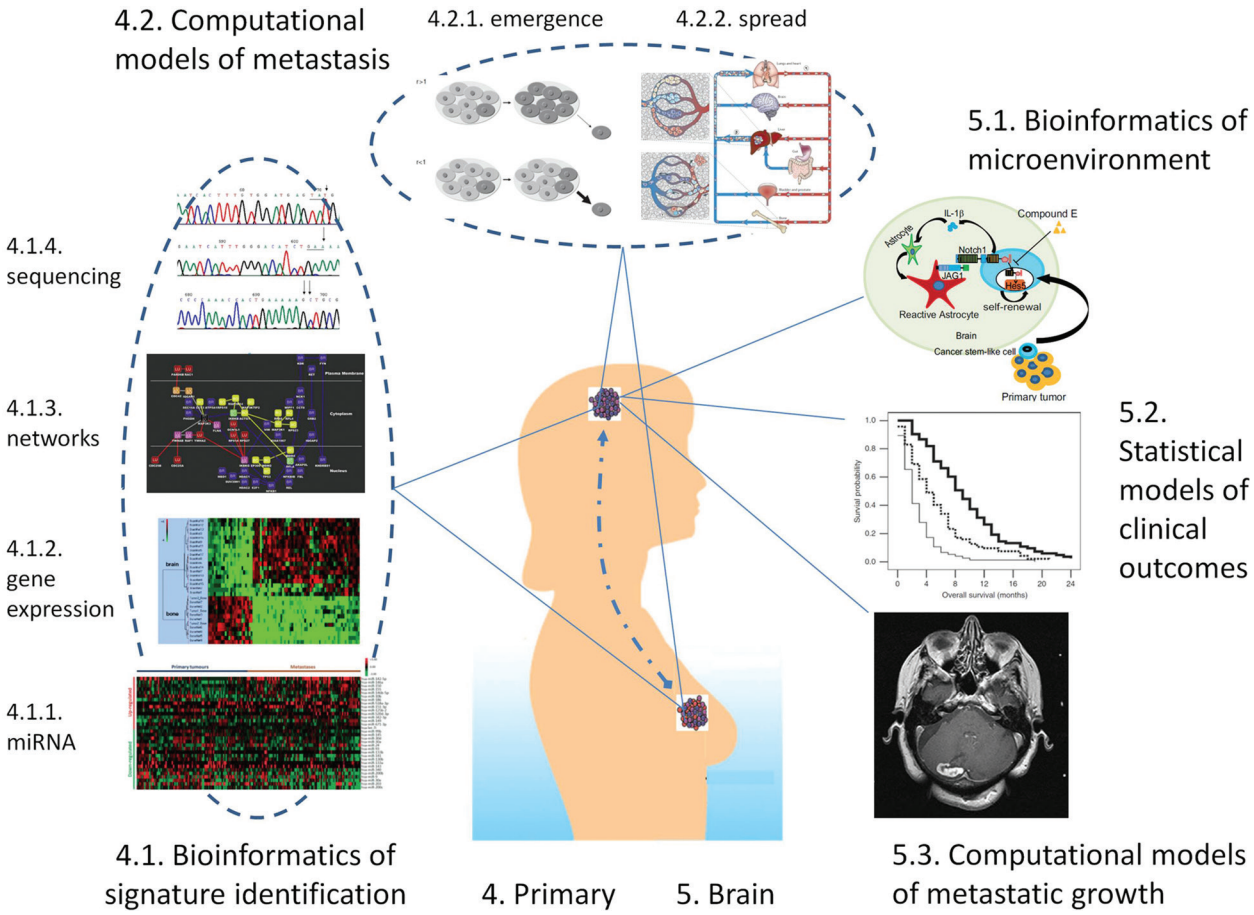

Figure 1. Diagram of computational systems biology of cancer brain metastasis (breast cancer as an example). Indicated numbers are the indexes of sections organized in this review.

transition (EMT) during which cells change its morphology from epithelial to mesenchymal shape. EMT enhances motility of cancer cells during invasion. In addition, cancer cell also secrete molecules that degrades the extracellular matrix and modulates environment of the primary tumor. These changes lead to invasion of local stroma followed by extravasation of cancer cells into the nearby blood vessels. Cancer cells in the circulatory system, also known as Circulating Tumor Cells (CTCs), have to survive Anoikis before its successful colonization into the distant organ. CTCs are known to survive in the circulation by activating survival pathway such as PI3-AKT-mTOR pathway (9). These surviving CTCs then reach the capillary of the distant organ before extravasating into the foreign tissue. Cells have to acquire specific properties to extravasate from the circulation system depending on the site of extravasation. Cancer cells generally mimic leukocyte by expressing secreted molecules (chemokines and cytokines) that allows permeability into the blood vessels. For special organ such as brain that is protected by blood brain barrier, extravasation is quite challenging. One recent study has shown that breast cancer cells secrete MMP1 to degrade the tight junction proteins in the endothelial cells of the blood brain barrier to extravasate into the brain (10). Another mechanism known for CTCs extravasation is the growth of cancer cells within the microvessels. The increasize in the size of tumor ultimately leads to the breakage of the endothelial wall leading to colonization at the distant site (11). However, survival at the distant organ is still challenging as various foreign tissue factors leads to apoptosis of the cancer cells. Only cells that can better accommodate in the foreign tissue environment can survive and grow as secondary tumor. The surviving cells must be capable of interacting with the microenvironment to induce its own survival. This phenomenon of survival of some but not all cells can be explained by “seed and soil” theory (12). Cancer cells surviving in the foreign tissue are the genetically distinct seeds that have acquired appropriate properties to grow in the foreign soil. Furthermore, this theory also explains why some seeds preferentially metastasize to particular soil. For example, it is known that bone one of the major site of metastasis for breast and prostate cancer (13, 14). Similarly, liver, lung and brain are other preferred site for metastasis of breast cancer (14), indicating that

microenvironment at the distant site plays a significant role in metastasis of cancer cells.

4. SYSTEMS BIOLOGY ON METASTATIC DRIVERS IN PRIMARY TUMOR

Studies have demonstrated that systems biology approaches are powerful in identifying metastasis-specific gene signatures as well as predicting disease outcome. In this section, we review the systems biology approaches used in studying metastatic drivers in the primary tumors. We will first introduce how bioinformatics approaches can be applied to identify key signatures of brain metastasis by analysis of various types of genomic data and then present the computational models used to deal with other issues like metastasis patterns by analyzing clinical and other data.

4.1. Bioinformatics approaches for mechanism identification

Mechanisms identification of cancer metastasis is largely dependent on the available data. Bioinformatics approaches are powerful in analyzing data and elucidate the mechanisms of cancer metastasis. Here we focus on reviewing the application of bioinformatics approaches in analysis of mircoRNA (miRNA) profiling, gene expression profiling, signaling transduction network and sequencing data.

4.1.1. miRNA profiling analysis

miRNAs have been known to drive cancer metastasis via regulation of pro-metastatic genes and the regulatory genes for optimizing tumor microenvironment. Profiling miRNA expression can be used to screen relevant miRNAs for tumor metastasis and identify gene signatures. The gene signatures can be used for classifying cancer subtypes as well as predicting metastasis-free survival outcome. For example, Lerebours *et al.* (15) profiled miRNA expression in patient samples and identified a miRNA signature, which could predict disease phenotype in inflammatory breast cancer (IBC). To identify the potential factors governing this disease, the authors performed a global expression profiling of miRNAs. Thirteen out of 804 miRNAs were differentially expressed in the IBC, when compared with the non-IBC tumors. Among 13 of them, a signature set with 5 miRNAs was found highly predictive of IBC. Baffa *et al.* (16) compared miRNA expression profiles between primary tumors and their matched metastases to identify miRNA signatures involved in the metastatic progression and organotropism. Several miRNAs were identified in that study and some of them have already been known to play a role in specific cancers, suggesting that miRNA expression profiling could be useful in determining cancer origin based on expression pattern analysis across different organs of origin because miRNA signatures were markedly tissue-specific, especially in the cases for which the origin of primary tumor is unknown. Brain metastasis is a major cause of mortality among melanoma patients. A molecular prognostic analysis can be used to evaluate the risk of developing brain metastasis. Hanniford *et al.* (17) performed a retrospective, cohort-based study to analyze genome-wide miRNA expression profiling for primary melanoma tumors from three patient groups with extensive clinical follow up. They used Cox regression analysis to establish miRNA-based signatures. Combination of prognostic analysis of miRNA expression signatures with the currently used staging criteria may improve the diagnostic accuracy of primary melanoma and predictive ability for development of brain metastasis. It will aid clinical management of patients, including selection for adjuvant treatment or clinical trials of adjuvant therapies. miRNAs have a diverse range of biological functions, such as temporal regulation of development, cell death and proliferation, hematopoiesis and tumourigenesis. miRNAs regulate molecular pathways in cancer by targeting various oncogenes and tumour suppressors. Generally, one miRNA can regulate hundreds of target genes as predicted (18). As a result, miRNAs can be used as a better classifier than messenger RNA. In the study by Nasser *et al.* (19), the authors combined validated miRNA expression values with imaging features to classify NSCLC brain metastasis from primary tumors and identify possible biomarkers of brain metastasis. This study involved comprehensive profiling miRNA expression, evaluation of normalisation techniques and imaging feature extraction of FDG-PET/CT and CT scan. The biomarkers were validated using an independent data set to predict potential brain metastasis.

4.1.2. Gene expression profiling analysis

Gene expression profiling analysis has been widely applied in the detection and quantification of key driver genes in order to understand the complex phenomenon of cancer brain metastasis (20). The molecular basis for breast cancer metastasis to the brain is largely unknown. Brain relapse typically occurs years after the removal of a breast tumor, suggesting that disseminated cancer cells must acquire specialized functions to take over the distant organ. Massagué *et al.* (21) showed that breast cancer metastasis to the brain involves mediators of extravasation through non-fenestrated capillaries, complemented by specific enhancers of blood-brain barrier crossing and brain colonization. They isolated cells that preferentially infiltrate the brain from patients with advanced disease. Through gene expression analysis of these cells and clinical samples, the authors identified several genes as mediators of cancer cell passing through the blood-brain barrier. Metastatic colonization in different target organs is a highly selective process that depends on specialized properties of tumor cells. Recent research has highlighted this process. Massagué and colleagues built on their earlier success in functional genomic analysis of breast cancer metastasis to bone and lung and reported the

identification of breast cancer brain metastasis genes, highlighting the importance of the stromal environment in the development of organ-specific metastasis (22). Breast cancer can spread to many different organs, with the most common sites being bone, regional lymph nodes, lung, liver, and brain. The detailed mechanism of organ specific metastasis is poorly understood. Klein *et al.* (23) looked into the genes associated with brain or bone metastasis of primary human breast cancer. They generated gene expression profiles of 18 brain and eight bone metastases derived from primary breast tumors and found that 73 genes were differentially expressed between brain and bone metastases. Visualization of the differential gene expression profiles by correspondence and cluster analyses showed that the metastases clearly separate into two distinct groups as an exact reflection of their site of metastasis. Moreover, the analysis of this gene set in primary breast tumors relapsing to either bone or brain allowed accurate categorization of the tumors according to their metastatic site. The identified genes may prove to be excellent markers in predicting metastatic site in breast cancer patients and lead to tailor-made therapy to an individual patient. Metastasis remains the most common cause of death in most cancers and limited therapies can be used for combating these disseminated disease. Microenvironment is an important regulator of cancer progression. It is less well understood how different tissue environments affect primary tumor metastasis. Cancer cells survival and colonization are influenced by non-cancerous stromal cells in the local microenvironment. Sevenich *et al.* (24) analyzed tumor-stroma interactions that modulate organ tropism of brain, bone and lung metastasis in xenograft models. They identified a number of potential modulators of site-specific metastasis and found that cathepsin S was a regulator of breast-to-brain metastasis. Organ-specific homing of malignant cells involves cell-cell interactions mediated through cell adhesion molecules and their receptors on the cell surface. Identification of gene markers that mimic these receptor-ligand interactions is critical for analyzing the functional role of these proteins and is therapeutically significant for targeting or blocking organ-specific homing of tumor cells. Sadanandam *et al.* (25) conducted three cycles of *in vivo* biopanning of a phage display peptide library in mice and identified 11 unique gene markers that were specific for homing to lung, liver, bone marrow, or brain. Bioinformatics analysis of the identified organ-specific gene markers indicated that cell adhesion molecules (26) were critical in tumor cell migration, invasion, and metastasis.

4.1.3. Network analysis

Network analysis is often used for studying the interactions of genes or proteins and is a powerful tool for demonstrate how the signaling network involves/drives in a specific disease. Hu *et al.* (27) analyzed two independent human breast cancer datasets and three different mouse populations and showed that gene networks could predict metastasis-free survival in human breast cancer cohorts. Interestingly, the data in this study suggest that different gene networks could predict the outcome for the different subsets of breast cancer. Specifically, it was shown that Estrogen receptor-positive breast cancers rely on tumor autonomous factors while Estrogen receptor-negative breast cancers were influenced by host-derived stroma. Identifying the genes within these networks will not only allow for further analysis on how they contribute to the metastatic process but it also indicated the complex interplay of various cell types. Zhao *et al.* (28) developed a computational model to derive specific downstream signaling pathways that reveal previously unknown target-disease connections and new mechanisms for specific cancer subtypes. The model enables us to reposition drugs based on available patient gene expression data. The authors applied this model to repurpose known or shelved drugs for brain, lung, and bone metastases of breast cancer based on their specific signaling mechanisms. The brain offers a unique microenvironment that plays an important role in the establishment and progression of metastasis. However, the molecular determinants that promote development of melanoma brain metastases are largely unknown. Nygaard *et al.* (29) analyzed cultivated metastatic tissues and their corresponding host tissues collected from melanoma metastatic mouse models and identified molecular events associated with melanoma brain metastases. Analysis of the host tissue uncovered a cooperative inflammatory microenvironment formed by activated host cells that permitted melanoma growth at the host organism. Importantly, the identification of essential molecular networks that operate to promote the brain-adaptive phenotype is of clinical relevance, as they can lead to the identification of novel therapeutic targets. From a genomic point, breast cancer can be divided into several subtypes. Few studies have described patterns of metastasis according to the major breast cancer intrinsic biologic subtypes. The subtypes of breast cancer may involve activation of a host organ-specific signaling network in metastatic cells. To test this possibility, Burnett *et al.* (30) measured gene expression patterns in MDA-MB-231 cells and its mammary fat pad tumor, lung-metastasis, bone-metastasis, adrenal-metastasis and brain metastasis variants. Pathway-analyses revealed that activation of specific signaling networks would enable cancer cells to adapt to organs of metastasis such as drug detoxification/oxidative stress response/semaphorin neuronal pathway in brain metastasis. Biological and clinical outcomes are not based on a single protein, but modules of proteins embedded in the protein networks. A fundamental question is how the proteins within each module contribute to the overall module activity. In the study led by Dutkowski and Ideker (31), the authors studied the modules underlying three representative biological programs related to tissue development, breast cancer metastasis, and progression of brain cancer, respectively. A new method called Network-Guided

Forests was applied for identifying predictive modules together with logic functions which tie the activity of each module to the activity of its component genes. The resulting modules implement a diverse repertoire of decision logic which cannot be captured using the simple approximations. Protein interactions and the structure of interacting surfaces (interfaces) have an important role in predicting the genotype-phenotype relationship. In the study by Engin *et al.* (32), the authors have built the phenotype specific sub-networks of protein-protein interactions (PPIs) involving in the relevant genes responsible for lung and brain metastasis from primary breast cancer. Functional analyses performed on these sub-networks revealed the potential relationship between immune system-infectious diseases and lung metastasis progression, but this connection was not observed significantly in the brain metastasis. Brain metastases are the most common fatal complication of systemic cancer, especially of lung (40-50%) and breast (20-30%) cancers. In this era of personalized therapy, there is a critical need to uncover the signaling architecture of brain metastases; however, little is known about what signaling pathways are activated in the context of the brain microenvironment. In the study by Improta *et al.* (33), using a unique study set of 42 brain metastases from patients with breast or non-small cell lung cancer (NSCLC), the phosphorylation/activation states of 128 key signaling proteins involved in cancer signaling were measured in laser capture microdissected tumor epithelium using reverse phase protein microarray technology. Protein pathway activation mapping revealed heterogeneity of signaling networks in brain metastases that would require a prior stratification to targeted therapies, as well as the requirement of direct analysis of the metastatic lesion.

4.1.4. Sequencing analysis

Gene mutation has been widely considered as an important driving factor in cancer cell metastasis. New generation technology in genomic research like whole-genome, whole-exome and deep sequencing technology has allowed cancer researchers to check the copy number alteration, chromosome rearrangement and peptide point mutations simultaneously in order to explore the global genome alteration profiles responsible for cancer brain metastasis. For example, to understand clonal selection, Ding *et al* (34) used second generation sequencing to analyze a single patient's peripheral blood, primary basal-like breast tumor, and matched brain metastasis. The authors found a wide range of mutations in the primary tumors supporting genetic heterogeneity within a sample. Subsequent analysis of the metastasis showed an enrichment of a subset of mutations, suggesting this particular cell population within the primary tumor metastasized to the brain. The xenograft derived from patient's primary tumor had a mutational profile that overlapped with the metastasis, which further supported the notion that a minority population of cells arose within the primary tumor with an enhanced metastatic capability. In another example very recently published, using whole-genome sequencing Gundem *et al* (26) sought definitive evidence for the existence of polyclonal seeding in human malignancy and to establish the clonal relationship among different metastases in the context of androgen-deprived metastatic prostate cancer. Integrated analyses of subclonal architecture, by characterizing multiple metastases arising from prostate tumors in multiple patients, revealed the patterns of metastatic spread in unprecedented detail, which elucidated in detail the complex patterns of metastatic spread and further our understanding of the development of resistance to androgen-deprivation therapy in prostate cancer. This work is the seminal work that documents the branched evolution of metastases using sequencing of primary and metastases.

4.2. Computational models of metastasis

Besides the signature identification by bioinformatics approaches based on genetic or genomic data discussed above, there are other two important topics regarding the mechanistic understanding of metastasis from primary site to metastasis site: emergence and spread pattern of metastasis. Clinical data could be very helpful to the study on these two topics. We here review computational models in which the clinical data were utilized to reach the purpose in the context of the above two topics.

4.2.1. Emergence of metastasis

The emergence of metastatic disease has largely been attributed to cells gaining functions specific to intravasation. This gain of function has been linked to genetic mutation, with large numbers of specific genes being implicated. To this end, a number of statistical models proposed by Michor and her colleagues (35-40) have employed a stochastic description called the Moran process (41) to study the genetic landscape of a tumor's cellular population over time. To study the dynamics of the emergence of the metastatic phenotype, Michor *et al.* (36) proposed a model of tumor growth, based on the Moran process, which took into account of mutation to a metastatic phenotype. The authors modeled a heterogeneous tumor made up initially of cells without the ability to metastasize (called type-0 cell, with fitness r0). At each discrete time step, a cell is randomly chosen to divide (biased by fitness) at which time the type-0 cell has a probability u of producing mutated offspring that has the ability of metastasis (called type-1 cell) with fitness r1 (where a fitness of 1 is neutral). This mutated offspring also now has a probability q of being 'exported' from the population to initiate a metastatic tumor of their own. The model predicted that metastatic clones are most likely the result of advantageous mutations that will occupy the majority of the primary tumor. Of importance, through this model the dynamics of metastases arising from a primary tumor of constant size could be investigated and

the expected number of metastatic cancer cells over time could be calculated. This model was based on a basic hypothesis in which a single mutation is necessary to confer metastatic abilities to a cancer cell, as proposed in another study (35). To further investigate an alternative hypothesis in which two mutations are necessary to confer metastatic abilities to a cancer cell (37), Michor *et al.* proposed an alternative model by adding a new type-2 (with fitness r2) representing the cancer cells with twice mutations (mutated from type-1). In this alternative model, only type-2 cell has the ability to metastasize. Subsequently, Michor and her colleagues (38) examined a branching process model of tumor metastases which was updated from the previous model proposed in (36) and investigated the effect of the export of metastatic cells from the primary site on the growth of the primary tumor. These models (36-38) did not allow an expanding of the cancer cell population. To further extend the model to a more clinically grounded context, in a following study (39), a stochastic mathematical model was designed and used to simulate the evolution of tumor metastases in an expanding cancer cell population. The probability of metastasis, the total number of cancer and metastasized cells at a particular time during tumorigenesis were calculated. Furthermore, they investigated the effect of drug administration and tumor resection on these quantities and predict the survival time of cancer patients. The model presented in that study can be used to determine the probability and number of metastases at diagnosis and identify the optimum treatment strategy to maximally prolong survival in cancer patients. Most recently, these authors applied their model proposed in (39) to the case of pancreatic cancer (40) for analyzing a large number of clinical data, i.e. image data of metastasis. In the study, they analyzed the effects of different treatment modalities and explored which therapies could efficiently reduce the growth rate of cells earlier in the course of treatment appear to be superior to upfront tumor resection. These predictions can be validated in the clinic. The authors pointed out that their interdisciplinary approach could provide insights into the dynamics of pancreatic cancer metastasis and identify optimum therapeutic interventions.

4.2.2. Metastatic pattern of primary tumors

Understanding the patterns of spread of a particular primary tumor can help guide clinicians in their decision making for therapy and is also useful for follow up purposes to give special attention to the organs most likely at risk for early detection of recurrence. A large number of statistical models have been proposed in the previous works for analyzing population level data of metastatic spread and predicting the most likely routes of spread, such as logistic regression model, Bayesian model, Markov model and so on. For example, in the study led by Hess *et al.* (42), the authors analyzed clinical data from a large number of patients with histologically confirmed, distant-stage adenocarcinoma to evaluate metastatic patterns. The primary and metastatic sites were cross-tabulated in various ways to identify patterns, and the authors developed algorithms by using multinomial logistic regression analysis to predict the locations of primary tumors based on the metastatic patterns. Cerebral metastases are the main determining factor in the failure of locally advanced NSCLC management. Wang *et al.* (43) assessed the risk factors of brain metastases in patients with postoperative, locally advanced NSCLC. Two hundred twenty-three patients treated with surgical resection for stage III-N2 NSCLC were retrospectively analyzed and a mathematical model based on multivariate logistic regression used for predicting brain metastases risk. The Bayesian network (BN) is a promising method for modeling cancer metastasis under uncertainty. BN is graphically represented using bioinformatics variables and can be used to support an informative medical decision/observation by using probabilistic reasoning. In the study by Wang *et al.* (44), the authors proposed a BN to describe and predict the occurrence of brain metastasis from lung cancer in which a nationwide database of clinical data in Taiwan was involved. Three statistical measures, including namely, the accuracy, sensitivity, and specificity, were applied to evaluate the performances of the proposed BN model. Comparing with the other three competitive approaches, including naive Bayes (NB), logistic regression (LR) and support vector machine (SVM), the proposed BN has advantages in interpreting how brain metastasis develops from lung cancer. This model is efficient in modeling non-linear situations, capable of solving stochastic medical problems, and handling situations where in information are missing in the context of the occurrence of brain metastasis from lung cancer. Unique metastatic patterns cited in the literature often arise from anecdotal clinical observations and autopsy reports. In the studies by Newton *et al.* (45,46), a stochastic Markov chain model for metastatic progression of primary lung cancer was developed based on a network construction of metastatic sites with dynamics modeled as an ensemble of random walkers on the network. In the study led by (47), the authors used a large database of Medicare claims to study the large-scale clinical pattern of metastases. They introduced the concept of a cancer metastasis network, in which nodes represent the primary cancer site and the sites of subsequent metastases, connected by links that measure the strength of co-occurrence. The authors analyzed the data by calculating a time-dependent hazard as a function of the primary and metastatic site to observe how certain metastatic lesions developed over time for a given primary tumor in a certain location. The same group also formulated a statistical model to predict the location of the primary tumor given a sequence of metastatic sites, and reversely to predict the most typical sequence of metastatic sites given a certain primary cancer site. In a recent theoretical work (48), Anderson and his colleagues examined the self-seeding hypothesis and showed that direct self-seeding (i.e. the

primary tumor shedding cells that directly returned to the primary) was many orders of magnitude less likely than 'secondary seeding', a process by which cells from the primary metastasize to a secondary location, grow and then re-shed progeny into the vasculature which then return to the primary. Although this distinction is difficult or currently impossible to measure in the clinic, it is of chief importance, as it suggests that there are levels of detail about extant disease that are not captured in the previous models.

5. SYSTEMS BIOLOGY ON METASTATIC GROWTH IN BRAIN

In the previous section, we have reviewed the systems biology approaches used to study the mechanisms driving primary cancer cells moving towards the brain. The brain is a common site of metastatic disease in cancer patients, which has few therapeutic options and commonly poor outcomes. The brain is generally a more complex system than tissues where the primary cancer arose. Once a metastasis is newly established in the brain, the specific niche or microenvironment will promote the tumor metastatic growth, and the tumor cell itself evolves and adapts to the new niche. The mechanism of metastasis is vital for drug design and therapeutic strategy selection. As a tool to deal with a complex system like the brain, systems biology is quite powerful. Here we review the application of systems biology approaches to deciphering the process of brain metastasis formation. First, we will review the bioinformatics approaches used to identify the molecular mechanisms that drive metastatic growth through mutual interactions between tumor and its surrounding microenvironment. Then, we will introduce the statistical models used to identify the prognostic factors for clinical use and further to predict the clinical outcome such as overall survival or therapeutic efficacy. Finally, we discuss the computational models used to simulate tumor metastatic growth in the brain.

5.1. Bioinformatics approaches for mechanism identification

Due to the complexity of the brain, the mechanisms of formation and growth of brain metastases are poorly understood. There are few reports addressing the mechanism of metastatic growth in brain using bioinformatics approaches so far. One of the reasons might be due to the difficulty for sample collection of brain metastasis from patients. Secondly, it is impossible to do any *in vivo* experiments in humans to study the mutual interaction between cancer cells and other agents in brain microenvironment. Here we summarize our review in two aspects. One is the study on microenvironment and the other is the study on molecular events in tumor metastasis.

To study the microenvironment of brain metastases, *in vivo* animal model or *in vitro* cell co-culture experiments have often been used. Bioinformatics approaches have been widely employed for identifying the key molecular factors or pathways. In the brain microenvironment, an astrocyte is a star-shaped glial cell which has been one of the most intensively studied. Astrocytes are among the most important host cell types in the brain microenvironment, closely communicating with metastatic cancer cells and apparently promoting brain metastasis growth, as reported by both Fidler's group (49, 50) and Watabe's group (10, 51). Fidler *et al.* reported that co-culture of human breast cancer cells or lung cancer cells with murine astrocytes led to an increased expression of survival genes in the tumor cells, including GSTA5, BCL2L1, and TWIST1 (49). In this study, gene expression profiles were first used to identify genes in tumor whose expression patterns were altered on interaction with astrocytes, and then a few survival genes among the altered genes were validated by Western blot and other biological experiments. In another parallel study (50), Fidler *et al.* investigated the influence of brain microenvironment on human breast cancer cells by independently extracting the data of cancer and host cells when human cancer cells were xenografted into different organ sites of immune compromised mice. In this study, both gene expression profiles and methylation profiles were generated and used in the statistical analysis for comparison. The data showed that the brain microenvironment induced a complete reprogramming of metastasized cancer cells and highlighted the outstanding function of astrocytes upon cancer cells' reprogramming. Dr. Watabe's group (10, 51) used a co-culture system to explore the detailed molecular mechanism on interaction between astrocytes and human breast cancer cells. This studies were based on a stem cell hypothesis, i.e. cancer stem cells (CSCs) play a key role in the progression of cancer brain metastasis. They also validated their results using mouse model in their studies. The bioinformatics approach, such as metastasis-free Kaplan-Meier survival analysis, was employed to stratify patients by individual genes and select the molecular candidates associated with cell-cell interaction. Several detailed cell-cell interaction pathways among astrocytes and CSCs have been identified for their role in promoting CSCs self-renewal and breast cancer metastatic growth in brain microenvironment. Their studies represented a novel paradigm for the understanding of how metastatic breast CSCs re-established their niche for their self-renewal in a brain microenvironment.

To study the molecular events or biomarkers of metastatic tumor in brain, the brain metastasis tissue samples from patients are vital. However, the acquisition of such samples is generally difficult. At this time, few have reported the use of human samples of brain metastases for identifying molecular markers. The successful use of such samples would employ a bioinformatics approach for comparative analysis. The first example showed how the gene expression profiles

of the brain metastasis samples can be used to identify the tissue origin of metastatic brain tumors (52). In this study, Wu *et al.* evaluated the performance of the Tissue of Origin Test in the diagnosis of primary sites for metastatic brain cancer patients. The Tissue of Origin Test (Pathwork Diagnostics, Redwood City, CA, USA) is a gene expression test to aid in the diagnosis of metastatic, poorly differentiated and undifferentiated tumors. In their study, gene expression profiles from 15 fresh-frozen metastatic brain tumor specimens of 9 known origins were processed using the Tissue of Origin Test. The comparison result demonstrated a high accuracy of the Tissue of Origin Test when applied to predict the tissue of origin of metastatic brain tumors, which suggests this test could be a very useful bioinformatics tool for classifying metastatic brain cancers based on gene expression profiles. The second example showed how multiple data types of the breast cancer brain metastasis samples can be integrated together to identify common and rare events that underlie breast cancer brain metastasis (53). In this study, Salhia *et al.* performed a deep genomic profiling, which integrated gene copy number, gene expression and DNA methylation datasets on a collection of breast brain metastases. Gene set enrichment analysis (GSEA) and hierarchical clustering were combined together with network analysis for the data analysis. The genomic and epigenomic profiling of breast brain metastases in this study provided insight into the somatic events underlying this disease, which have potential in forming the basis of future therapeutic strategies. A third example showed how immunohistochemistry (IHC) data combining with gene expression profiles of brain metastasis samples can be used as early stage prognostic gene markers and the related signaling pathways in cerebral metastases of lung adenocarcinomas (54). In this study, Bleckmann *et al.* first identified the specific biomarkers using IHC experiments, and analyzed a microarray dataset containing 19 adenocarcinoma brain metastases of the lung using a bioinformatics approach. Pearson's correlation test and hierarchical clustering and Cox proportional hazards regression model were used to establish novel gene signatures and the related pathways in their study.

5.2. Statistical models for clinical outcome prediction

As we have discussed in the section 5.1, the mechanisms associated with cancer metastatic growth in the brain environment have not been well studied systemically. However, integration of clinical data using statistical models can be applied for identification of key prognostic factors and predictions of clinical outcomes, particularly overall survival and the development of new brain metastases. The major statistical models used in the published studies are Cox regression-based models because of the ability to assess the time to an event such as death or new brain metastases. Because of the competing risk of death from brain metastases and extracranial disease, these models are often imperfect. Some studies also used other types of models such as Bayesian network model and General Linear Mixed model as so on.

Cox regression-based models are commonly applied for clinical prediction of treatment outcome or overall survival. Broadbent *et al.* reported the outcome of total 474 patients with brain metastases from multiple types of solid tumors treated with whole brain radiotherapy (WBRT) (55). In their study, survival was calculated using the Kaplan-Meier method and Cox regression modeling was used for multivariate analysis. Staudt *et al.* reported the survival of total 265 patients with brain metastases from cutaneous melanoma (56). In their study, Kaplan-Meier analyses were performed to estimate and compare overall survival and Cox modeling was used to identify independent determinants of the overall survival, which were used in explorative classification and regression tree analysis to define meaningful prognostic groups. The independent prognostic factors for these patients were the level of serum lactate dehydrogenase, administered therapy, the number of brain metastases and presence of bone metastasis. Marko *et al.* reported the survival of total 261 female breast cancer patients with brain metastases (57). A Cox proportional hazards regression with a nomogram representation was proposed to predict the survival. Vern-Gross *et al* investigated the variance in patterns of failure after Gamma Knife radiosurgery for 154 patients with brain metastases based on the subtype of the primary breast cancer (58). Kaplan-Meier method was used to estimate survival times and multivariable analysis was performed using Cox regression models. The results, through specifically analyzing breast cancer population, showed that Her2 status affects survival and development of new metastases. Ayala-Peacock *et al.* reported the outcome of total 464 patients with brain metastases from multiple tumors at our institute in recent ten years (59). The patients were treated with Gamma Knife stereotactic radiosurgery (SRS) for the brain metastases without whole brain radiation therapy. Kaplan-Meier method was used to estimate rate of distant brain failure and multivariate analysis was performed using Cox proportional hazard regression with a nomogram representation. Systemic disease, number of metastases, and histology were identified as key factors for prediction of distant failure rate after primary radiosurgical management of brain metastases. Most recently, Lucas at el described competing risk analysis of who actually dies of brain metastases (60). This analysis explores why brain metastasis survival analyses are difficult because patients do not always die of brain metastasis.

A few other models have also been used for demonstrating key prognostic factors and predicting clinical outcomes. Makond *et al.* reported the survival of total 438 patients with brain metastases from lung cancer (61). To predict substantially short survivability

in patients, Makond *et al.* proposed a probabilistic model using Bayesian network. The Bayesian network was constructed based on total seven clinical variables including age, gender, region, site, treatment, interval and survivability. They utilized synthetic minority over-sampling technique to solve the imbalanced property embedded in the proposed network. Freedman *et al.* reported the outcome of over 600 female breast cancer patients with brain metastases treated with or without fulvestrant (62). In their study, General Linear Mixed modeling, which adjusts for clustering within individual cancer centers, was applied to identify the factors significantly associated with the usage of fulvestrant and to subsequently develop the prediction model to identify those patients who could potentially derive the most clinical benefit.

5.3. Computational models of brain metastatic growth

When tumor cells metastasize, tumor cells need to gain access to the circulation first, survive during circulating, pass through the microvasculature of the adopted organs, extravasate into the organ parenchyma, and colonize at the secondary site. We have reviewed the bioinformatics approaches for exploring underlying molecular mechanisms of tumor progression and the mathematical modeling studies that focused on the early stages of metastasis, i.e. leaving the primary tumor, and circulating in the blood and lymphatic systems. In this section, we review the existing work related to the tumor growth at the metastatic sites.

The computational cancer models are a mathematical representation of the biological system (the tumor and its micro-environment) in question and often used for analyzing a class of subjects which share similar biophysical and biochemical properties. Brain metastatic tumor growth from particular origins has been experimentally studied using *in vitro* and *in vivo* models (63-67). In the experimental metastatic models, the tumor cells are directly inoculated into circulation and colonize into the brain, which only accounts for the late steps of metastasis: survival in the circulation, extravasation, and colonization in the target organs (68, 69). Despite useful for specific experimental conditions, an experimental model system does not allow animal-to-animal variability to be circumvented since identical initial configurations could not be used for a whole series of wet-lab experiments, which is exactly the strength of the computational models (70, 71).

However, there were scarce computational models applied for investigating metastatic tumor growth in other specific organs. Many efforts have been devoted to studying tumor metastatic growth for general cancers. These mathematical models, especially those developed for solid tumors which share similar biophysical environment with the brain, could be similarly applied to the case of brain metastasis. Generally, the existing models for tumor metastatic growth can be categorized into two classes, including stochastic dynamics models and determinative models. The stochastic dynamics models use agent-based discrete model configured with update rules and the determinative models leverage growth formulae or equations to describe the tumor expansion in question (72).

Michor *et al.* (40, 73-75) employed a stochastic model, called Moran process, to describe the dynamics of metastasis formation and expansion. The hypothesis is that the metastatic behavior of cancer cells is promoted by mutation in one or multiple genes, which confer a fitness advantage for the selected colony. In their models, the steps for a metastatic tumor formation are controlled by a series of probabilities which depend solely on the somatic mutation(s) of cells acquired at each time point. All of these stochastic events together predict the eventual metastasis number and size (40). Kimmel *et al.* applied statistical methods to study tumor size-metastasis relationship in solid cancers (76). The metastatic spread profiles can be inferred from the size of a primary tumor. Taylor *et al.* developed a two-state Markov Chain Monte Carlo model to simulate micro-metastatic proliferation and death based on stochastic survival probability (77). A narrow survival probability window that allowed for dormancy across a range of starting cell numbers was identified through this simulation. Kansal *et al.* developed a versatile three-dimensional (3D) cellular automaton (agent-based) model of brain tumor growth, showing that macroscopic tumor behavior can be realistically modeled using a few microscopic parameters (78). Their model recapitulated the clinical outcomes related to the dynamic composition of the tumor.

On the other hand, the deterministic tumor growth models use population growth formulae or ordinary (partial) differential equations (ODEs/PDEs) to depict the growth dynamics of metastatic tumor. The Gompertz curve and logistic regression model have been widely used to deal with tumor (primary or metastatic) growth. Norton *et al.* built a Gompertz model to simulate the breast cancer growth and revealed a growth pattern with largest growth rate in the middle period and a flattened growth at the beginning and late stages (79). Bosl *et al.* developed logistic regression model of metastatic testicular cancer and predicted the prognoses of patients using several clinical traits (80). ODEs/PDEs are the most popular way to describe tumor growth in the deterministic models. Iwata *et al.* developed a dynamical model by PDEs to estimate the colony size of metastatic tumors and predict spreading of the colonies (81), and Barbolosi *et al.* conducted a thorough mathematical analysis of this model, including the numerical solution for further extension and refinement (82). Very recently, Hartung *et al.* adapted a top-down model to estimate the risk of metastasis when no clinical evidence is available (83). By

calibration and validation using experimental data, their model was proved to be powerful in predicting metastatic spreading during the early stage of cancer progression.

Hybrid models are also used for describing tumor growth by integrating the stochastic and deterministic properties. Anderson presented a hybrid mathematical model of tumor cell invasion in healthy tissue (84). This model considered the nutrition and a series of secreted protein diffusing and reacting within the local tumor micro-environment and modeled them by a system of PDEs. On the other hand, tumor cells are simulated by a cellular automaton which treats each cell as an individual. Based on this modeling framework, our group extended this model by introducing the cancer stem cell (CSC) concept, incorporating the angiogenesis and necrosis process, and visualizing the whole system in 3D space with specific cell composition and molecule distribution (85, 86). On our modeling platform, we were also able to simulate the tumor response to various drug treatment regimens and identify important mechanisms related to the cancer stem cell-initiated tumor progression. Since our model simulated the tumor growth dynamics after stem cell seeding, which mimics the event of cancer cell homing and establishment in remote sites, it is well applicable to the metastatic tumor growth scenarios.

6. DISCUSSIONS AND FUTURE DIRECTIONS

Although brain metastasis has been studied for decades, our understanding of metastatic process is still poor. Various potential mechanisms of metastasis have been suggested over the years, each of which provided experimental evidence for the possible signaling pathways. The birth of genomic technologies has allowed for a more global study of the genes and pathways involved. With the increased ability to observe tumor and metastatic cancer cells on a whole genome level, it has advanced our understanding of a disease that includes many different aspects such as tumor and population specificity. Systems biology can be used to study and understand the various mechanisms of cancer brain metastasis. The ability to perform high throughput sample analyses has provided many insights into the various mechanisms of metastasis. It is now clear that cancer brain metastasis is not only a consequence of somatic mutations, but it also involves in the interplay of cells within the tumor microenvironment in the brain. Furthermore, studies have demonstrated that an individual's genetic make-up can also influence metastatic susceptibility. A systems biology approach can also be employed for gene expression profiling as well as network analyses to identify gene signatures for diagnosis and outcome prediction. These studies can assist scientists to identify new biomarkers for metastatic disease. The remaining challenge in this area mainly lies in that how to link genetic data analysis with epigenetic data analysis to understand deeper regulatory mechanisms (87-90), and how to effectively integrate all the components associated with cancer brain metastasis together in order to consider this complex biological process in a complete system. Multi-scale modeling may be a potential solution for this kind of complicated and highly integrative research, which could make connections at multiple scales of biological system while reflecting valuable information of spatial and temporal scales (91-99). For example, Liotta and colleagues made the first attempt to model the whole metastatic process in a manner of multi-compartment and multi-scale modeling (100). In their work, a mouse model was used to generate experimental data for the process of pulmonary metastases from thigh muscle in order to train a mathematical model, which was then used to predict the effect of perturbations to the metastatic process including tumor resection, vessel growth inhibition and so on. This type of modeling work has potential to give significant insight into the mechanisms driving the response to perturbations on metastatic process including brain metastasis. However, *in vivo* experimental data is usually difficult to be generated which is vital for model training and model validation and involves multiple biological scales and different organs as well as complex mechanisms. Another future direction lies on how system biology can be used to model or simulate the clinically relevant process of dormancy and recurrence in the distant microenvironment such as brain. As we know the mechanisms and timing of distant recurrence of cancers after treatment remain challenge for clinical study. Systems biology approaches may help for the study. For example, Taylor *et al* (77) developed a two state Markov Chain Monte Carlo model simulating dormancy of micrometastasis. Another promising model type is based on cancer stem cell hypothesis. For example, Enderling and colleagues (101) proposed a stochastic model of cellular hierarchy within a tumor to show that single cancer stem cell-driven solid microtumors may undergo long periods of dormancy in spite of complex cellular activity. However these models are still far away from the complete understanding of the mechanisms of dormancy and occurrence. In conclusion, scientists working alone are not able to make as much progress as when they work together in studying cancer brain metastasis. Going forward, scientists from disparate fields, including the mathematical/theoretical disciplines, must open and foster dialogues between one another and work together to understand and interrupt this complex and nonlinear process of cancer brain metastasis (102, 103).

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