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## The molecular genomics of metastatic brain tumours

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### Abstract

**Introduction**—Metastatic brain tumours remain an intractable clinical problem despite notable advances in the treatment of the primary cancers. It is estimated that 30–40% of breast and lung cancer patients will develop brain metastases. Typically, brain lesions are not diagnosed until patients exhibit neurological symptoms because there are currently no tests that can predict which patients will be afflicted. Brain metastases are resistant to current chemotherapies, and despite surgical resection and radiotherapy, the prognosis for these patients remains very poor with an average survival of only 6–9 months. Cancer is ultimately a genetic disease, involving patient genetics and aberrant tumour genomics; therefore the pursuit of an explanation for why or how brain metastases occur requires investigation of the associated somatic mutations.

In this article, we review the current literature surrounding the molecular and genome-based mechanistic evidence to indicate driver oncogenes that hold potential biomarkers for risk, or therapeutic targets for treatment of brain metastases.

**Conclusion**—Patients afflicted with metastatic brain tumours are in dire need of more effective therapies, and clinicians need predictive laboratory tests to identify patients at risk of developing metastatic brain tumours. The as yet unrealized comprehensive analysis of metastatic brain tumour genomics is necessary to meet these needs. Moreover, without improved understanding of the genomic aberrations that drive metastatic brain tumours, development of biomarkers and molecularly targeted therapies will remain stalled and patient outcomes will continue to be dismal.

### Introduction

#### Incidence and current therapeutic options

There are approximately 200,000 newly diagnosed cases of brain metastases annually in the United States, 10-fold greater than primary brain cancer<sup>1,2</sup>. Current estimates indicate that up to 40% of patients with a systemic primary cancer will develop a metastatic brain tumour (MBT)<sup>3</sup>. A majority of brain metastases come from lung cancer (50–60%); secondarily from breast cancer (20–30%); third melanoma (5–10%) and various cancers, including gastrointestinal, oesophageal, prostate and ovarian that combined contribute 5–10%<sup>3–14</sup>

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(summarized in Figure 1). Improved treatments for primary cancers are allowing patients to live longer, leading to increased incidence of MBTs<sup>4,15,16</sup>.

Brain metastases are among the most feared complications for cancer patients because of diminished quality of life and a lack of effective chemotherapies. There are no predictive biomarkers to identify patients at risk to develop brain metastasis, and the healthcare system cannot bear the burden to screen all cancer patients for early detection of MBTs. Metastatic lesions are discovered when a patient presents with neurological symptoms; then, if left untreated, median survival is 1 month. The standard course of treatment for patients with controlled systemic disease is microsurgical resection plus radiation (whole-brain radiation therapy and/or stereotactic radio-surgery), extending median survival to 9–10 months<sup>17,18</sup>. Patients receiving radiation alone have a median survival of 4–6 months<sup>17,18</sup>.

Chemotherapeutic agents are not effective, partly attributed to their limited ability to penetrate the blood–brain barrier (BBB)<sup>19,20</sup>. Although new therapies are in development to penetrate the BBB<sup>21,22</sup>, recent evidence also suggests that the BBB may not be intact in patients with brain tumours. Promising preliminary studies indicate that molecularly targeted therapies (small-molecule kinase inhibitors against activated oncogenes) can cross the BBB<sup>23,24</sup> and correlate with modest improved outcomes<sup>25–28</sup>. Selected independent case studies report marked tumour regression and sustained response<sup>29–31</sup>.

The vast majority of MBTs develop from primary lung and breast cancers. The frequency at which lung and breast cancer patients develop brain metastases is approximately 25% and approximately 30%, respectively, but differs more specifically according to the histology or molecular subtypes of the systemic disease (described in detail in Figures 2 and 3)<sup>9,32–40</sup>. Stephen Paget first posited that the non random pattern of metastasis linked to specific primary cancers was not due to chance and that formation of metastatic tumours depends on interactions between the microenvironment of the metastatic site and the metastatic cancer cells<sup>41–43</sup>. Herein, we focus our review on this broadly accepted concept and what is known about the stages of metastasis as they relate to the understanding that cancer is ultimately a genetic disease with pathological phenotypes driven by oncogenic, somatic mutations.

## Discussion

### Stages in the metastatic process

Metastatic biological processes, including the role of microenvironment and the ability of cancer cells to transverse the BBB, have been extensively reviewed in great detail elsewhere<sup>44–46</sup>. Briefly, the stages of metastasis comprise, in temporal order of occurrence, local invasion, survival in the circulation, intravasation, extravasation, micrometastasis formation and metastatic colonization. Invasion depends upon an epithelial to mesenchymal transition (EMT) where epithelial cell traits are suppressed and mesenchyme cell traits are activated, losing cell polarity and cell–cell adhesion. Underlying EMT is a gene expression program regulated by epigenetic changes across the genome and aberrant expression of key transcription factors and micro-RNAs<sup>45,47,48</sup>. New evidence describes further complexity, where a reversal of the EMT process is required for colonized metastatic cells to proliferate<sup>49,50</sup>. This potential phenotypic plasticity is consistent with epigenetic principles inherent to the hierarchically organized cancer stem cell model, where cancer stem cells

undergo irreversible epigenetic changes to produce phenotypically diverse cancer cells<sup>51</sup>. Additional work does suggest that with the EMT process, cancer cells also acquire stem cell-like traits<sup>52</sup>. The resulting ‘cancer stem cells’ represent a minor subset of cells within a tumour. They maintain the capability for unlimited self-renewal, have the ability to seed new tumours and are a potential cell type from the primary tumour that initiates a metastatic lesion<sup>52</sup>.

Relatively large numbers of circulating tumour cells can be detected in the blood of carcinoma patients, including those who do not clinically develop distant metastases<sup>53</sup>. Of those numbers of circulating cells, approximately 80% undergo extravasation. However, once implanted at distant sites, fewer than 3% of cancer cells survive to form micrometastases and fewer than 0.1% persist to metastatic macro-colonization<sup>54,55</sup>. Macro-colonization is described as the rate-limiting step in metastatic tumour formation<sup>45</sup>. A related mechanistic hypothesis, consistent with the clinical observation of long latency periods for distant relapse following initial diagnosis<sup>56</sup>, posits that during the latent period, cancer cells, and likely also the surrounding microenvironment<sup>57</sup>, undergo gradual evolution to acquire molecular aberrations advantageous for metastatic colonization either because micrometastatic cell growth and death are equal or by discontinuous growth and periods of quiescence<sup>45,58</sup>. However, for brain metastasis from lung and breast cancers, the latency period is relatively short. The median latency from breast cancer diagnosis to MBTs is 34 months (with HER2 positive developing more quickly) and lung cancer-derived MBTs are detected within months of the initial diagnosis<sup>9,59–61</sup>. This suggests a different course for MBTs in which only a subset of cells (possibly stem cells) in the primary tumour has the molecular prerequisites for all metastatic stages. Alternative to a stem cell hypothesis, selection pressures within progressing primary tumours give rise to clonal evolution of tumour cells with acquired genetic variations and increased malignant or metastatic potential<sup>51,62</sup>. In line with this hypothesis, metastatic potential and inherent resistance against selective pressures are coincident with heightened genetic instability<sup>63</sup>.

The unique environment of the brain compared to other organs presents an optimum environment for metastatic progenitor cells arising from particular tissues, fitting with the nonrandom pattern of metastasis. While tropism and anatomical limitations are also thought to be pre-determinants, strong evidence suggests that the dominant influence is the nature of compatibility between the primary cell of origin and the site of metastatic colonization. et al. established this with a rodent xenograft model of human metastatic melanoma in which the same number of micrometastases was identified in the parenchyma of lung and kidney tissues embedded side by side; but metastatic tumours only developed in the lung fragment and not in the kidney fragment<sup>43</sup>.

Details of the biology of brain metastases are still emerging, but it appears that multiple widely expressed mechanisms in cancer cells are able to support intravasation, survival in circulation, extravasation and micrometastasis. However, a more select oncogenic mechanism drives colonization and growth. Putative oncogenic drivers of brain metastasis and whether they exist in the primary tumour or are acquired in disseminated cells are further addressed below.

## Oncogenes and tumour suppressors

Decades of research have contributed to the understanding that carcinogenesis is governed by the activation of oncogenes and inactivation of tumour suppressor genes. Oncogenes are activated by various mechanisms including gain-of-function sequence mutations, overexpression due to genomic amplification or deregulated epigenetics and genomic rearrangements<sup>64,65</sup>. Tumour suppressors, factors that otherwise slow normal cell growth processes or promote tissue differentiation, are inactivated by genomic lesions resulting in loss-of-function. Thus, within the tumour genome, the causes for initiation and progression of the disease can be found. Important clinical advances in primary cancer treatments are attributed to molecularly targeted treatments tailored to target activated oncogenes, e.g. HER2, EGFR, MET and BRAF<sup>64</sup>. Their aberrant presentation in the primary tumour genome presents a biomarker to indicate the efficacy of the targeted treatment. Moreover, oncogenic factors often possess kinase or ligand-binding function that present 'druggable' protein motifs<sup>66</sup>. The benefits of targeted treatments over traditional chemotherapy are clear, resulting in greater efficacy and less debilitating side effects<sup>67-69</sup>.

The success of driver oncogene-targeted therapies against specific primary cancers can also be attributed to the fact that oncogene-directed signalling promotes the many phenotypes that define the pathobiology of cancer cells including unrestricted growth, immune system evasion, metabolic transformation, invasive potential and cancer cell tumorigenicity (local and metastatic)<sup>70,71</sup>. This wide array of biological impacts suggests that somatic genome mutations effecting activated oncogenes and inactivated tumour suppressors are likely causally implicated in all stages of MBT development.

## Genomic mutation landscape of metastatic brain tumours

Early studies indicate that molecularly targeted therapies in the form of small-molecule kinase inhibitors and monoclonal antibodies against activated oncogenes can elicit a response in the treatment of metastatic tumours and potentially reduce the rates of metastatic recurrence<sup>25-30,72,73</sup>. On the other hand, increased incidence of MBTs in patients with controlled primary cancer and peripheral metastasis has also been attributed to the inefficiency of targeted drug transfer across the BBB<sup>16</sup>. Although further investigation is required, each of these data indicates that an oncogene mechanistically linked to the primary cancer is also implicated in driving the incidence of MBTs. This scenario may apply to the mutated expression of BRAF oncogene in metastases from melanoma<sup>27</sup>, aberrant levels of HER2 oncogene in breast cancer brain metastases<sup>30</sup> and mutated EGFR oncogene in lung cancer brain metastases<sup>28</sup>.

The potential importance of BRAF, HER2 and EGFR for brain metastasis was identified based on knowledge of their driver oncogene status and frequency of mutation in the related primary cancer. Their specific oncogenic role and frequency of mutation in MBTs remain to be ascertained. Additionally, it is unclear if for a given oncogene certain types of activating mutations are more often represented in brain metastases, as early evidence suggests<sup>74,75</sup>. Studying these unknowns will explain why brain metastases develop in cases where systemic disease is treated with targeted therapies and why treating MBTs has had only modest effects and no impact on therapy options. An important starting point is

comprehensive genomic characterization of MBTs compared to their primary cancers, particularly the well-characterized molecular breast and lung cancers subtypes. Despite the large number of patients afflicted, the characterization of significant cancer-linked genomic copy number aberrations, rearrangements and sequence mutations in MBTs is only in its infancy.

**Gene coding mutations**—Most studies to date that have examined somatic DNA coding mutations in MBTs have been biased towards the activating mutations in oncogenes or tumour suppressors previously associated with the primary cancer. A limited study that included DNA from 10 melanoma metastases measured a panel of BRAF, NRAS, AKT, PIK3CA and KIT activating mutations using a highly sensitive primer extension, and mass spectrometry approach showed that BRAF activating mutations were the most frequent and secondarily NRAS, similar to primary melanoma<sup>76</sup>. A later study using Sanger sequencing identified BRAF and NRAS mutations in both primary melanoma and matched brain metastases from 44 patients at 80% consistency<sup>77</sup>. Another study measured mutations in 19 oncogenes, including BRAF, KRAS, NRAS and PIK3CA, by high-resolution DNA melting analysis of specimens from matched brain metastases and primary colorectal cancer. The study recapitulated that KRAS mutations were a more common event in colorectal cancer and that 9 of 10 matched brain metastases were 100% concordant with mutations observed in the primary cancer<sup>78</sup>. A breast cancer study that analysed 39 matched pairs of primary breast cancers and brain metastases using primer extension mass spectrometry investigated mutations in EGFR, HRAS, KRAS, NRAS and PIK3CA<sup>79</sup>. NRAS and PIK3CA mutations were identified independently in samples from two patients, in both the primary and metastatic specimens. An EGFR mutation was identified in breast cancer from a single patient, but not in the matched metastasis<sup>79</sup>.

From studies of Japanese populations, the frequencies of EGFR mutations in MBTs from non-small cell lung cancer (NSCLC) are similar to those reported for the primary tumour in the same population<sup>80,81</sup>. Results from studies investigating EGFR mutations in NSCLC-derived MBTs from Caucasian patients indicate that the mutation frequency in EGFR is 2%<sup>82-84</sup>, markedly less frequent than the estimated 10% in NSCLC primary tumours<sup>85</sup>. However, where a mutation was discovered in the metastasis, it was concordant with a mutation in the primary cancer. These results indicate that coding mutations in the investigated oncogenes are either equivalent or less represented in the MBT compared to the primary tumour, although the body of work is inadequate to draw any conclusions with certainty. However, evidence suggests that the incidence of mutations in tumour suppressor genes may increase in metastatic cancer<sup>86</sup>.

Whether or not an implicated driver oncogene is discovered in both the primary and metastatic cancer or solely in the brain metastasis is an important distinction that provides rationale for adapting targeted treatments, or developing predictive biomarkers for MBTs. Ding et al. directly investigated whether processes leading to macrometastases are driven by mutations that pre-exist in primary tumour cells or arise at the distant site using massively parallel DNA sequencing methods to analyse three related specimens: a primary triple negative basal-like breast cancer, the tumour derived from a mouse xenograft model and the matched MBT that developed within 8 months of primary diagnosis<sup>75</sup>. For coding

mutations, 48 of 50 validated somatic gene mutations were detected in DNA isolated from all three tumour samples, supporting the notion that coding mutations are consistent for primary and metastatic cancer. The mutation allele frequency for 26 of those was significantly increased in the metastases or xenograft specimens, and the allelic frequency of two mutations was decreased. The observed levels of enrichment and loss of enrichment (but still detectable) for specific coding mutations in the metastasis and xenograft specimens may indicate that a subset of primary tumour cells carrying the array of mutations in the primary, not just a single clone, were contributing<sup>75,87</sup>. Unlike coding mutations, copy number aberrations were gained in the metastasis and xenograft specimens, such that 19% and 39%, respectively, were unique compared to the primary cancer<sup>75</sup>. Comparable work also using massively parallel sequencing to investigate an oestrogen receptor alpha-positive breast tumour arrived at a differing conclusion. Here, the metastasis was detected 9 years after the initial diagnosis<sup>88</sup>. Of the 32 non-synonymous coding mutations detected in the metastatic cancer, 19 were absent in the primary tumour, 5 were prevalent in the primary tumour and 6 were present at lower allelic frequency levels than the primary tumour<sup>88</sup>. The list of mutated genes identified in these studies of individuals has little translational value because a substantial number of specimens are required to elucidate specific, recurring causally implicated gene mutations. However, these two studies intimate highly valuable insights surrounding the potential for sequence and structural mutations and the potential for varied mechanisms driving metastatic cancer associated with short versus long latency.

**Somatic copy number variations**—The observation made by Ding et al. that the incidence of copy number alterations is increased in metastasis and xenograft specimens<sup>75</sup> lends itself to the re-interpretation of other studies. For example, low-resolution arrayed, comparative genomic hybridization (aCGH) studies have highlighted recurring, broad copy number variation in primary cancers that develop brain metastases, positing that the specific loci represent risk markers of functional consequence<sup>89,90</sup>. Further validation is required as to whether the broad regions harbour true driver lesions, or if they represent greater genomic instability underlying other structural mutations with a role in metastasis. Nevertheless, studies do show significant frequencies of copy number aberrations in specific known cancer genes in brain metastases. Two studies of breast cancer brain metastasis suggest that gains in EGFR oncogene and loss of PTEN tumour suppressor are more frequent in metastases, one using aCGH methods and the other fluorescent in situ hybridization (FISH)<sup>91,92</sup>. Two additional studies reporting on FISH analysis of lung cancer brain metastases suggest a marginally higher frequency of EGFR copy number gain (by amplification or polysomy) in brain metastases compared to the matched primary cancer<sup>82,83</sup>.

Markedly high rates of brain metastases are observed for patients with HER2-amplified breast cancers<sup>93</sup>. Indications are that the frequency of HER2-positive status is increased in brain metastases over other peripheral metastases, although this may be confounded by the use of HER2-targeted treatments that may be more effective in the treatment of peripheral metastases and restricted in reaching brain tumours<sup>16,92</sup>. Additional work by Palmieri et al. that analysed HER2 expression in 124 specimens reinforces that the rate of HER2 overexpression largely associated with gene amplification is 36% in brain metastases, a

10% increase over rates of amplification and overexpression reported in primary breast cancers<sup>94,95</sup>. The functional consequence of HER2 amplification and overexpression was demonstrated by this same group using a human cancer cell line model of forced HER2 overexpression, which when introduced into mice yielded threefold greater numbers of brain macrometastases compared to the parent cell line xenografts<sup>95</sup>.

**Whole-genome expression studies**—The variety of possible structural and coding mutations and epigenetic deregulation establish the foundation for aberrant gene expression that is the biomolecular link from the aberrant genome to transformed cancer cell function. Whole-genome and transcriptome studies of primary cancers are used to identify genes or transcripts displaying significant cancer-specific expression and are especially useful to elucidate cancer cell-specific systems and pathway level dysfunction. Additionally, diagnostics based on gene expression signatures have proven effective at predicting lymph node metastases and improving patient outcomes<sup>96</sup>. Limited recent gene expression data also suggest mechanisms underlying brain metastasis. A study from Da Silva et al. compared 15 matched primary triple negative, basal breast cancers and their matched brain metastatic cancers using microarrays that simultaneously analysed 512 cancer-linked genes. Twenty-seven genes differed significantly, notably HIF1A and HER3, which were markedly upregulated<sup>79</sup>. This suggests a role for the epidermal growth factor receptor family in even the triple negative breast cancer subtype. Also, the HIF1A transcription factor activates genes encoding glucose transporters and glycolytic pathway genes, and an important role for HIF1A is supported by independent work showing more frequent overexpression in patient brain metastases compared to primary breast cancer tissues and enhanced aerobic glycolysis in cells migrating to or implanted in the brain versus parent cells in mouse models of human cancer cell line xenografts<sup>97,98</sup>. Multiple lines of whole-genome investigation in cell lines and patient specimens by Bos et al. identified upregulated expression of an EGFR ligand HBEGF and cyclooxygenase PTGS2 and the ectopic expression of the brain-specific gene  $\alpha$ 2,6-sialyltransferase ST6GALNAC5 in breast cancer cells as factors contributing to their ability to cross the BBB<sup>99</sup>. Increased PTGS2 expression is also identified as an important factor in brain metastasizing melanoma<sup>100</sup>.

While the described studies highlight what is unique to the metastatic cancer and do not address when or how the features arise, multiple studies present evidence for many, potentially coexisting possibilities. Studies combining analysis of methylation and gene expression to elucidate recurring features in breast cancer specimens associated with the incidence of brain metastasis do suggest pre-existing features supportive of metastasis, particularly related to genes implicated in EMT<sup>101–103</sup>. Analysis of substantial numbers of primary and metastatic breast tumours using whole-genome expression assays was successful in identifying gene expression signatures preserved in both primary and metastatic cancers<sup>104,105</sup>, suggesting that the bulk of tumour cells in the primary lesion and the metastasis are similar. Work by Park et al. following a xenograft model of metastases presents specific evidence that metastatic breast cancer cells acquire expression patterns found in neuronal cells, thus indicating that an evolutionary process underlies MBT development<sup>106</sup>.

## Conclusion

### Outlook for developing novel therapeutic strategies

The current lack of insight into brain metastases spawns many questions (depicted graphically in Figure 4), including whether or not the primary cancer drivers are also the drivers of the related MBT. Conversely, early data suggests that unique drivers may be responsible for MBT development, raising additional key questions. Does a new driver/s emerge with the gain of mutations at the metastatic site? Or alternatively, do cells derived from a minor subclone in the original tumour harbour lesions with the capacity to evade primary adjuvant therapy and seed the metastatic occurrence. Much research is still needed to fill the void and answer these critical questions.

Patients afflicted with MBTs are in dire need of more effective therapies, and clinicians need predictive laboratory tests to identify patients at risk of developing MBTs. The as yet unrealized comprehensive analysis of MBT genomics is necessary to meet these needs. Moreover, without improved understanding of the genomic aberrations that drive MBTs, development of biomarkers and molecularly targeted therapies will remain stalled and patient outcomes will continue to be dismal.

### Abbreviations list

<b>aCGH</b>	arrayed, comparative genomic hybridization
<b>BBB</b>	blood–brain barrier
<b>EMT</b>	epithelial to mesenchymal transition
<b>FISH</b>	fluorescent in situ hybridization
<b>MBT</b>	metastatic brain tumour
<b>NSCLC</b>	non-small cell lung cancer

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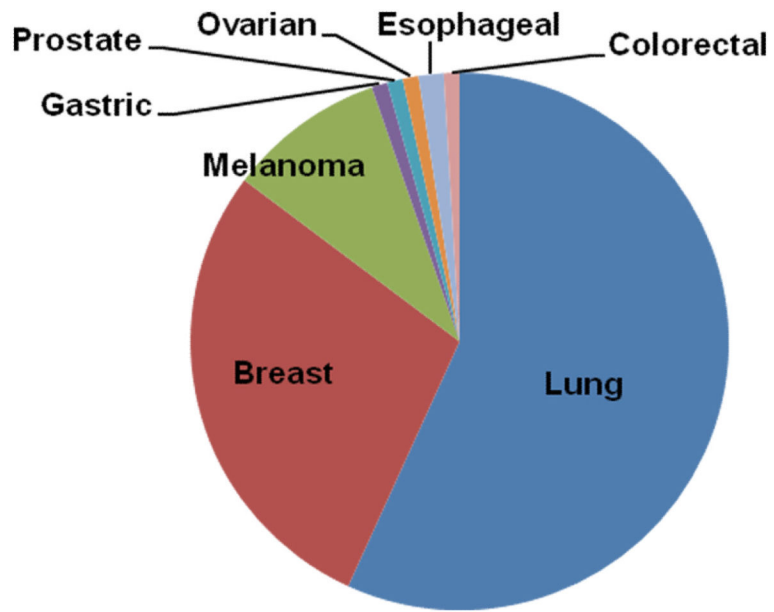
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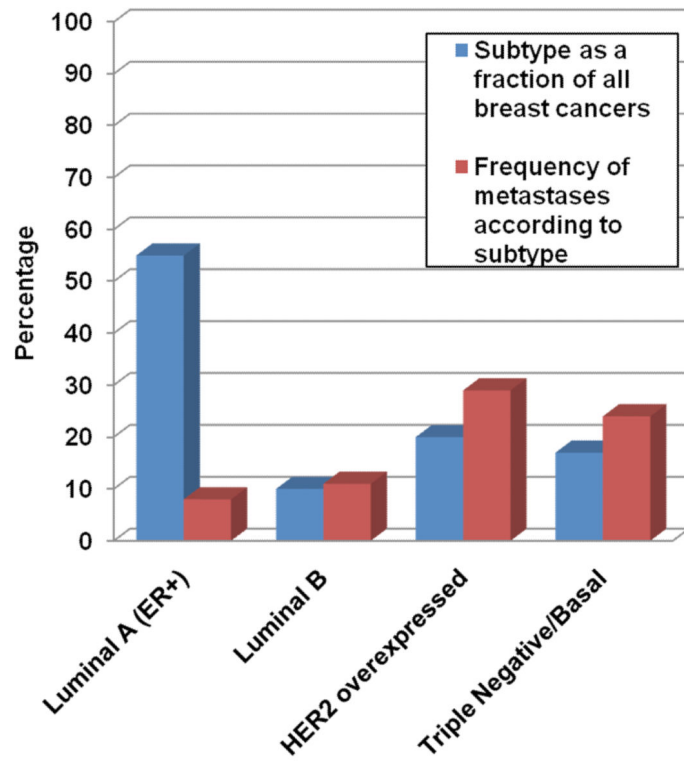
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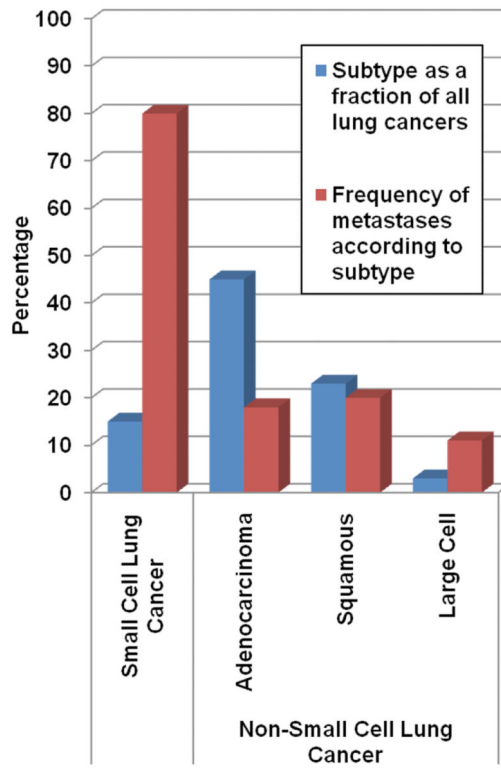


**Figure 1.**  
Breakdown of brain metastatic tumors according to originating cancer.

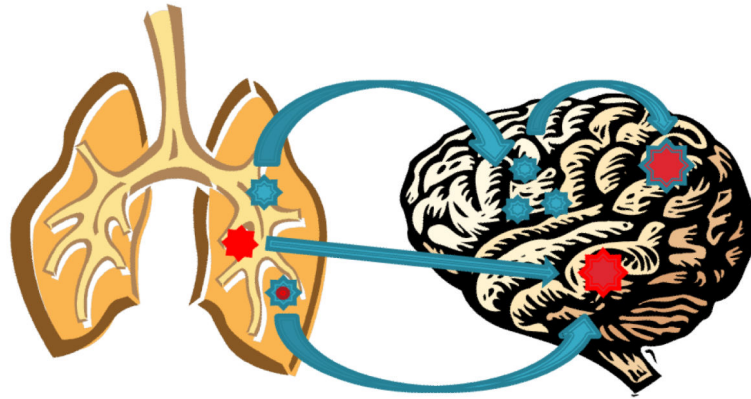


**Figure 2.** Frequency of occurrence for metastatic brain tumors according to breast cancer molecular subtype.





**Figure 3.** Frequency of occurrence for metastatic brain tumors according to lung cancer subtype.



**Figure 4.** Potential pathways from cancer cell somatic mutations to metastatic colonization and metastatic tumor growth. Upper arrows in sequence depict how disseminated cancer cells may acquire further metastatic growth promoting mutations. Middle and lower arrows depict how cells from a major or minor subclone fraction in the original tumor may harbor mutations that specifically support metastatic tumor growth.