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Understanding susceptibility to breast cancer metastasis: the genetic approach

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SUMMARY

Metastasis is a complex phenotype that is not discrete, is polygenic, varies in range over the entire population and follows non-Mendelian inheritance. Recent evidence indicates that inherited susceptibility affects not only the development of the primary tumor, but is also an important factor in progression and metastasis. Since metastasis accounts for the majority of breast cancer deaths, identification and understanding of the genetic modifiers of metastasis underlies success of personalized therapy. Studies from our laboratory and others have now characterized several metastasis susceptibility factors. While an important step forward, these certainly do not describe the entire metastatic phenomenon and efforts continue to expand this knowledge. Here we review the complex metastatic process and current knowledge on the genetics of breast cancer metastasis, including germline polymorphisms that have been associated with the disease.

Breast cancer is the most common form of cancer among women in the USA, and is the second leading cause of cancer-related deaths worldwide. In the USA in 2013 alone, approximately 40,000 women were predicted to die from breast cancer [1]. Cure rates, however, are remarkably high when the tumor remains confined to breast tissue. By contrast, those patients with disseminated tumors show a significant decrease in survival. Better understanding the mechanisms of metastasis and new treatment strategies to target the vulnerabilities of the secondary tumors would therefore provide the greatest decrease in patient morbidity and mortality. One of the many difficulties in executing this strategy, however, is the fact that breast cancer metastases can remain dormant for long periods of time and surface as incurable lesions long after the primary tumor has been removed. At present our ability to predict those patients who are likely to develop metastatic disease is imperfect. As a result, a significant fraction of patients currently receive adjuvant therapy who do not benefit from this antimetastatic treatment strategy. Improvements in antimetastatic therapies will therefore hopefully not only prolong patient survival, but also spare some patients the unnecessary morbidities associated with current adjuvant therapy.

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Metastasis is an evolutionary process that results in departure of tumor cells from their original location and their establishment at a secondary site. Metastasis has been likened to seed and soil, where the tumor cells shed from the primary tumor are like seeds that are disseminated into the bloodstream [2]. Similar to successful implantation and germination, the tumor ‘seeds’ must fall on fertile soil, an environment that allows them to establish themselves and grow. Similar to seeds, many tumor cells die after they are disseminated and before they reach a distant organ, while some of them have the capacity to overcome host defense and colonize another site. Efforts over many years have revealed that this process encompasses more than simple tumor autonomous events. Factors that determine this capability range from environmental, both micro- and macro-, somatic mutational events and even inherited genetic susceptibilities.

The metastatic process

The metastatic cascade is a complicated process that involves multiple tumor-associated events and, as recent progress has revealed, incorporation of nontumor tissue factors as well [3]. Within the tumor tissue metastasis is associated with a combination of mutations, genomic instability and epigenetic alterations. Genetic changes that help initiate metastasis aid proliferation, angiogenesis, survival and invasion, some of which are the same prerequisites as those for primary tumor establishment. Gene-expression studies of cells that are able to establish themselves as distant metastases reveal genes that are coexpressed in the primary tumor as well as some that are not detected in the primary tumor [4]. A 54-gene signature associated with breast cancer metastasis to the lung includes epiregulin, *CXCL1*, *COX2* and *MMP1* that are expressed in both primary tumor and metastatic cells, as well as other genes such as *SPARC*, *MMP2*, *VCAM1* and *IL13RA2* that are largely expressed in lung metastatic populations [5]. A brain metastasis signature reveals ST6GALNAC5, a sialyltransferase that is specifically expressed in brain metastases of breast tumor cells. While it does not impact growth of tumor cells or distant metastasis to the lung, it specifically enhances tumor cell migration across the blood–brain barrier and infiltration into the brain [6]. These results indicate that some factors that promote metastasis might be the same as the ones that aid primary tumorigenesis, while others may be distinctly different. Some cells of the primary tumor that are endowed with these special features are able to attract vasculature, overcome restraints of gap and tight junctions and intravasate into capillaries. Tumor cells that lodge themselves in host capillaries render these vessels leaky and disseminate into host circulation. This allows them to be carried to secondary organs. Epithelial to mesenchymal transition of these cells enhances their plasticity, motility and invasive capability that helps them extravasate out of the vascular circulation or lymphatics and into the secondary tissue parenchyma.

Once disseminated, tumor cells must land on congenial soil for them to thrive. They must exit the vasculature they are embedded in and survive the hostility of the secondary organ. Different tumor types preferentially metastasize to different organs. The target organ of choice or organ tropism depends on circulation routes and genetic factors that render them competent to thrive in a secondary location. Gene expression signatures have correlated certain primary tumors with organ-specific metastasis, indicating that tumor-derived transcriptional programs play a role in target site selection. Breast tumors typically

metastasize to the lung, bone and lymph nodes but may differ from one another in their capacity to metastasize, the extent of metastasis as well as in their metastatic site of preference in a genetically dependent manner. Estrogen receptor-positive mammary tumors have higher propensity to metastasize to bone, while estrogen receptor-negative tumors more readily metastasize to visceral organs. Luminal breast tumors preferentially metastasize to the bone [7].

In addition, there is an important stromal component to metastatic colonization. Recent evidence suggests that primary tumors mobilize bone marrow-derived cells that arrest in secondary organs prior to the arrival of disseminated tumor cells [8]. It is thought that the tumor cells that arrest at or near these bone marrow-derived cells are the probable origin of the clinically relevant metastatic lesions. The bone marrow-derived cells are believed to provide a more hospitable local microenvironment, the premetastatic niche that promotes tumor cell survival and eventual expansion [9].

As mentioned earlier, dormancy, or the residency of tumor cells at the secondary site in a subclinical state, remains a major problem for elimination of metastatic disease. In breast cancer these cells can remain in a subclinical state for years or decades before growing into detectable lesions. Since metastases can arise even after adjuvant therapy, these cells are either inherently resistant to the current adjuvant therapies and/or are in a nonproliferative state and are therefore not susceptible to antiproliferative agents.

The conversion of single disseminated tumor cells to proliferative lesions represents one of the most important targets for control of metastatic disease. Evidence suggests that tumor cells disseminate from the primary tumor well before the clinical manifestation of the primary tumor [10]. Thus, the metastatic seed has already been sown before the initial treatment and resection of the tumor. Identification of the events that result in conversion to clinically relevant lesions are, however, probably the most difficult to directly visualize. Since these cells remain resident in tissues for years or decades, and because, fortunately, only a tiny fraction of disseminated cells are capable of completing the metastatic cascade, it is not currently possible to know which cell the progression occurs in, or when the important events happen.

Genetics of breast cancer metastasis

Meiotic genetics provides one method for indirectly visualizing the mechanisms associated with metastatic disease. Meiotic genetics has played an important role in our understanding of inherited susceptibility to cancer. Early studies of mouse inbred strains demonstrated that genetic background plays an important role in tumor susceptibility. Subsequently, studies of high-risk cancer families led to the identification of tumor suppressor genes in humans. For example, breast cancer *BRCA1* and *BRCA2* gene mutations are the most common cause of hereditary breast cancer [11–14]. These mutations confer a breast cancer risk of 60–85% [15]. Genetic screening for *BRCA1* and *BRCA2* mutations is in common current use to identify individuals at risk of developing breast cancer [16]. *p53*, *PTEN*, *CHEK2*, *ATM* and *STK11* mutations are some other significant risk factors [17]. Besides these, single nucleotide

polymorphisms (SNPs) in *TGF- β 1*, *PGR* and *CASP8* have been associated with breast cancer risk [18].

Since mortality from breast cancer largely results from metastasis, identifying genetic determinants of breast cancer metastasis is of potential great importance, both for clinical prognosis as well as treatment. As various studies are increasingly revealing, pure metastasis effector genes exist, those that do not necessarily impact primary tumor growth, indicating that processes other than proliferation (extravasation, colonization) limit metastasis. Genetic alterations, such as mutations, deletions, chromosomal rearrangements, epigenetic changes, gene silencing, microRNA-mediated effects, post-transcriptional and post-translational modifications, may participate in the many steps of the metastatic process. Although the genetics of metastasis is less well studied than that of primary breast tumorigenesis, some metastasis modifier genes are known. *NM23*, which encodes a histidine kinase, was the first *bona fide* metastasis suppressor gene cloned [19]. *KISS1*, *BRMS1* and *MKK4* have also been identified as metastasis suppressor genes. HER2, MMP1, EREG and COX2 are potent promoters of breast cancer metastasis [3, 20]. Upregulation of HER2 is a classical oncogenic event, while MMP1, EREG and COX2 aid vascular remodeling, angiogenesis and extravasation [21]. EZH2, a member of the polycomb repressor complex 2, promotes histone methylation and is associated with invasive growth in metastatic breast cancers [22]. Investigations into these as well as other metastasis-associated genes are providing new insights and potential targets for clinical intervention.

Role of host polymorphisms in inherited metastasis susceptibility

However, studying families with a high risk of developing cancer, such as those that harbor mutations in *BRCA1*, *BRCA2*, *p53*, *PTEN* and other genes as discussed above, is only one use of meiotic genetics to better understand tumor initiation and progression. Studying how the polymorphisms that make us all unique individuals is another way to study cancer development using meiotic genetics. Polymorphisms are short stretches of nucleotides that differ between members of a species or paired chromosomes in humans. A SNP is a DNA sequence variation that arises due to a difference of a single nucleotide. SNPs may occur in coding or noncoding regions of genes or in intergenic regions. While SNPs in coding regions are often not consequential due to degeneracy of the genetic code, those in noncoding regions may alter transcription factor binding, splicing, methylation or mRNA degradation and may lead to altered gene expression.

With the exception of identical twins, in nature each individual has their own unique set of SNPs. Many of these polymorphisms effect gene function or expression levels in subtle ways, as compared with the gain or loss of function for the genes associated with high-risk cancer families. The sum of all of the polymorphisms is what makes each of us unique individuals, with different morphometric characteristics as well as disease resistances or susceptibilities.

Identifying those polymorphisms that influence disease onset or progression therefore provides another lens through which to examine the underlying mechanisms. In addition, since polymorphisms exist in all tissues of an individual the effect of the variant can be on

any cellular component associated with disease. In the case of cancer, for example, the variants can impact tumors in an autonomous manner by changing proliferation rates, or in a nonautonomous manner influencing the ability of cytotoxic T cells to effectively eliminate disseminated cells. Furthermore, since the polymorphisms are present in an individual from birth, they can impact the disease anywhere along the process. Moreover, functional polymorphisms can be identified using end point assays or retrospective data, since they serve as a genomic ‘fingerprint’ of what was important during the course of the disease, without the investigator actually having to directly observe it. A recent study that reports the sequencing and analysis of mRNA and microRNA of a large human cohort from five populations including the CEPH, Finns, British, Toscani and Yoruba populations discovers widespread genetic variation affecting regulation of most genes [23]. Once the polymorphic variants and their associated genes are identified, investigators can reconstruct the probable mechanism based on gene ontology, functional clues and direct experimentation, much like reconstruction of crime scenes from various clues.

Identification and analysis of inherited polymorphisms that affect metastatic progression forms the focus of our laboratory [24,25]. The genetic basis of breast cancer metastases has been best demonstrated by Lifsted *et al.* by varying the background on which the tumors from a common oncogenic event arose [26]. Male FVB mice carrying the *MMTV-PyMT* oncogene were crossed to females of various inbred strains. The FVB/N–*MMTV-PyMT* is a highly aggressive metastatic mouse model that develops palpable tumors in approximately 5 weeks of age, large tumors and pulmonary metastases in about 100 days of age [27]. Interestingly, varying the maternal genotype significantly altered the metastasis capacity of this tumor model. Lifsted *et al.* illustrated that compared with the standard FVB/FVB F1 generation, *MMTV-PyMT*-driven mammary tumors had lower metastatic density when crossed to some inbred strains like LP/J, NZB/B1 NJ, DBA/2J and C57BL/6JNCr, while it was enhanced in other cases such as the C57BL/10J and AKR. Since the oncogenic event, activation of the *PyMT* transgene, remained the same, this genetic dependence of metastasis is attributed to polymorphisms.

We subsequently bred strains of low metastatic potential, such as the DBA and the NZB, with strains of high metastatic potential, such as FVB and AKR, in recombinant inbred back crosses and used these panels to identify quantitative trait loci (QTLs) that control metastasis. Using this strategy, we identified metastasis QTLs on chromosomes 6, 7, 9, 17 and 19. Haplotype mapping revealed *SIPA1* as a candidate metastasis modifier gene with a polymorphism in its PDZ domain across the genotypes investigated above [28]. *SIPA1* has RAP-GTPase enzymatic activity, while the amino acid change associated with the SNP abrogates this activity. Overexpression of *SIPA1* enhanced pulmonary metastasis in an orthotopic transplantation assay, while knockdown of *SIPA1* reduced the number of lung metastases, indicating that *SIPA1* is a metastasis promoter. Pilot epidemiology studies performed in cohorts from Orange County (CA, USA) [29] and Rotterdam (The Netherlands) [30] suggest variants in *SIPA1* are also associated with distant metastasis-free survival in human breast cancer patients.

Another QTL analysis was carried out to determine if the loci driving expression of extracellular matrix (ECM) genes (that are often dysregulated in cancer and predict survival)

and those that control metastasis susceptibility are the same. This revealed several genes, one of which was RRP1B, a protein that interacts with SIPA1 and appears to be coregulated [31]. RRP1B interacts with the polymorphic PDZ domain of SIPA1 and in so doing disrupts the RAP-GTPase activity of SIPA1. A gene-expression signature derived from ectopic expression of RRP1B in mouse breast cancer cell lines includes several ECM genes and predicts survival in human datasets [32]. RRP1B associates with various nucleosome-binding proteins and transcription factors and with markers of heterochromatin and euchromatin, indicating that RRP1B might regulate gene expression in a dynamic way. Recent studies also reveal that different polymorphisms of RRP1B differentially modulate the expression of several transcription factors, a mechanism that could account for the role of RRP1B polymorphism in metastasis susceptibility [33]. Analysis of two human breast cancer cohorts demonstrates an association between a nonsynonymous RRP1B SNP, rs9306160 (1421G→A) and improved outcome.

A significant metastasis suppressor that emerged from an expression QTL analysis for ECM gene expression in the AKXD recombinant inbred panel (F2 progeny from a cross between the highly metastatic AKR and low metastatic DBA strains) was BRD4 [34]. BRD4 is a member of the BET (bromodomain and extra terminal) family that is characterized by the presence of two tandem bromodomains in its members [35]. BRD4 has two alternatively spliced isoforms that have a distinct 3' UTR. While both isoforms have the same N-terminal region containing the bromodomains, the C termini are distinct. Ectopic expression of the shorter isoform enhances metastasis, as opposed to the ectopic expression of the long form that inhibits cell invasion and motility *in vitro*, alters expression of some ECM genes and reduces both primary tumor growth and metastatic capability of mouse mammary cancer cell lines *in vivo* [33]. Gene-expression signature derived from these cells predicted survival in multiple human breast cancer datasets. Similar to RRP1B, BRD4 isoforms also interact with SIPA1. The short isoform has been recently shown to interact with SIPA1, RRP1B, as well as with members of the LINC complex at the inner nuclear membrane, and may thus alter cellular mechanotransduction (Figure 1) [36]. BRD4-long form decreases the Rap-Gap activity of SIPA1 [37]. It is intriguing that while RRP1B and BRD4-long form have opposite effects on the RAP-GAP activity of SIPA1, they both suppress metastasis. The detailed mechanism of their action remains to be determined, but perhaps one or both proteins function independently of RAP-GAP activity in altering metastasis. BRD4 may also function as a metastasis suppressor by promoting a differentiated state. This is revealed by analysis of a C-terminal mutant of BRD4 that displays a highly mesenchymal morphology and high expression of stemness markers [38]. BRD4 has two alternatively spliced isoforms that have a distinct 3' UTR. While both isoforms have the same N-terminal region containing the bromodomains, the C termini are distinct. Ectopic expression of the shorter isoform enhances metastasis, as opposed to the reduced colonization with the long form of BRD4. How the two variants lead to these unique effects is still under investigation. Nevertheless, BRD4 holds great therapeutic promise. A small molecule inhibitor of BRD4, JQ1 that displaces both isoforms of BRD4 from chromatin (and therefore creates a BRD4 knockout environment) has been shown to display antiproliferative and differentiating effects [39].

ARID4B, AT-rich interactive domain 4B, is a metastasis modifier gene, also associated with polymorphisms in the AKXD recombinant inbred panel of mice [40]. *ARID4B* is a metastasis promoter, with ectopic expression resulting in enhanced primary tumor growth *in vivo* and enhanced cell motility and invasion *in vitro*. Knockdown of *ARID4B* decreases pulmonary metastasis, while high expression of *ARID4B* is associated with increased risk of metastasis in human breast cancer patients. Similar to *RRP1B*, *ARID4B* interacts with several chromatin-associated factors, including the Sin3A HDAC complex.

While new metastasis susceptibility genes continue to be identified both in our laboratory and in others, a common mechanism appears to be emerging associated with chromatin interactors and modifiers. Changing the state of chromatin could alter the ratio of heterochromatin and euchromatin, move the cells into a more 'stem' form, affect DNA repair and gene transcription and alter physicochemical properties of the cells that would cause abnormal proliferation, invasiveness and survival. It is not then unexpected that chromatin modification emerges as a common theme for metastasis modifiers. Histone modifications correlate with patient disease outcome and are potential biomarkers of different forms of cancer. Agents to reverse the modifications identified as causal to cancer, such as histone demethylases, offer potential therapeutic strategies, although we are far from their implementation for this purpose.

Conclusion & future perspective

Despite this knowledge, metastasis remains the major cause of solid tumor-related deaths. Environmental effects further confound the scenario already complicated by genetic complexity and diversity. However, rapid technological advances make us more hopeful today that the mammoth task of identifying all polymorphisms that underlie metastasis is achievable. Advanced QTL analyses now utilize recombinant and congenic inbred strains, heterogenous crosses, chromosome intercross lines, genome tagged mice and advanced intercross lines to identify complex trait modifiers. Sophisticated statistical and bioinformatics analyses help better inform biological experiments. A new resource called the Collaborative Cross (CC) initiated by a community effort in 2004 has increased the genetic diversity for mapping studies [41]. The CC, developed by the complex trait community, is a large panel of new inbred mouse strains that provide a large genetic diversity to study a trait of interest. The CC is an eight-way cross of mouse strains comprising A/J, C57BL/6J, 129Sv/ImJ, NOD/LtJ, NZO/H1J, CAST/EiJ, PWK/PhJ and WSB/EiJ strains, which together constitute more than 90% of the genetic diversity seen in laboratory mice and capture approximately two-times the number of SNPs found in humans. A further advance from the CC cross is the Diversity Outbred (DO) mice, being developed by the Jackson laboratory [42]. It utilizes breeding lines from the CC cross as its founder strains, but the DO mice, unlike the CC lines, are maintained as outbred through random mating. Each of the DO mice has a unique genome and thus represents the human population more closely. However, it also means they are not reproducible and that entire genomes of all mice under study have to be genotyped. The CC and DO mice have a much greater resolution than previously used crosses, of the order of 1 Mb, and are currently being used in several systems genetics-based population studies to unveil QTLs underlying various phenotypes. These and even better tools will eventually educate us suitably to enable better

prognosis and design of individualized therapies and perhaps alleviate some of the mortality that arises from the sudden emergence of long-dormant metastases.

References

Papers of special note have been highlighted as:

▪ of interest

1. American Cancer Society. Cancer Facts and Figures 2013. USA: American Cancer Society, GA; 2013.
2. Paget S. The distribution of secondary growths in cancer of the breast 1889. *Cancer Metastasis Rev.* 1989; 8(2):98–101. [PubMed: 2673568]
3. Nguyen DX, Massague J. Genetic determinants of cancer metastasis. *Nat. Rev. Genet.* 2007; 8(5): 341–352. [PubMed: 17440531]
4. Minn AJ, Gupta GP, Padua D, et al. Lung metastasis genes couple breast tumor size and metastatic spread. *Proc. Natl Acad. Sci. USA.* 2007; 104(16):6740–6745. [PubMed: 17420468]
5. Minn AJ, Gupta GP, Siegel PM, et al. Genes that mediate breast cancer metastasis to lung. *Nature.* 2005; 436(7050):518–524. [PubMed: 16049480]
6. Bos PD, Zhang XH, Nadal C, et al. Genes that mediate breast cancer metastasis to the brain. *Nature.* 2009; 459(7249):1005–1009. [PubMed: 19421193]
7. Kennecke H, Yerushalmi R, Woods R, et al. Metastatic behavior of breast cancer subtypes. *J. Clin. Oncol.* 2010; 28(20):3271–3277. [PubMed: 20498394]
8. Kaplan RN, Riba RD, Zacharoulis S, et al. VEGFR1-positive haematopoietic bone marrow progenitors initiate the pre-metastatic niche. *Nature.* 2005; 438(7069):820–827. [PubMed: 16341007]
9. Psaila B, Kaplan RN, Port ER, Lyden D. Priming the ‘soil’ for breast cancer metastasis: the pre-metastatic niche. *Breast Dis.* 2006; 26:65–74. [PubMed: 17473366]
10. Klein CA. The systemic progression of human cancer: a focus on the individual disseminated cancer cell—the unit of selection. *Adv. Cancer Res.* 2003; 89:35–67. [PubMed: 14587870]
11. Hall JM, Lee MK, Newman B, et al. Linkage of early-onset familial breast cancer to chromosome 17q21. *Science.* 1990; 250(4988):1684–1689. [PubMed: 2270482] ▪ First identified the association of *BRCA1* and breast cancer.
12. Futreal PA, Liu Q, Shattuck-Eidens D, et al. *BRCA1* mutations in primary breast and ovarian carcinomas. *Science.* 1994; 266(5182):120–122. [PubMed: 7939630]
13. Miki Y, Swensen J, Shattuck-Eidens D, et al. A strong candidate for the breast and ovarian cancer susceptibility gene *BRCA1*. *Science.* 1994; 266(5182):66–71. [PubMed: 7545954]
14. Wooster R, Bignell G, Lancaster J, et al. Identification of the breast cancer susceptibility gene *BRCA2*. *Nature.* 1995; 378(6559):789–792. [PubMed: 8524414]
15. Bradbury AR, Olopade OI. Genetic susceptibility to breast cancer. *Rev. Endocr. Metab. Disord.* 2007; 8(3):255–267. [PubMed: 17508290]
16. Gage M, Wattendorf D, Henry LR. Translational advances regarding hereditary breast cancer syndromes. *J. Surg. Oncol.* 2012; 105(5):444–451. [PubMed: 22441895]
17. Njiaju UO, Olopade OI. Genetic determinants of breast cancer risk: a review of current literature and issues pertaining to clinical application. *Breast J.* 2012; 18(5):436–442. [PubMed: 22957996]
18. Breast Cancer Association Consortium. Commonly studied single-nucleotide polymorphisms and breast cancer: results from the Breast Cancer Association Consortium. *J. Natl Cancer Inst.* 2006; 98(19):1382–1396. [PubMed: 17018785]
19. Steeg PS. Metastasis suppressors alter the signal transduction of cancer cells. *Nat. Rev. Cancer.* 2003; 3(1):55–63. [PubMed: 12509767]
20. Slamon DJ, Leyland-Jones B, Shak S, et al. Use of chemotherapy plus a monoclonal antibody against HER2 for metastatic breast cancer that overexpresses HER2. *N. Engl. J. Med.* 2001; 344(11):783–792. [PubMed: 11248153]

21. Gupta GP, Nguyen DX, Chiang AC, et al. Mediators of vascular remodelling co-opted for sequential steps in lung metastasis. *Nature*. 2007; 446(7137):765–770. [PubMed: 17429393]
22. Alford SH, Toy K, Merajver SD, Kleer CG. Increased risk for distant metastasis in patients with familial early-stage breast cancer and high EZH2 expression. *Breast Cancer Res. Treat.* 2012; 132(2):429–437. [PubMed: 21614565]
23. Lappalainen T, Sammeth M, Friedlander MR, et al. Transcriptome and genome sequencing uncovers functional variation in humans. *Nature*. 2013; 501(7468):506–511. [PubMed: 24037378]
 - First sequencing analysis of mRNA and microRNA in human populations revealing diversity in the regulatory genome.
24. Hunter K. Host genetics influence tumour metastasis. *Nat. Rev. Cancer*. 2006; 6(2):141–146. [PubMed: 16491073]
25. Hunter KW. Mouse models of cancer: does the strain matter? *Nat. Rev. Cancer*. 2012; 12(2):144–149. [PubMed: 22257951]
26. Lifsted T, Le VT, Williams M, et al. Identification of inbred mouse strains harboring genetic modifiers of mammary tumor age of onset and metastatic progression. *Int. J. Cancer*. 1998; 77(4): 640–644. [PubMed: 9679770]
 - Landmark study that describes the effect of maternal genotype on breast cancer metastasis.
27. Guy CT, Cardiff RD, Muller WJ. Induction of mammary tumors by expression of polyomavirus middle T oncogene: a transgenic mouse model for metastatic disease. *Mol. Cell Biol.* 1992; 12(3): 954–961. [PubMed: 1312220]
28. Park YG, Zhao X, Lesueur F, et al. *SIPA1* is a candidate for underlying the metastasis efficiency modifier locus Mtes1. *Nat. Genet.* 2005; 37(10):1055–1062. [PubMed: 16142231]
 - Describes the identification of the first polymorphic metastasis susceptibility gene *SIPA1*.
29. Crawford NP, Ziogas A, Peel DJ, Hess J, Anton-Culver H, Hunter KW. Germline polymorphisms in *SIPA1* are associated with metastasis and other indicators of poor prognosis in breast cancer. *Breast Cancer Res.* 2006; 8(2):R16. [PubMed: 16563182]
30. Hsieh SM, Look MP, Sieuwerts AM, Foekens JA, Hunter KW. Distinct inherited metastasis susceptibility exists for different breast cancer subtypes: a prognosis study. *Breast Cancer Res.* 2009; 11(5):R75. [PubMed: 19825179]
31. Crawford NP, Qian X, Ziogas A, et al. *Rrp1b*, a new candidate susceptibility gene for breast cancer progression and metastasis. *PLoS Genet.* 2007; 3(11):e214. [PubMed: 18081427]
32. Crawford NP, Yang H, Mattaini KR, Hunter KW. The metastasis efficiency modifier ribosomal RNA processing 1 homolog B (RRP1B) is a chromatin-associated factor. *J. Biol. Chem.* 2009; 284(42):28660–28673. [PubMed: 19710015]
33. Crawford NP, Alsarraj J, Lukes L, et al. Bromodomain 4 activation predicts breast cancer survival. *Proc. Natl Acad. Sci. USA.* 2008; 105(17):6380–6385. [PubMed: 18427120]
34. Crawford NP, Walker RC, Lukes L, Officewala JS, Williams RW, Hunter KW. The diasporin pathway: a tumor progression-related transcriptional network that predicts breast cancer survival. *Clin. Exp. Metastasis.* 2008; 25(4):357–369. [PubMed: 18301994]
35. Dey A, Chitsaz F, Abbasi A, Misteli T, Ozato K. The double bromodomain protein Brd4 binds to acetylated chromatin during interphase and mitosis. *Proc. Natl Acad. Sci. USA.* 2003; 100(15): 8758–8763. [PubMed: 12840145]
36. Alsarraj J, Faraji F, Geiger TR, et al. BRD4 short isoform interacts with RRP1B, SIPA1 and components of the LINC complex at the inner face of the nuclear membrane. *PLoS ONE.* 2013; 8(11):e80746. [PubMed: 24260471]
37. Farina A, Hattori M, Qin J, Nakatani Y, Minato N, Ozato K. Bromodomain protein Brd4 binds to GTPase-activating SPA-1, modulating its activity and subcellular localization. *Mol. Cell Biol.* 2004; 24(20):9059–9069. [PubMed: 15456879]
38. Alsarraj J, Walker RC, Webster JD, et al. Deletion of the proline-rich region of the murine metastasis susceptibility gene Brd4 promotes epithelial-to-mesenchymal transition- and stem cell-like conversion. *Cancer Res.* 2011; 71(8):3121–3131. [PubMed: 21389092]
39. Filippakopoulos P, Qi J, Picaud S, et al. Selective inhibition of BET bromodomains. *Nature.* 2010; 468(7327):1067–1073. [PubMed: 20871596]

40. Winter SF, Lukes L, Walker RC, Welch DR, Hunter KW. Allelic variation and differential expression of the mSIN3A histone deacetylase complex gene *Arid4b* promote mammary tumor growth and metastasis. *PLoS Genet.* 2012; 8(5):e1002735. [PubMed: 22693453]
41. Churchill GA, Airey DC, Allayee H, et al. The Collaborative Cross, a community resource for the genetic analysis of complex traits. *Nat. Genet.* 2004; 36(11):1133–1137. [PubMed: 15514660] ■ Describes the development of the eight-way cross between various mouse strains called the Collaborative Cross that together constitute more than 90% of the genetic diversity found in laboratory mice.
42. Svenson KL, Gatti DM, Valdar W, et al. High-resolution genetic mapping using the Mouse Diversity outbred population. *Genetics.* 2012; 190(2):437–447. [PubMed: 22345611] ■ Describes the Diversity Outbred mice, that has significantly enhanced the resolution of genetic mapping.

Practice Points

- Metastasis is the major cause of breast cancer-related mortality.
- Breast cancer metastases can remain dormant for many years and resurface as incurable lesions; therefore, prognosis via genetic tools is of immense significance.
- Metastasis is a complex, non-Mendelian trait.
- Host polymorphisms influence metastasis susceptibility.
- *NM23*, *KISS1*, *BRMS1* and *MKK4* function as metastasis suppressor genes, while *HER2*, *MMP1*, *EREG* and *COX2* promote breast cancer metastasis.
- Single nucleotide polymorphisms in *SIPA1*, *RRP1B*, *BRD4* and *ARID4B* determine susceptibility to breast cancer metastasis.
- An eight-way cross of mouse strains called the Collaborative Cross is a powerful tool to understand genetic modifiers of complex traits.

Figure 1. A proposed model showing the localization of BRD4 isoforms and other proteins identified to be in a complex

INM: Inner nuclear membrane; ONM: Outer nuclear membrane.