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Many Breast Cancer Mutations Parallel Mutations in Known Viral Cancers

Bernard Friedenson

Department of Biochemistry and Molecular Genetics, College of Medicine, University of Illinois Chicago, Chicago, IL, USA.

ABSTRACT: Infections may play a larger role in breast cancer than previously believed, especially if normal breast cell architecture and immune defenses have been weakened by gene mutations. Mutated genes in 289 breast cancers were compared with mutated genes in model viral and non-viral cancers. DNA sequences were obtained from publicly available data. Mutated genes in breast cancers were just as likely to be found in viral cancers as in non-viral cancers. Breast, viral, and non-viral cancers all had damage to genes encoding essential immune functions and physical barriers to cell infection. Potentially, damage to these protective genes can suppress control of cancer-associated viruses and open easier routes for infectious particles. Genes that provide protection against cancer viruses and form barriers against infection were damaged in every breast, viral, and non-viral cancer tested. Breast and other cancer cells may already harbor infections. Gaps in immunity or in infection barriers caused by distinct individual sets of mutations make cancer cells more susceptible than normal cells to infections that can exploit the cancer mutations. Other infections may be less likely because mutations have altered host proteins the other infection requires. Understanding gaps in cell defenses may enable therapy that more specifically destroys cancer cells and preserves normal cells.

KEYWORDS: breast cancer causes, breast cancer treatment, breast cancer virus, breast cancer infection, breast cancer mutations, breast cancer immunotherapy, breast cancer cure, breast cancer

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CORRESPONDENCE: molmeddoc@yahoo.com; bernief@uic.edu

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Background

The risk factors for breast cancer are well known, but how they relate to causing the disease is not well understood. Some studies implicate specific tumor viruses, such as mouse mammary tumor virus (MMTV) in a human version (HMTV), human papilloma virus (HPV), or Epstein Barr virus (EBV).[1] Evidence is abundant that multiple viruses with known potential to cause cancer are present in both normal and malignant breast tissues. Some studies demonstrate additional correlations between viral presence and breast cancer risk. HMTV, for example, occurs in both normal and malignant human mammary epithelium; antibodies have been reported; HMTV–breast cancer associations correlate with geographical areas where some wild mouse species are prevalent; relocation changes breast cancer risks; HMTV particles can be isolated from cultures of human breast cancer cells; 5% of breast milk samples contain viral sequences; and the mouse virus can infect human mammary epithelial cells.[2,3] MMTV causes breast cancer in mice. Despite the seemingly convincing associations in these reports, other studies find no association between breast cancer and MMTV.[4,5] Thus, a relationship between breast cancer and HMTV remains controversial. Conflicting conclusions have also been reached by studies of associations between HPV and breast cancer.[6–11] Similarly, EBV infection in breast carcinoma tissues has been detected by many studies but agreement among investigators has again been inconsistent.[12] As a herpes virus, primary infection with EBV persists for life and must be controlled by host immune surveillance.

Acquired defects in immunity may be a lurking variable that could confound breast cancer viral association studies.

The DNA sequences of breast cancers from 21 different women all had mutations in genes associated with immunity, especially against viruses.[13] Mutations often targeted innate immunity and its ability to signal to the adaptive immune system. However, the entire individual sets of genes mutated and the subsets of immune system genes were different. There were large differences in the numbers of genes mutated and only a few genes mutated in more than one breast cancer.[13]

Cervical cancer is well studied and can be used as a model for viral cancer. In contrast to controversial contributions of viruses to breast cancer, cervical cancer does not develop without HPV infection. Over 100 different types of HPV exist, about 40 different types infect the mucosal epithelium of the cervix and about 15 cause cancer. Most women with HPV are able to clear the infection without developing cervical cancer, so cervical damage such as by "micro-abrasions" may be required to develop cervical cancer. HPV or other microbial infections may be more likely to cause cancer if mutations occur within immune defense mechanisms protecting the cervix.[13]

HPV virions infect epithelial tissues only if they are damaged (eg by micro-abrasions) enabling entry of the virions into basal epithelial cells. Even if the infection clears, latently infected stem cells may be stimulated by trauma or the infection might be activated if mutations impair the immune system.[13] Crowded packing of both external and internal host cell structures makes transit required by infection difficult, even for infectious particles as small as a virus or viral nucleic acids. Infections in model systems travel many times slower than those in aqueous media alone.[14] Cell microenvironments can be filled with tightly packed cells and there are dense tight structures packed into cell interiors. Extracellular structures, host cell membranes, and nuclear barriers that are missing in model systems would slow transit even further. Along with increasing the spread of infection, damage to these barrier structures would facilitate cancer invasion whatever the initial cause.

Some immune system interactions in the breast and some in the cervix are organ specific. However, at the molecular and cellular levels, there are notable similarities in barriers to infection and the responses to it. Some structures involved in cell adhesion or in internal cell barriers are formed from molecules related to those in the cervix such as collagens, integrins, actins, and laminins. Intercellular connections such as desmosomes, tight junctions, and adherens junctions exist in both organs and use similar constructions encoded by similar genes. Immunity in both organs uses many common pathways.

There may be extensive similarities in defenses against viruses capable of causing cancer. The following work was done to test the hypothesis that host cell barriers and immunity that block viral infection and transit in the cervix would also be damaged by mutation in breast cancers. Do the protective functions damaged in breast cancers have anything in common with those damaged in known viral cancers of the cervix vs non-viral small cell cancers of the lung?

Methods

Data inclusion/exclusion. Samples were chosen from publicly available data that were suitable for intensive analysis. Initially, breast cancer samples used represented breast cancers with about 5% being hereditary and the remainder being sporadic.[15,16] Because infiltrating ductal cancer is the most common form, studies selected were heavily weighted for ductal cancers. Cervical cancer genomes were from samples with the most complete data available at the time. Additional comparisons were made with a set of 104 triple-negative breast cancers[17] to 100 breast cancer exomes[18] and up to 677 small-cell lung cancers as prototype non-viral cancers.

Characteristics of breast cancers used for comparisons to viral and non-viral cancers. Breast cancers included as whole genome sequences were stage III (18/21) and stage II (3/21). Of these 21 breast cancers, 11 were estrogen receptor positive, 7 were progesterone receptor positive, and 4 were positive for Human Epidermal growth factor Receptor 2 (HER2). Most mutations were probably somatic and not germ line except for 5 *BRCA1*-associated (PD3890a, PD3905a, PD4005a, PD4006a, and PD4107a) and 4 *BRCA2*-associated (PD3904, PD3945, PD4115, and PD4116a) breast cancers with whole genome sequences.[15] Twenty of the 21 breast cancers with whole genome sequences were ductal, with only one being lobular. There were a total of 10,685 mutations or breakpoints in these 21 breast cancers. Number of coding mutations varied from 12 to 710, noncoding mutations varied from 175 to 1109, and breakpoints varied from 2 to 217.[15,19] About 3000 mutations affected coding sequences, and the types of genes and their likely effects on immune responses in these genomes were previously reported.[13] Whole exome sequences that were most extensively studied came from 103 matched sporadic breast cancer/normal pairs from Mexico (54 tumors; median age, 54) and Vietnam (49 tumors; median age, 48).[16] Eighty-seven of these 103 sporadic breast cancers were invasive ductal with the remaining 16 cancers being tubular, medullary, mucinous, mixed, lobular, and Ductal Carcinoma In Situ (DCIS). Over 60% of the breast cancers were stage II, but about 20% were stage III. Eight (14%) of the cancers from Mexico and 3 (6%) from Vietnam were stage I. Nine (17%) of the breast cancers were stage 0 (DCIS). These breast cancer exomes had nearly 5,000 candidate somatic gene mutations.[16]

To check the generality of the conclusions, some comparisons were added based on validated mutations in 65 patient samples derived from a set of 104 triple-negative breast cancers (negative for estrogen, progesterone, and EGF receptors).[17] Ninety-three of the 104 triple-negative breast cancers were infiltrating ductal and 83/104 did not have distant metastases. Patients varied in age from 25 to 90 years with a mean age of 55.9 years. Forty-seven of the 104 cancers were known basal carcinomas.[17] Exome sequences including three of whole genome breast cancers (PD4103a, PD4107a, and PD4109a) as part of 100 exome sequences were also used for brief comparisons.

Seventy-nine of these cancers were estrogen receptor positive and 21 were negative.[18]

DNA sequences from 14 different squamous cell cervical cancers used for comparison were obtained from the COSMIC database of genome sequences (Study ID COSU415). Non-viral small-cell lung cancer data also came from the COSMIC database (Study ID not found).

Types of mutations. The 14 cervical cancers had 69 changes in two consecutive bases and 5,666 single base changes. Of the single base changes, 208 mutations did not encode for a change in amino acid (synonymous substitutions) and 324 were nonsense mutations. In the breast cancer whole genomes, there were 183,916 somatic base substitutions including 117 nonsense mutations. There were 2,233 deletions out of 2,869 indels identified. Sixteen deletions were homozygous. A total of 1,192 rearrangements were found and 14 regions had increases in copy number.[15] The breast cancer exomes contained 4,985 somatically acquired substitutions including 1,157 synonymous and 242 nonsense mutations, 194 deletions, and 110 insertions.[16] The triple-negative breast cancers had 2,414 single nucleotide variants validated "with targeted deep sequencing to a median of 20,000X coverage".[17] Validated somatic mutations were from 65 different patients out of 104 patients. Only 36% of the mutations were expressed.[17] The small-cell lung cancer mutations were about 25% nonsense substitutions and 70.5% missense substitutions. There were about 10% insertions and 27.5% deletions. An added set of breast cancer exomes identified 7,241 somatic point mutations: 6,964 were single-base substitutions, of which 4,737 were predicted to generate missense and 422 nonsense mutations.[18]

Method to compare breast cancer with known viral and non-viral cancers. Using Microsoft Access and Excel, genes mutated in viral and non-viral cancers were compared to genes mutated in all the breast cancers examined. Functions of mutated genes were determined from many original publications, by PubMed searches; from Entrez system searches of many molecular, immunology, and other literature databases; from OMIM, Google and Wikipedia. Information contained in many images, drawings, and YouTube videos was also incorporated.

Considerable evidence supports the view that the human genome is normally stable. A study of all the mutations in the human Y chromosome found only four mutations after 13 generations.[20] To eliminate prior prejudice, potential functional implications of all mutations were thoroughly evaluated. DNA sequence data from the initial breast cancer whole genomes and exome sets had a similar sequencing depth, at minimum depth ~30.[15,18] Multiple issues in library preparation, sequencing and data processing can resemble somatic nucleotide events. Therefore, all somatic variations considered were validated by some methods in the original article.

The mutations do not include intronic variants unless they were part of a deletion. Synonymous variants were included but represent a minority of the mutations. These were included because they are not necessarily silent or neutral because they may affect gene regulation or splicing. Both common and rare variants were considered because cancer is typically heterogeneous and because breast cancer is thought to originate from rare stem cells. Mutations were stratified according to whether they damaged known immune functions or barriers to infection created by cellular architecture. To facilitate further studies, the results of these functional analyses were collected into a growing database of immune system and barrier genes.

Results

Numbers of different shared genes with mutations and negative control genes. There were 4,802 different genes with acquired somatic mutations in the Mexico–Vietnam breast cancer exomes[16] and in the whole genome sequences.[15] In all the cervical cancers, 4,010 different genes were mutated, giving a total of 8,812 different genes mutated in both types of cancers. Of the 8,812 different genes mutated, 1,175 were found in both types of cancers.

Shared genes with mutations affecting the immune system and barriers to infection. Of the 1,175 shared genes that had mutations in the initial set of breast and cervical cancers, approximately 385 genes (33%) could be placed as encoding a function impacting the immune system and another 435 different genes could be assigned to some cell barrier function (37%). Thus, damage to immunity and barrier functions account for the bulk of the mutations (70%) that occurred in shared genes. Other mutations in shared genes encoded for functions involved in cell division, metabolism, energy provision, and cell homeostasis. Many of these other genes are essential for immunity or for cellular barriers that prevent infection.

Defects in immunity in breast and cervical cancers. Because of its secretory functions to maintain life, some breast–immune system interactions are specific and may not be shared with the cervix. However, the need to prevent infections is broadly required by different organs, so many innate immune and anti-viral functions are general and found both in the breast and the cervix. For example, defects in cell surface antigens or in the ability to present antigens to the immune system via major histocompatibility complex antigens are damaged in cervical cancer cells and in breast cancer cells as well.[8] Based on data from 21 different breast cancer whole genomes and 14 cervical cancers, Figure 1 illustrates genes mutated that are essential for innate immunity pathways. While the individual cancers in the two different organs may differ in the exact genes and pathways damaged, the overall effect of gene damage appears to be similar. In the data presented below, a total of 303 breast and cervical cancers have been examined. All 303 cancers had mutations in multiple genes that could cause some deficit not only in immune responses but also in cellular barriers to infection and cancer spread.[21,22]

Structural barriers to infection. Figure 2 is a cross-sectional model of a breast duct. It pictures breast duct cells

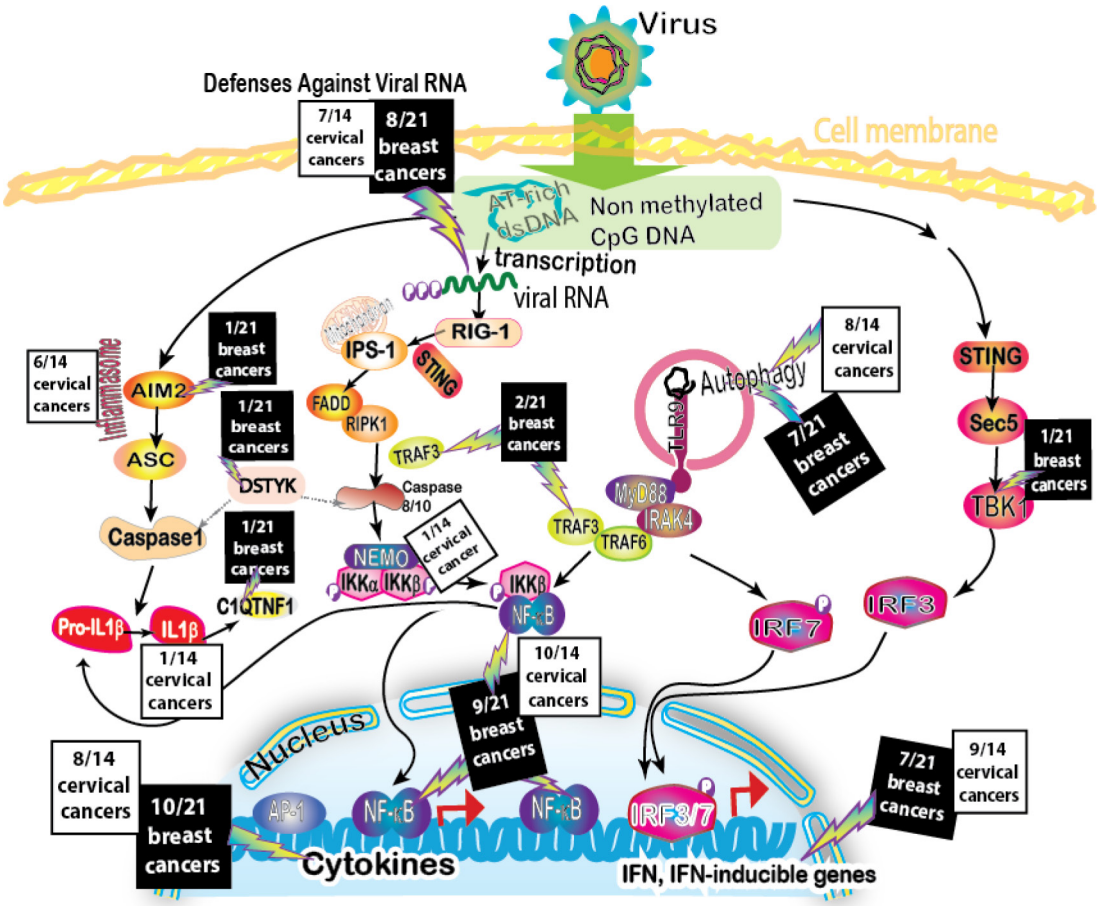

Figure 1. Similar functions impacted by mutations in innate immunity in breast and cervical cancers. The basic innate immunity pathways shown are based on Mogensen's[88] and Barber's[89] studies. Many intermediate steps are omitted for clarity but omitted steps provide further opportunities for mutations in additional genes to interfere with the immune response. Breast cancers with mutations affecting the function are indicated in black boxes. The 21 breast cancer whole genomes were used for this analysis and all 21 are represented. The black and white boxes show the fractions of breast and cervical cancers with mutations affecting a given gene or a general type of gene.

surrounded by protective structural barriers. The diagram is based on multiple sources including the following.[23–27] Tables 1–5 specifically list about 450 instances of a whole genome or Mexico–Vietnam breast cancer with mutations in barrier functions. Mutated genes in Tables 1–5 are representative examples and are distributed over about 300 different genes and isoforms.

Cell–cell and cell–matrix attachments provide extracellular barriers to protect tissues from pathogens (Fig. 2). All 21/21 breast cancer whole genomes, 102/103 exomes, and 12/14 cervical cancers have mutations affecting cell attachments to each other, to the extracellular matrix (ECM), or to ECM stability (Figs. 2 and 3). Attachments among cells are formed by tight junctions, adherens junctions, desmosomes, and hemidesmosomes (Fig. 2). Damage to genes encoding molecules essential for these barriers (Fig. 3) would predispose to infection and damaged barriers would favor spread of infection and malignancy.

Despite differences in the anatomy of breast duct cells vs cervical epithelium, similar genes are used to encode for analogous structural barriers in both breast and cervix. In almost all the breast cancers, there are mutations in genes required for the synthesis of basement membrane, including collagens, fibrillins, and laminins. For example, 33 different collagen genes are mutated in 27 different breast cancer exomes (Table 1). Loss of collagen receptor genes has been implicated in breast cancer invasion and metastasis.[28] Tables 1–5 and Figure 3 show that mutations in the kinds of genes encoding for cell structural barriers are common in both breast and cervical cancer.

Breast epithelial cells are enclosed by a layer of myoepithelial cells that are not continuous and contain gaps (Fig. 2). Myoepithelial cells contract to expel milk through the duct when stimulated by oxytocin. A basement membrane surrounds the myoepithelium and separates it from connective tissue. Myoepithelial cells sense and adjust to their microenvironment because of integrin-binding properties. Integrins are receptors for extracellular ligands and directly participate in cell adhesion. The same integrin gene is frequently mutated in both a breast and a cervical cancer (Table 1). Other genes mutated in breast and cervical cancers alter regulation or expression of

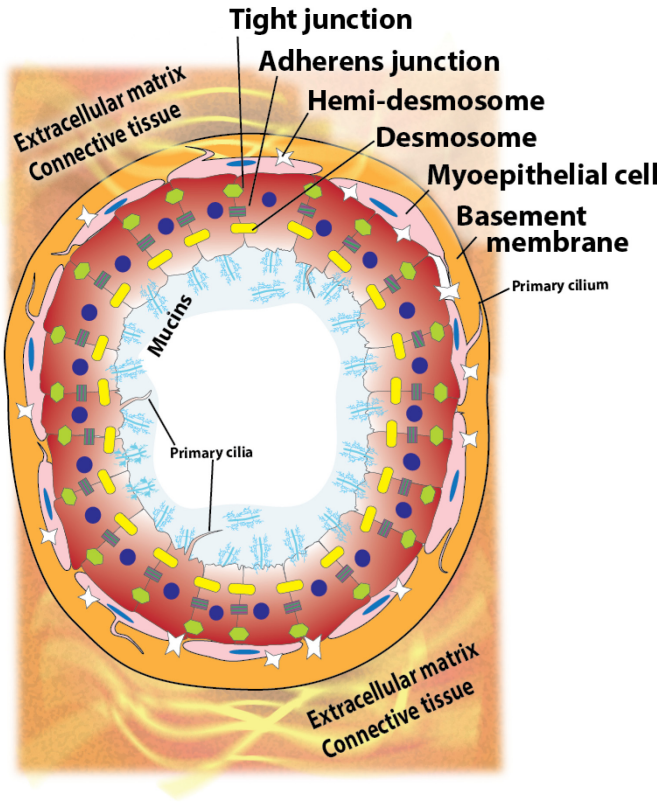

Figure 2. Diagram of a breast duct cell in cross section. Microbial infections would have to pass through the barriers shown. Damaged barriers would also favor cancer invasion. The genes encoding proteins that make up these barriers are mutated in breast cancers. Attachments among cells are formed by tight junctions, adherens junctions, desmosomes, and hemi-desmosomes. Epithelial cells are surrounded by myoepithelial cells that contract to expel milk through the duct when stimulated by oxytocin. A basement membrane surrounds the myoepithelium and separates it from connective tissue. Basement membrane of breast ducts adheres strongly to epithelial cells because of binding both to integrin and non-integrin receptors. Primary cilia are more numerous in the myoepithelial layer than in epithelial cells.[26] Desmosomes are probably involved in junctions between myoepithelial and epithelial cells[27] but are not shown in the diagram.

integrins (Table 1). Various integrins may compete for binding to fibronectin and other substrates. In breast carcinoma, major changes in cell–cell and cell–ECM occur and can affect cellular morphology.[29] Defects or deregulation of integrin binding disrupts normal breast duct organization and can allow cells to leave their normal microenvironment. Some integrins are formally listed in the innate immunity database.[21]

In normal mammary epithelial cells, CDH1 (E-cadherin) participates in tightly connecting epithelial cells by forming an adhesion band under the apical surface of cells (Figs. 2 and 3). *CDH1* is expressed at normal levels in ductal carcinoma in situ but a complete or partial loss of *CDH1* is associated with invasive ductal carcinoma.[30] Examples of breast cancers with *CDH1* gene mutations are listed in Table 1. CDH1 has tumor suppressing activity and is a prototype for a large superfamily of related proteins.[31] The normal breast has 13 different cadherins and cadherin gene alterations in breast cancer are well known. Interference with organized cell adhesion disrupts contact inhibition, alters cell migration, and interactions with the stroma. Several cervical cancers had damage to genes that regulate or alter expression of CDH1 (Table 1). For example, CBX4 (mutated in cervical cancer DS-A0VN-01) attenuates repression of *CDH1*.

Protocadherins participate in attachments between molecules in one cell to identical molecules in an adjacent cell. Unlike cadherins, protocadherins are not linked to the actin cytoskeleton. Table 1 shows that most cervical cancers (12/14) have a damaged protocadherin gene and that 25 breast cancers also have mutations in a protocadherin gene.

Desmosomal cadherins are part of structures that enable cells to adhere to each other. This cellular adherence forms restrictive endothelial barriers that prevent infection. In the breast, desmosome structures connect myoepithelial cells to breast duct epithelium and to basement membrane (Figs. 2 and 3). These connections also involve keratin-based filaments, and genes required for these filaments are mutated in many breast cancers.

Matrix metalloproteinases (MMPs) can degrade all the molecules in the ECM. At least 25 proteases belong to this family and are subdivided into collagenases, membrane-type MMPs, and several other enzymes. *MMP* genes mutated in breast cancer encode for collagenases (*MMP1*, *MMP8*, and *MMP13*), gelatinases (*MMP2* and *MMP90*), or membrane types (*MMP15*). MMPs together with inhibitors such as TIMP2 regulate the composition of the ECM and its remodeling and repair. This is widely associated with cancer cell invasion.[32] MMP1 is highly expressed in one subgroup of DCIS.[33] Strong MMP activity may break down the ECM and basement membrane spreading infection or conversely allow infiltration of cells from the immune system to combat infection.

Other mutations (data not shown in Table 1) disrupt cell attachments by damaging the products of diverse genes. In some breast and cervical cancers, *MAML2* mutations damage notch signaling that normally allows cells to organize themselves. *SIGLEC* genes are also mutated. *SIGLEC* genes normally encode sialic acid-specific cell receptors contributing to cell adhesion and host innate immune responses.[34]

Cilia and molecular motors. Table 2 lists breast cancer genomes and exomes with mutations in genes encoding motor proteins essential for cilia function.[35] Primary cilia are nonmotile, microtubule-based structures within and extending out from the outer cell membrane. Both myoepithelial and epithelial cells in the breast contain primary cilia, but they are more numerous on myoepithelial cells. Primary cilia are absent or decreased in numbers in breast and other cancers.[26] Many mutations found in breast and cervical cancers could affect the function of primary cilia (Table 2). Unlike motile cilia found on some cervical cells, breast primary cilia usually do not beat but sense environmental conditions and maintain cell orientation. Extracellular signals

Table 1. Examples of related genes mutated in breast and cervical cancers encoding functions in ECM and cell adhesion.

FUNCTION	MUTATED GENE	BREAST CANCER	CERVICAL CANCER WITH THE SAME OR RELATED GENE MUTATED
Collagens, components of basement membrane, extracellular matrix, and underlying connective tissue	27 different collagens in 33 breast cancer exomes. 5 different collagens and 1 regulatory protein in 5 breast cancer whole exomes, 13 different collagens in 13 different triple negative breast cancers	BR-M-037, BR-M-041, BR-M-050, BR-M-055, BR-M-076, BR-M-085 BR-M-095, BR-M-098, BR-M-106, BR-M-110, BR-M-120, BR-M-122, BR-M-123, BR-M-126, BR-M-174, BR-M-191, BR-M-193, BR-V-002, BR-V-009, BR-V-017, BR-V-022, BR-V-024, BR-V-026, BR-V-027, BR-V-034, BR-V-037, BR-V-043, BR-V-044, BR-V-045, BR-V-047, BR-V-064, BR-V-067, BR-V-071, PD3890, PD3905, PD4107, PD4120, PD4199	17 different collagens in 8 cervical cancers
Regulation of cell junctions	DNMBP/TUBA/KIAA1010	BR-V-009, PD3905	DR-A0ZM-01
Desmosomes are the most common type of intercellular junction in epithelial cells	DSP/desmoplakin	PD4006	DR-A0ZM-01
Fibrillin: an essential component of the basement membrane and extracellular matrix. Involved in collagen–elastin interaction, an essential component of connective tissue	FBN1, 2, 3	BR-M-191, BR-V-012, BR-V-027, BR-V-030, BR-V-043, PD4006, PD4107, PD4198	
Fibronectin, fibronectin domain containing. Binds to integrins to mediate EC cell attachments. Fibronectin signals may be required for mammary gland proliferation	FSD1, FSD2, LRFN2, NEO1/ NGN; IGDCC2, NRCAM, OBSCN isoforms, TNN, USH2A, BOCANKFN1, EGFLAM, FANK1	BR-M-166, BR-M-167, BR-V-002, BR-V-014, BR-V-019, BR-V-033, BR-V-054, BR-M-074 PD4005, PD4120, PD4199	DR-A0ZM-01, DS-A0VK-01, DS-A0VL-01, BI-A0VR-01
Based on homology to a similar protein in *Caenorhabditis elegans* HMCN1 may stabilize germ-line syncytium and organizing cell attachments to the ECM in the epidermis	HMCN1/hemicentin1/fibulin-6	PD4006, PD4120, BR-V-002, BR-V-048, BR-M-045, BR-M-076	DR-A0ZM-01
Integrins: Integrins are heterodimer membrane proteins involved in cell–cell and cell–matrix attachments. There are 18 different integrin alpha chains and 8 different beta chains. Integrins are key receptors for cell adhesion and migration that involves the ECM. They provide extracellular barriers to protect tissues from pathogens and transmit signals from the ECM to the cells. They also function in immunity, tissue repair, and cancer metastasis. Some are formally listed in innate immunity databases. Fibronectin, vitronectin, collagen, and laminin are ligands that bind integrins. ITGB3 is a receptor fibronectin attachment to the ECM.	ITGA2, ITGA2B	BR-M-027	
	ITGA3	BR-V-023	
	ITGA4		DS-A0VK-01
	ITGA7	PD4107	DS-A0VK-01
	ITGA8	BR-M-116	DR-A0ZM-01
	ITGA11	BR-V-012	
	ITGAE	BR-V-030	DS-A0VK-01
	ITGAL	BR-V-034	DS-A0VM-01
	ITGAX	BR-V-028	DS-A1OC-01
	ITGB1	PD4109	
	ITGB2/LFA-1	PD4086	
	ITGB3	PD4006	DR-A0ZM-01 isoform
	ITGB1BP2	BR-M-193	
	ITGB4	BR-V-037	DR-A0ZM-01
	ITGBL1	PD4120	
Laminins: major noncollagen component of basement membranes, bind integrins	LAMA1-6	BR-M-037, BR-M-095, BR-M-129 BR-V-014, BR-V-023, PD3904, PD4006, PD4086, PD4107, PD4120	BI-A0VR-01 DR-A0ZM-01 DS-A0VM-01

Matrix remodeling-associated protein	MXRA5	BR-V-003, BR-V-037, BR-V-043	FU-A23K-01, DS-A0VM-01
Mediates cell adhesion and migration (70)	PLCG1	BR-M-079	DR-A0ZM-01
Plectin links the cytoskeleton to connections in the outer plasma membrane that join different cells. Plectin links actin microfilaments, microtubules, and intermediate filaments. This helps maintain the mechanical integrity of tissues	PLEC	BR-V-036, BR-M-037, BR-M-073 BR-M-191, BR-M-028	DS-A0VN-01 DR-A0ZM-01
Extracellular matrix protein. Maintains tumor dormancy in the smallest blood vessels of endothelium	POSTN	BR-M-076	DR-A0ZM-01
Prototype for a superfamily of cell adhesion proteins. Cervical cancers listed have mutations in genes encoding proteins that regulate or alter CDH1 expression. Other cadherins are mutated both breast and cervical cancers. CDH1 mutations can cause proliferation by releasing CDH1 inhibitory interactions with EGF and beta catenin	Cadherin 1/E-cadherin/CDH1	BR-V-022 , BR-M-126, BR-M-166, PD4194	BI-A0VS-01, DR-A0ZM-01, BI-A0VR-01, DS-A1OC-01, DS-A0VN-01
Cadherin cell adhesion molecules are major determinants of tissue patterning in cooperation with the actin cytoskeleton (71). Although referred to as neural cadherin, CDH2 is commonly found in cancer cells and is involved in trans-endothelial migration and intercellular adhesion	CDH2/N-cadherin	PD3905, PD4005, BR-M-041, BR-M-129	DS-A0VL-01, DR-A0ZM-01
Claudins are small (27 kd) transmembrane proteins involved in tight junction formation between cells. Control apical composition of space between cells in epithelium	CLDN6, 9, 11, 14, 16, 22	BR-M-116, BR-M-120, PD4088, PD4199	DR-A0ZM-01, DS-A0VM-01, DSA10C-01, DSA10D-01
Homophilic cell adhesion (may have receptor or other functions as well) Not linked to the actin cytoskeleton	Proto-cadherins	35 breast cancers: PD4103, PD4120, BR-M-026, BR-M-028, BR-M-037, BR-M-045, BR-M-047, BR-M-055, BR-M-073, BR-M-079, BR-M-080, BR-M-094, BR-M-095, BR-M-105, BR-M-110, BR-M-116, BR-M-121, BR-M-122, BR-M-126, BR-M-129, BR-M-174, BR-M-184; BR-M-191, BR-V-002, BR-V-014, BR-V-020; BR-V-022, BR-V-023, BR-V-034, BR-V-036, BR-V-037, BR-V-039, BR-V-043, BR-V-067, BR-V-070	12 cervical cancers: BI-A0VR-01, BI-A0VS-01, DR-A0ZL-01, DR-A0ZM-01, DS-A0VL-01, DS-A0VM-01, DS-A1OC-01, DS-A1OD-01, DS-A0VK-01, DS-A0VN-01, EA-A1QT-01, FU-A23L-01
Adhesion recognition of integrins	PSG3		DS-A1OC-01
Adhesion molecule extracellular matrix remodeling	PXDN	BR-M-191	DR-A0ZM-01 FU-A23L-01
Secreted ECM protein controls cell–cell interactions SNP associated with breast cancer (72)	RELN	BR-V-033, BR-V-064, BR-M-122	DS-A0VL-01
Homophilic cell adhesion	SDK1, SDK2	BR-V-027, BR-M-094, BR-V-048, BR-V-050	BI-A0VR-01, DR-A0ZM-01
Cell adhesion	SHANK1, 2	BR-V-011	DS-A0VL-01 DS-A1OC-01 DR-A0ZM-01
Cell adhesion. Carbohydrate recognizing cell receptors; contribute to host innate immune responses; specifically recognize sialic acid; central role in mediating immune signaling (35)	SIGLEC1, 9–12	PD4103, BR-M-095, BR-V-019	DR-A0ZM-01 DS-A1OC-01 DS-A1OD-01 FU-A23L-01
ECM protein with adhesion modulating properties	TNN	BR-V-002, PD4199	DR-A0ZM-01
Regulates integrin ECM interaction	VASP	BR-V-049	DS-A1OC-01

Table 2. Examples of related genes mutated in breast and cervical cancers that encode functions essential for primary cilia and for mucins.

FUNCTION	EXAMPLES OF GENE MUTATED IN BREAST CANCER(S)		EXAMPLES OF GENE MUTATED AND ITS ASSOCIATION WITH CERVICAL CANCER
Cilia function. Axonemal dyneins, kinesins, and regulatory proteins. Dyneins (with heavy chains encoded by DNAH genes) are molecular motors required for cargo transport in cilia. Primary cilia mainly function in sensing the microenvironment and communicate extracellular signals into intracellular processes. Dyneins transport cellular cargo by moving along microtubules usually toward the cell nucleus	**Axonemal Dynein (DNAH) gene**	**Breast cancer with DNAH gene mutations**	**Cervical cancer with DNAH gene mutations**
	DNAH1	BR-M-028	DR-A0ZM-01
	DNAH2	BR-V-026, BR-V-070, BR-M-038, BR-M-150	DS-A0VK-01, DR-A0ZM-01, and FU-A23K-01
	DNAH3	BR-V-048, BR-M-191, whole genome PD4120	DR-A0ZM-01, DS-A0VN-01, DS-A1OC-01, DS-A1OD-01
	DNAH5	BR-V-027, BR-V-054 exomes and in PD4120 whole genome	DR-A0ZM-01, DS-A1OC-01
	DNAH6	BR-V-014	Not found
	DNAH7	[*Mutated in other breast cancer genome data*]	DS-A1OC-01
	DNAH8	BR-M-074, BR-M-105, BR-M-106, BR-M-191 and whole genomes PD4006, PD4248, PD4120	DR-A0ZM-01
	DNAH9	BR-M-191	Not found
	DNAH10	BR-M-038	DS-A0VL-01, DS-A1OC-01
	DNAH11	BR-V-022, BR-V-043, whole genome PD4109	DR-A0ZM-01, DS-A0VK-01
	DNAH12	Not found	DR-A0ZM-01
	DNAH17	BR-M-027 whole genomes PD4116, PD4194	DR-A0ZM-01
Kinesins and associated proteins	**Breast cancers with kinesin gene mutations**		**Cervical cancers with kinesin gene mutations**
A family of molecular motors that travel in direction opposite to dyneins along microtubule tracks. Cilium biogenesis and multiple other roles associated with centromere	BR-M-027, BR-M-028, BR-M-036, BR-M-037, BR-M-045, BR-M-047, BR-M-073, BR-M-079, BR-M-098, BR-M-116, BR-M-122, BR-M-186, BR-M-191, BR-M-193, BR-V-014, BR-V-027, BR-V-032, BR-V-039, BR-V-043, BR-V-054, PD3905, PD4006, PD4085, PD4086, PD4107, PD4109, PD4198, PD4199		BI-A0VR-01, DS-AOVN-1, DS-A1OC-01, DS-A1OD-01, DS-A0VM-01, DR-A0ZM-01
Mucins	**Mucin gene**	**Breast cancers with Mucin gene mutations**	**Cervical cancers with Mucin gene mutations**
Mucins are essential for a lubricating barrier that protects against infectious agents and are believed to play a crucial role in cervical cancer. Mucins are high molecular weight glycoproteins produced by many epithelial tissues. Any of several mucins have been found in breast cysts	MUC2	BR-M-005, BR-M-030, BR-M-047, BR-M-080, BR-M-083, BR-M-085, BR-M-098, BR-M-120, BR-M-167, BR-M-169	EA-A1QT-01. A MUC2 isoform is mutated in cervical cancer DS-A0VL-01
	MUC5B	BR-V-012, BR-V-033, BR-M-116	DR-A0ZM-01 DS-A0VM-01
	MUC16/CA125	BR-V-002, BR-V-043, BR-M-038, BR-M-045, BR-M-186, BR-M-191, BR-M-193	DR-A0ZM-01, DS-A1OC-01
	MUC17	PD4120	DR-A0ZM-01, DS-A0VL-01, DS-A1OD-01

are then sent to intracellular processes such as immunity. Both primary and motile cilia originate from a basal body containing hundreds of proteins in the cell membrane (Fig. 3).

Cargo and signaling. Intraflagellar transport (IFT) is a system that moves cargo from the cell body to the flagellar tip to assemble the cilium. Cilium biogenesis requires IFT proteins to deliver precursors to their sites of incorporation. Mutation in the *IFT88* gene (breast cancer PD4103) has been associated with a loss of cilia structure.[36] Four different breast cancer exomes also have mutations in *IFT* genes. IFT proteins participate in the assembly of immune synapses in hematopoietic cells that do not have cilia.[37] Mutations in kinesin genes in breast and cervical cancers (Table 2) may affect the kinesin function in transport of cargo away from the nucleus. Alterations in the *KIF4A* gene has been identified as one of the changes associated with progression of DCIS.[38]

Table 2 also includes mutations in genes encoding dyneins and regulatory protein. Dyneins (with heavy chains

Table 3. Examples of related genes encoding cellular actin functions mutated in breast and in cervical cancers.

FUNCTION	ACTIN RELATED GENE(S)	BREAST CANCERS WITH MUTATION	CERVICAL CANCERS WITH MUTATION
Alpha2 actin (alpha smooth muscle actin) is a major contractile protein essential for clathrin mediated traffic. A prominent protein in breast cancer MYH14 associated fibroblasts. Myoepithelial cells contain smooth muscle actin and have hemidesmosome connections to basement membrane	ACTA2	PD3904, PD4107, BR-V-034	Not found
ACTB is one of two non-muscle cytoskeletal actins and another major component of the contractile apparatus. ACTL8 is an actin-like protein	ACTB, ACTBL2, ACTL8	PD4107, BR-V-032, BR-V-002, BR-V-095	Not found
Actin bundling protein	ACTN4	BR-V-067	DS-A0VL-01
Actin related proteins T1, T2	ACTRT1, ACTRT2	BR-V-036	DS-A0VN-01, DS-A1OC-01
Adducin binds actin to stabilize cell-cell and membrane cortical cytoskeleton adhesion (73). Adducin occurs in many cells, caps rapidly growing actin filaments and attracts spectrin (74)	ADD2	BR-M-038	DR-A0ZM-01
AHNAK/Desmoyokin encodes a large protein essential for cell migration and invasion. The protein is found in desmosomes and mediates actin reorganization needed for pseudopodia	AHNAK, AHNAK2, AHNAK2 isoform	BR-V-033, BR-V-043, BR-V-053, BR-M-055, BR-M-189	DS-A1OD-01, FU-A23K-01, DR-A0ZM-01, DS-A0VN-01
Cytoskeletal proteins that promote actin remodeling, key regulators of RhoGTPases which regulate cytoskeletal dynamics, actin reorganization, F-actin dynamics, filopodia	ARHGAP6, 17, 21, 24, 30, 31, 44, ARHGAP17, 24 isoforms	PD4006, PD4107, PD4115, PD4120, BR-M-027, BR-M-037, BR-M-174, BR-V-019, BR-V-036	DR-A0ZL-01, DR-A0ZM-01, DS-A0VK-01, DS-A0VM-01, DS-A0VN-01, DS-A1OC-01, FU-A23K-01, FU-A23L-01
Coordinate microtubule integrity with reorganization of actin cytoskeleton, guides actin cytoskeleton, involved in filopodia, stress fibers, cell shape	ARGEF2, 3, 4, 6, 7, 11, 19	PD4107, PD4120, BR-V-023, BR-V-040, BR-V-043	DR-A0ZM-01, DS-A0VL-01, DS-A0VM-01, DS-A0VN-01
Change cell shape by regulating polymerization of actin filaments	CDC42EP4, CDC42EP2	PD4115, PD4116, BR-M-047	BI-A0VS-01
DAB1 and RELN gene products are members of the same signaling cascade that regulates Rho GTPases and modulates and stabilizes the actin cytoskeleton (75)	DAB1, RELN	PD4199, BR-M-122, BR-V-033, BR-V-064	DR-A0ZM-01, DS-A1OC-01, DS-A0VL-01
DOCK proteins are a family of guanine nucleotide exchange factors that participate in intracellular signaling networks. DOCK proteins interact with the Rho family of GTPases. The members of the Rho GTPase family in turn regulate intracellular actin dynamics (76)	DOC1-7, 10, 11	PD3904, PD3945, PD4086, PD4120, PD4199; PD4085, BR-M-037, BR-M-041, BR-M-110, BR-M-116, BR-M-191, BR-M-123, BR-V-022, BR-V-023, BR-V-032	BI-A0VR-01 DR-A0ZL-01, DR-A0ZM-01, DS-A0VM-01, DS-A0VN-01, DS-A1OC-01
Cortical actin and endocytosis. Encoded proteins couple endocytosis to the actin cytoskeleton, also involved in actin reorganization (77)	EHBP1, EHBP1L1	PD4116, BR-M-126	DR-A0ZM-01, DS-A1OD-01
An adaptor protein first identified in red cells, links transmembrane proteins to the cytoskeleton (78). EPB41 may be a linker between the cytokeratin and actin-based cytoskeleton (79) EPB41L3 is a cortical cytoskeleton gene	EPB41L3, EPB41, EPB41 isoform	PD4086, PD4107	DR-A0ZM-01 DS-A1OC-01 EX-A1H5-01
Actin remodelers, actin capping	EPS8, EPS8L2	PD3890, PD4120, BR-V-071	Not found
Actin remodeler, regulator with scaffold function. Formin related	FHOD3	PD4198, BR-M-191	DS-A1OC-01
Actin nucleating and remodeling proteins that can also regulate microtubule stability and organization, filopodia. Cytoskeletal organization, Golgi structure. FMN2 causes actin to concentrate around chromosomes. FMNL3 generates cytoplasmic projections (filopodia)	Formins (FMN2, FMNL1, 3)	PD3905, PD4109, BR-M-191, BR-M-034, BR-V-033	FU-A23L-01
Regulates actin polymerization	KANK1	PD4109	FU-A23L-01

(*continued*)

Table 3. (*Continued*)

FUNCTION	ACTIN RELATED GENE(S)	BREAST CANCERS WITH MUTATION	CERVICAL CANCERS WITH MUTATION
Binds actin, organizes cytoskeleton, cell morphology, pseudopodia (80) and other effects. KLHL proteins can be co-opted by virus after infection (81)	KLH, 6 KLHL genes	PD3905, PD4006, PD4116, PD4194, PD4199, PD4120, BR-M-186, BR-V-024, BR-V-033, BR-M-041, BR-M-110, BR-M-150, BR-V-071, BR-V-027, BR-M-191, BR-M-098, BR-V-022, BR-M-095	BI-A0VS-01, DR-A0ZM-01, DS-A0VK-01, DS-A0VM-01, DS-A1OC-01, EA-A1QT-01, EX-A1H5-01
Controls RhoGTPases, interacts with actin	MCF2, MCF2L isoform/DBL/ ARHGEF21	BR-V-002, BR-V-024, BR-V-047	BI-A0VS-01, DR-A0ZM-01
Myosins and myosin kinases, molecular motors and actin binding proteins, stress fibers, filopodia, cytoskeletal organization, maintenance and stability, cell shape, microvilli motion, endocytosis, actin remodeling	MYH, MYL, MYO genes	BR-M-037, BR-M-073, BR-M-076, BR-M-116, BR-M-167, BR-M-193, BR-V-021, BR-V-047, BR-V-036, BR-V-037, D4103	DR-A0ZL-01, DR-A0ZM-01, DS-A0VK-01, DS-A0VM-01, DS-A0VN-01, DS-A1OC-01, DS-A1OD-01
Site directed actin assembly	NCKAP1, NCKAP1L	PD3945	DS-A0VM-01 FU-A23K-01 DR-A0ZM-01
Dynamic remodeling of actin cytoskeleton to maintain Golgi complex, nucleation and elongation of actin filaments	FMN, FMNL1, 2, 3	BR-M-191, BR-M-034, BR-V-033, PD3905, PD4109	FU-A23L-01
Binds and stabilizes F-actin	NEB, NEBL	BR-M-191, BR-M-116, PD4103	BI-A0VS-01 DR-A0ZM-01 DS-A0VK-01 DS-A1OC-01
Protein activated kinases function in actin polymerization, organization (PAK3), arrangement (PAK6), cytoskeletal regulation (PAK5/7)	PAK1, 2, 3, 6, 7/5	BR-M-120, PD4103	DS-A0VM-01 BI-A0VS-01 DS-A1OD-01 DR-A0ZM-01
Regulates dynamic assembly of presynaptic F-actin scaffold protein	PCLO	BR-V-002, BR-M-174, PD4120	DR-A0ZM-01 DS-A1OC-01
Regulates chemokine induced cytoskeletal reorganization. Links actin to other members of the cytoskeleton. Links cytoskeleton to different cells	PLEC, 10 PLEC isoforms	BR-V-036, BR-M-037, BR-M-073, BR-M-169, BR-M-191, BR-M-028, BR-V-009	DR-A0ZM-01 DS-A0VK-01 DS-A0VN-01
Actin binding protein conserved in evolution expressed in most tissues	PLS1	BR-M-191, PD4120	DR-A0ZM-01
F-actin regulator	RAI14	BR-V-052, SA090	DS-A1OC-01
Assembly of microtubule and actin cytoskeletons, cytoskeletal architecture, actin associated protein	SHROOM2-4	BR-M-174, BR-V-037, BR-V-067 BR-V-042, PD4192, PD4085, PD4120	DS-A0VM-01 DS-A0VN-01 DR-A0ZM-01 DR-A0ZL-01
Required for embryonic actin filaments	SPIRE1, 2	PD4116, BR-V-012, BR-V-018	Not found
Actin cross linking, molecular scaffold. Non erythrocyte spectrin stabilizes plasma membrane and organizes organelles	SPTAN1	BR-V-043	DS-A0VN-01, FU-A23-01
Caps pointed ends of actin filaments	TMOD1, 2	PD4120, PD3851	DR-A0ZM-01
Contractile protein in cytoskeleton also found in nucleoskeleton	TTN	BR-V-008, BR-V-019, BR-V-022, BR-V-034, BR-V-036, BR-V-042, BR-V-050, BR-V-067, BR-M-005, BR-M-026, BR-M-037, BR-M-050, BR-M-076, BR-M-079, BR-M-166, BR-M-174, PD4085, PD4086, PD4109, PD4199	BI-A0VR-01, DR-A0ZL-01 DR-A0ZM-01, DS-A0VM-01, DS-A1OC-01, DS-A1OD-01, EA-A1QT-01 FU-A23K-01, FU-A23L-01
Actin skeleton, actin polymerization	WAS, WASF1-3, WASL	PD4107, BR-M-059 BR-M-192	DS-A0VL-01 DR-A0ZM-01

Table 4. Examples of related genes encoding barriers to the nucleus mutated in breast and cervical cancers including import/export proteins, the nuclear pore complex, the nuclear envelope, and the nuclear skeleton.

FUNCTION	IMPORTINS (IPO), NUCLEAR PORE COMPLEX, NUCLEOPORIN (NUP) GENE	BREAST CANCERS WITH MUTATIONS IN GENES	CERVICAL CANCERS WITH MUTATIONS IN GENES
Although nuclear transport proteins are not strictly physical barriers, some viruses must pervert host transport systems such as importins and exportins (encoded by IPO genes or XPO genes, respectively) to pass through the nuclear envelope barrier via pore structures. Nuclear transport and pore proteins can even stimulate uncoating of some viruses. Mutations in genes encoding various nuclear pore transport systems were found in only a few cancers, but these are included because they might select against some infections. Nuclear export is documented as abnormal in HPV18 mediated cervical cancer (82). SYNE1 and SYNE2 genes (Spectrin repeat containing Nuclear Envelope) encode links between the nuclear envelope and the cytoskeleton. The translocated promoter region nucleoporin gene (TPR) encodes nuclear pore complex intranuclear filaments. TPR maintains channels that are not blocked by chromatin near the nuclear pore complex. Titin (TTN) crosslinks proteins in the cytoskeleton and provides elasticity. TTN associates with chromatin in the nucleus where its functions are probably similar to those in the cytoplasm (83).	IPO4	BR-V-008	
	IPO5	PD3945	
	IPO7	BR-M-192	DS-A0VL-01
	IPO9		DS-A0VL-01
	IPO11	PD3945, PD4198	DS-A0VL-01
	IPO13		DR-A0ZM-01
	AHCTF1/ELYS	BR-V-037	DR-A0ZM-01
	NUP37	BR-M-037	
	NUP43	BR-V-022	
	NUP54	PD4116	FU-A23L-01
	NUP93	BR-M-116	
	NUP98	PD4248, BR-V-014	
	NUP107	BR-M-200	
	NUP133	PD4115, PD4116	
	NUP153	BR-M-154	
	NUP160	BR-M-189	
	NUP188	BR-M-045, BR-V-067	
	NUP205	BR-M-037, BR-M-045	
	NUPL1		BI-A0VR-01
	NUPL2	BR-M-155	
	NUP210L	BR-V-023	DR-A0ZM-01
	NUP214		DS-A1OC-01
	POM121C		DR-A0ZM-01
	RANGAP1, NUFIP1		DS-A0VN-01
	SYNE1, SYNE2 and alternatively spliced forms	BR-V-002, BR-V-033, BR-M-037, BR-M-116, BR-M-191, PD3905, PD4088, PD4116, PD4120	DR-A0ZM-01, EX-A1H5-01, BI-A0VR-01 FU-A23L-01
	TPR (nuclear pore complex intranuclear filaments)	PD4109, PD4120, BR-M-055, BR-M-105, BR-M-200	DR-A0ZM-01, DS-A0VK-01, DS-A1OC-01
	TTN	BR-V-008, BR-V-019, BR-V-022, BR-V-034, BR-V-036, BR-V-042, BR-V-050, BR-V-067, BR-M-005, BR-M-026, BR-M-037, BR-M-050, BR-M-076, BR-M-079, BR-M-166, BR-M-174, PD4085, PD4086, PD4109, PD4199	BI-A0VR-01, DR-A0ZL-01, DR-A0ZM-01, DS-A0VM-01, DS-A1OC-01, DS-A1OD-01, EA-A1QT-01 FU-A23K-01, FU-A23L-01
	XPO1	BR-M-048	
	XPO6	BR-V-027	DR-A0ZM-01
	XPO7	BR-M-116	
	XPOT	PD4107	BI-A0VR-01

encoded by *DNAH* genes) are molecular motors required for cargo transport in cilia. Dyneins transport cellular cargo by moving along microtubules usually toward the cell nucleus. In contrast to mutations in axonemal dyneins, mutations in cytoplasmic dyneins were not clearly identified in either breast or cervical cancers so mutations in these motors are not indicated in Figure 3. Cytoplasmic dyneins interact directly with some viruses such as adenovirus or herpes viruses and transport them to the host cell nucleus along microtubules (Fig. 3).[39]

Primary cilia use several signaling pathways, such as hedgehog (Hh) and platelet-derived growth factor (PDGF), which have many interconnected pathways. Many or all breast

Table 5. Barrier and immune functions in 40 genes with mutations found in the same breast–cervical cancer pair (BR-M-191 and DR-A0ZM-01).

GENE	FUNCTION
AARS2	Alanyl t-RNA synthase. Interacts with RNase L, a unique anti-viral RNA degrading enzyme induced by IFN (84)
AKT2	Helper T-cell response
ANPEP	Membrane integrity
GALNT14	Mucin oligosaccharide synthesis
GCN1L1	Histone kinase (chromatin barrier) Associates with a complex that controls expression of virtually all protein coding genes
GPR98	Transmembrane protein allows sampling and response to environment
HDLBP	Heterochromatin formation, Plaque macrophages
HYAL1	Proteoglycan, cell surface glycoprotein
IKBKAP	Known viral target associated with immune function
IPO13	Nuclear import. Controlled ability to traverse nuclear barrier for import and export of cargo
KDM3B	Response to inflammatory cytokines, demethylase and chromatin structure
MRVI1/IRAG	Viral integration site, homology to a membrane protein restricted to endoplasmic reticulum of lymphoid cells. Participates in myeloid cell growth or differentiation (85)
MYH15	Myosin associates with actin
NCAM2	Membrane protein Ig superfamily member
NELF	Selects B cells that have undergone hypermutation in germinal centers during T cell-dependent antibody responses (86). Guidance molecule for cell projections, neurophilic migration
NLRP1_ENST00000262467	Intracellular sensor of damage, innate immunity
NOD2	An intracellular receptor in the innate immune system recognizing bacterial products in monocytes Activation converts and transmits signals resulting in NFKB activation
NOS1AP	Nitric oxide synthase many relationships to the immune system, complement, NK cells
OS9	Membrane structures, immunity. Glycoprotein degradation, lysosomes, carbohydrate addition to proteins
P2RY4	Ion channels in outer membrane, keratinocyte proliferation
PLEC	Intermediate filament protein
PLS1	Actin binding protein
PM20D2	Unknown
PRKRIR	Type 1 IFN antiviral activity
PTCH1	Pro-apoptotic, increased inflammation susceptibility, HH receptor and primary cilium signaling
PXDN	ECM remodeling, cell adhesion
RB1CC1	Autophagy, cell migration, apoptosis, growth
SEC14L5	Unknown
SIK2	Immunity. Negatively regulates TLR4 signaling, stimulates export of HDACS, autophagy, macrophage function
SIPA1	GTPase activator involved with actin bundling protein
SLC16A14	Membrane transporter not extensively studied
SLC9A7	Regulates cell adhesion (87). Cell homeostasis
SORL1	Released from cell membrane of leukocytes on cleavage with disintegrin and metalloprotease (88)
SYNE1	Nuclear membrane
TECTA	Component of specialized membranes
TUBB3	Tubulin (see Fig. 3)
USH2A	Contains laminin-EGF motifs, and fibronectin motifs. Found in basement membrane
VPS8	Endosome function (immunity)
ZNF831	Associated with inflammation (inflammatory bowel disease). HIV-1 dependency factor

cancers have Hh or PDGF mutations, so response to extracellular signals may be frequently damaged. This supports data in Table 2 and Figure 3, even though genes encoding these pathways were not included.

Damage to Mucin Genes

Mucins. Before infecting breast cells, potential oncogenic viruses known to be present in the breast duct lumen encounter protective molecules and fluid on host breast epithelial cells

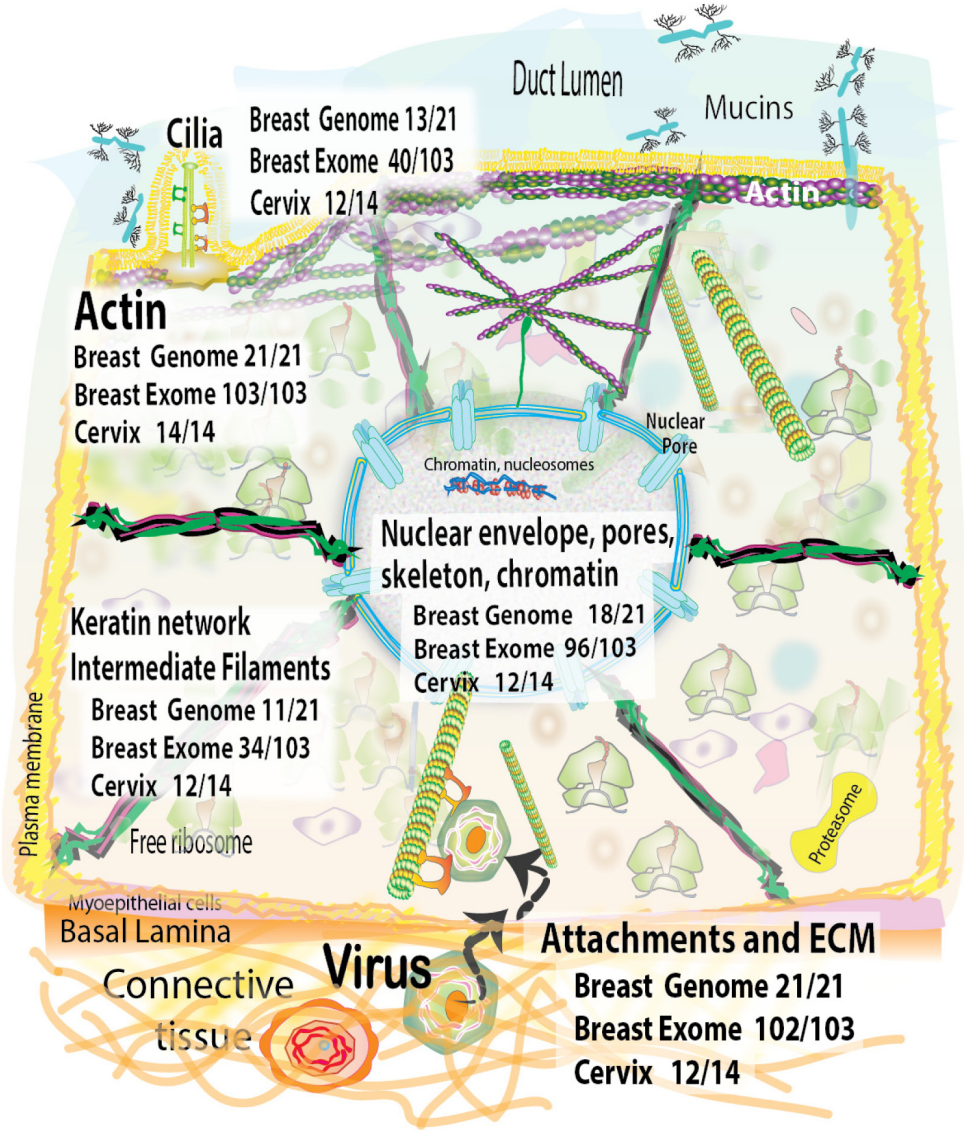

Figure 3. Breast cancer and cervical cancer cells both have mutations in genes encoding the same barrier structures. A breast duct cell is densely populated with structural obstacles to the passage or spread of infectious particles. Evidence for multiple different viruses (including but not limited to HPV) have been found in the ducts. Viruses may be activated to bind to a cell receptor for entry if there is damage to the immune system. Alternatively, a virus may enter the cell from the bloodstream passing through connective tissue barriers, the basal lamina, myoepithelial layer, and plasma membrane. Genes encoding infection-related barriers against viral cancers are damaged in breast cancers and many of the same or closely related genes are damaged in cervical cancers. Damage to genes essential for primary cilia prevents adjustment to environmental changes. The cell is filled with enzymes, proteins, and organelles involved in metabolism, protein synthesis, energy production, transport, etc. For clarity, only some of these structures are indicated but such structures are tightly packed within the cell. The fractions near each structure show the frequency of mutations found that could affect the barrier in breast cancer whole genomes, in breast cancer exomes, and in cervical cancers.

(Figs. 2 and 3). Cervical mucus forms a protective barrier to prevent infections from ascending into the uterus but thins during cyclical changes to allow sperm entry at ovulation.[40] In contrast, there is no similar mucus layer to prevent breast cell infection. However, there are a wide variety of carbohydrate containing molecules such as mucins and proteoglycans on the surfaces of breast duct cells. Mucins as an example of these carbohydrate-rich structures are highly hydrophilic and attract fluid into the breast duct lumen to maintain its hydration for protection and lubrication. Mucin-containing cysts occur in atypical ductal hyperplasia and in some forms of DCIS,[41] and some mucins are overexpressed in breast cancers. Overexpression of membrane mucins is thought to reduce cell–cell adhesion and protect abnormal cells from immune cell surveillance.[42]

Breast and cervical cancers with mutations in genes affecting mucins are listed in Table 2 and indicated in Figure 3. Genes for both secreted (*MUC2*, *5B*, *6*) and membrane-bound mucins (*MUC4*, *15*, *16*) were mutated in 22/103 breast cancer exomes and one whole genome. The gene for the most abundant mucin in cervical mucus (*MUC5B*, secreted) was

mutated in two cervical cancers. Other cervical mucin genes with mutations included *MUC2*, *5AC*, *6*, *13*, *16*, *17*, and *21* distributed half the cervical cancers (7/14).

Other mutations affect mucins in breast cancer whole genomes more indirectly. A substitution mutation in *PRR23C* (proline-rich protein) in breast cancer PD3904 changes the binding properties of mucin. In PD4088, a mutation in *GUCY2C* (guanylate cyclase 2C) facilitates mucosal wounding and inflammation. In PD4103, a mutation in *CFTR* (the chloride channel) changes mucins significantly. A total of 194 proteins were found in cervical mucus in addition to mucins, including DMBT1.[40] The *DMBT1* gene was mutated in two cervical cancers and DMBT1 protein is a component of breast milk. DMBT1 may have a role of innate immunity similar to IgA. Mothers upregulate DMBT1 in breast milk if their newborn has an infection.[43]

Expression of cell surface and secreted mucins can be increased by inflammatory cytokines such as interleukin (IL)-4, IL-6, interferons, and tumor necrosis factor-α. These cytokines relate mucins to innate mucosal immunity and to mucosal inflammation.[44] Insulin-like growth factors and transforming growth factor beta may also regulate mucins[45] and their genes are frequently damaged in the breast and cervical cancers. There are many examples of cytokine or growth factor genes damaged in breast[13] and in cervical cancers (data not shown).

Damage to plasma membrane barrier genes. The outer cell membrane is an initial barrier to intracellular infection by pathogens. Genes encoding transmembrane proteins or integral membrane proteins required for the multiple functions of cell membranes are damaged in many breast and cervical cancers. These proteins include interferon-induced transmembrane proteins, ion channels, cell surface antigens, lectin receptors, G-protein-coupled receptors, and cadherins. Defects in lipid metabolism, signaling, and biosynthesis may well effect the control of both infection and invasion by the physical barriers of the cell membrane. Mutated genes in breast and cervical cancers encode enzymes that may be required for cell membranes such as lipases, mono-oxygenases, and acyl transferases. However, these effects were difficult to predict and are not specifically listed.

Damage to Internal Cell Barriers

Actin. All the cervical and breast cancer genomes and exomes have mutations in genes encoding actin interacting proteins or regulating actin polymerization (Table 3). Every virus known interacts with the actin cytoskeleton.[46] There are many striking similarities in actin-related genes mutated in breast and cervical cancers. If the identical gene is not mutated in the two cancers, then mutation occurs in a closely related gene or in a gene that disables the same function. In Table 3, this occurs many times in the different cancers.

A mesh-like barrier composed largely of cortical actin exists just beneath the outer host cell membrane (Fig. 3). The physical barrier of cortical actin must be breached and cell shape altered to accommodate infection. Breast cancer cells can have highly abnormal cell morphology. Starting with viral entry into the host cell through assembly and exit, cellular actin affects all stages of viral infection. Countless different viral strategies have evolved for changing actin and reorganizing the cytoskeleton.[46] Actin occurs elsewhere in the cytoplasm as filaments (Fig. 3), woven bundles, networks, and free globular monomers.[23] Actin filaments are important features of adherens junctions (Figs. 2 and 3).

Keratins. At least 9/21 breast cancer whole genomes, 40/103 different breast cancer exomes, and 12/14 cervical cancers have mutations in keratin or keratin-related genes. Alteration of keratin intermediate filaments has been reported in other breast cancers as well.[47] Keratin monomers form polymers within cells to become structural intermediate filaments, connecting luminal cells to myoepithelial cells, and in turn connecting myoepithelial cells to basement membrane (Fig. 3). HPV disrupts normal organization of keratin networks[48] leaving clear space in the cytoplasm. Many mutations in genes encoding keratin and keratin-associated proteins in cervical cancer may facilitate this disturbance or remove a barrier to other infections. For example, five different breast cancer exomes and two cervical cancers have mutations in *PLEC* or *PLEC* isoforms. The *PLEC* gene encodes plectin-1, a 500-kD intermediate filament-(Keratin) binding protein. Plectin 1 gives mechanical strength to cells and tissues by cross-linking the cytoskeleton.

Nuclear, nucleosome barriers. The nucleus itself presents a formidable barrier to infection due to its structure. A double-membrane envelope encloses the nucleus (Fig. 3). The size and shape of the nucleus is an important determinant of nuclear grade in cancer pathology. Table 4 lists 13 breast cancer whole genomes with damaged nuclear structures or transport mediators (importins or exportins). Nuclear structural damage was found in at least 96/103 breast cancer exomes. Nuclear envelope breakdown is essential for HPV16 infection.[49] Twelve of 14 different cervical cancers had mutations in proteins essential for nuclear barrier functions (Fig. 3). *SYNE1* and *SYNE2* (nesprins) encode nuclear outer membrane proteins that bind cytoplasmic F-actin.[24] This binding helps tether the nucleus to the cytoskeleton and reinforces the structural integrity of the nucleus (Fig. 3). Five breast cancer whole genome and five Mexico–Vietnam exome sequences show mutations in *SYNE1* or *SYNE2*.

Nuclear pore complexes are direct pipelines between the nucleus and the cytoplasm and they control essential exchanges and transport. The nuclear pore complex is composed of 30 nucleoporin proteins (Nups). Genes encoding some Nups have acquired mutations in various breast cancers and less frequently in cervical cancers (Table 4).

Histones and nucleosomes create additional barriers to nuclear DNA that shape and control pathogen access to host DNA. Multiple additional factors affect these barriers.[50] Chromatin and the mechanisms that regulate and repair chromatin regulate the infectious cycles of many DNA viruses. Similar to

the control of cellular functions, chromatin affects the progression of infection, viral latency, and reactivation for a wide range of virus pathogens.[51,52]

The titin gene (*TTN*) encodes a large contractile protein found both in the nucleus and in the cytoplasm. *TTN* is mutated in 20 breast cancers and in most (9/14) cervical cancers (Tables 2 and 4). In contrast, mutation in *TTN* was not found in two different DCIS samples. *TTN* may help shape nuclear structure, binding histones and lamin structural filaments.[53]

Shared Mutated Genes in Triple-negative and Other Breast Cancers

The shared mutated genes are probably not a common set of genes associated with the cancer process, based on additional comparisons and with 104 triple-negative breast cancers[17] and with 100 breast cancer exomes.[18] The set of triple-negative breast cancers had only 143 mutated genes in common with the set of breast cancer exomes from Mexico and Vietnam. The 65 triple-negative breast cancers had 2,045 genes with validated mutations, but less than 1,800 encoded known functions. At least 65% of the testable mutations in the triple-negative breast cancers could be related to some barrier or immune function. Although pleiotropic functions are common, most mutated genes in the triple negatives damaged some immune and barrier functions even if the particular gene damage was not shared with viral or with other breast cancers. This suggests that breast cancer mutations can disable immune and barrier functions in many different ways. Nonetheless, these functions are probably always damaged. In 19 ductal breast cancers, 73–100% of mutations from a total of nearly 1000 mutations occurred in genes that could be associated with immunity or barriers to infections (submitted for publication). Over two-thirds of triple-negative cancers were reported by Shah et al to contain "one or more mutations in actin/cytoskeletal functions group of genes".[17]

Although damage to immune and architectural functions was always found, the numbers of identical shared mutations were variable. Four hundred ninety-one mutated genes in the triple-negative breast cancer sets were also mutated in the viral cancer sets. Of these 491 genes, only 239 were found among the 1,175 mutated genes that breast cancer exomes/whole genomes share with viral cancers. These 239 mutated genes were still heavily enriched for functions essential for immunity, for barriers, or for functions underlying defenses against infection. The 100 breast cancer exomes[18] had 1,144/5,098 mutated genes in common with cervical cancer and only slightly more (1,379/5,098) in common with the Mexico–Vietnam exomes.

Controls: Comparisons to Viral and Non-viral Cancers

As a positive control, breast cancer DNA exome sequences were compared to hepatocellular carcinoma, another known viral cancer. Hepatocellular carcinoma is caused by hepatitis B or C viruses, especially in underdeveloped countries. Of the 3,807 different genes with mutations in breast cancer DNA from Vietnam and Mexico, a high percentage (53%) were also mutated in hepatocellular carcinoma. A surprisingly high rate of concordance was also found by comparing a small set of genes mutated in Burkitt's lymphoma to the breast cancer exomes. Over 50% of genes mutated in Burkitt's lymphoma were mutated in one or more breast cancer exomes. The two viral cancers with more data available (cervical and hepatocellular) had 856 different mutated genes in common. Although data were limited, only a few genes mutated in the breast cancers and in the viral cancers were also mutated in breast diseases that raise the risk of breast cancer or in cervical dysplasias—precursor forms of cervical cancer (cervical intraepithelial neoplasias).

Small-cell lung cancer as a "non-viral cancer" negative control. The cause of small-cell lung cancer is commonly listed as tobacco smoking, and never-smokers are rarely diagnosed with small-cell lung cancer. Small-cell lung cancer was therefore chosen as a prototypical "non-viral" cancer. Comparisons were then made to breast and cervical cancers stated above.

Small-cell lung cancers are heavily mutated with 9,571 genes with acquired mutations based on 56 to 677 samples tested. Figure 4 is a plot of the ratios of genes mutated in breast

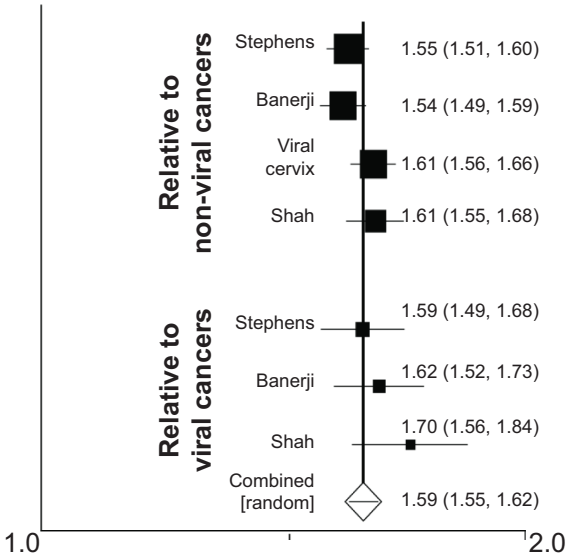

Figure 4. Relative ratios of shared gene mutations in breast cancer datasets to non-viral and viral cancer datasets. Ratios of shared genes mutated in breast cancer per total genes mutated in each dataset are compared to the ratios of genes mutated per whole genome in "non-viral" small-cell lung cancers (top) and to the ratios in viral cervical cancer datasets (bottom). The size of the boxes indicates the weight given each set of data relative to all others. The 95% confidence intervals are shown by the numbers to the right of the graph and indicated by the length of lines going through the boxes. References are indicated by first author only and are as follows: Stephens et al,[18] Banerji et al,[16] Shah et al.[17]

cancers relative to shared genes mutated in non-viral cancers and to shared genes mutated in viral cancers. There is no statistically significant difference in these ratios. Breast cancers all share about the same percentage of their mutations with both viral and non-viral cancers.

The "non-viral" lung cancers had 24 different detoxification genes (cytochrome *P450s*) with mutations and at least 35 damaged genes encoding DNA repair functions. Damaging these genes may facilitate mutations in many genes essential for immunity and barriers. Alternate or related forms of genes that probably encode similar functions were frequently mutated in the lung cancers. When all these were added together, at least 40% of the mutations in the "non-viral cancer" could be accounted for by mutations in the immune system or in barriers to infection. This is a minimal estimate, because the result is based on an evolving database of immune and barrier system mutations.

Genes that do not mutate in viral, non-viral, and breast cancers. The percentages of genes without mutations in viral, non-viral, and breast cancers were about the same. Viral (cervix) cancer data had 24,835 entries and the "non-viral" lung cancer data had 19,183 entries. Of the genes without mutations, 17,412 were shared between the viral and non-viral cancers. Thus, 90% of the genes without mutations in the non-viral lung cancer are also without mutations in the viral cervical cancer. Similarly, based on data from 28 to 54 breast cancers in the COSMIC database, ductal breast cancers had 26,267 "genes without mutations." Of these, 22,981 (87%) were also found in known viral cancers of the cervix.

Ability to Distinguish Chance Events

Biologic arguments. Many gene mutations in breast, cervical, and lung cancer could contribute to carcinogenesis by damaging essential functions that prevent or control cancer-related infections. In Figure 3, 135/138 (98%) of all cancers had a mutation that involved cell adhesion or the ECM. Genes encoding other cell barriers such as the cytoplasm and the nucleus were also damaged in high percentages of both cervical and breast cancers. If damage or deregulation of a gene or a host cell function is not lethal and prevents cell destruction by immunity, facilitates cell survival, or growth after infection, then the odds that the gene will be mutated in other cancer populations may increase.[54] The only Mexico–Vietnam breast cancer exome with no mutated genes in common with the 14 cervical cancers was BR-V-051, which had 12 mutated genes. Only 10 genes had known functions and all 10 had some relationship with immunity (5/10) or cell barriers (5/10).

Even if a given gene was mutated in breast cancers but not in cervical or lung cancers, there were often closely related genes with mutations encoding architectural, immune, or supporting functions. For example, breast cancer BR-V-019 had a mutation in membrane-bound *MUC4*. *MUC4* was not mutated in cervical cancer, but there were mutations in membrane mucin genes *MUC16*, *17* and isoforms in five different cervical cancers. The "non viral" lung cancers had mutations in *MUC2*, *4*, *5AC*, *6*, *13*, *16*, *17*, and *21*.

Forty identical pairs of genes were mutated in a breast–cervical cancer pair: BR-M-191 (breast cancer) vs DR-A0ZM-01 (cervical cancer). Information about the functions of all the 40 genes with shared damage are given in Table 5. The functions of three genes were not known or poorly understood, but the remaining 37 shared genes with damage (100%) are involved in the immune response or in maintaining cell barriers to infection (Table 5).

Probability calculations. Even if mutations may occur at random, their consequences to the cell are not random. Mutations that are lethal are not sustained. Other mutations may not be found because the same essential function is already disabled by different mutations. If each of the ~27,000 genes in the human genome has an equal likelihood of becoming mutated in cancer, then the probability is quite low (3.7×10^{-5}) that the same gene would be mutated in two different cancers. In the initial set of breast cancers (103 Mexico–Vietnam exomes + 21 whole genomes), there are ($14 \times 124 = 1{,}736$) possible different breast–cervical cancer pairs. The probability of finding the same gene mutated in one of these pairs purely by chance rises to 1,736/27,000 = 0.06. However, breast cancer exome BR-M-037-01 had seven mutated genes in common with DS-A0VM-01 ($P = 2.8 \times 10^{-9}$), 10 mutated genes in common with cervical cancer EA-A1QT-01 ($P = 6 \times 10^{-13}$), and 29 mutated genes in common with cervical cancer DR-A0ZM-01 ($P = 3.6 \times 10^{-36}$). Forty identical pairs of genes had mutations in breast–cervical cancer pair BR-M-191 vs DR-A0ZM-01 ($P = 1.3 \times 10^{-49}$). None of these observations are consistent with chance occurrences.

APOBEC may provide a mechanism for some mutations. Apolipoprotein B (APOBEC 3) represents a family of mRNA editing enzymes that can cause cytosine deamination, resulting in cytosine conversion to thymine or guanine.[55] Viruses or retrotransposons can activate APOBEC and may cause these cytosine conversions as collateral damage, representing a mutation signature related to viral infections. Other mutagens such as UV light[56] can also cause the same conversions in high yield and can complicate analysis. Base changes of 34.6% vs 34.9% in Mexico–Vietnam breast and in cervical cancers, respectively, were of C > T/C > G mutations. The *PIK3CA* proto-oncogene has been suggested as a specific target of viral APOBEC 3-induced mutagenesis.[57] Three of 21 whole genome sequence breast cancers, 28/103 different exomes, and 6/104 triple-negative breast cancers had a mutation in *PIK3CA*. Two of 14 cervical cancers had mutations in *PIK3CA* isoforms.

Discussion

Evidence above predicts that cancer cells are either already infected or should be more susceptible to some infections than normal cells because mutations create gaps in immunity and

damage to infection barriers. Other infections may be less likely because mutations have altered host proteins required by the infection. Analysis of genes mutated in breast, viral, and non-viral cancer genomes reveals extensive similarities (Tables 1–5). Damage to immunity and architectural barriers to infection may enable infections to play a larger role in breast cancer than previously believed. Damage to genes essential to protect against breast cancer cell infection accounted for substantial percentages of all coding mutations. Mutations in genes that control cell growth or genome stability can also disable the immune system because genes essential for immunity are not only widely dispersed throughout the genome, but also depend on overall metabolic integrity. For example, mutations encoding functions essential for protein synthesis, transcription, splicing, energy production, cell volume regulation, lipid, transport, metabolism, and others may all have profound effects on the ability to control infection.

Acquired deficits in immunity may release oncogenic infections that are normally controlled in breast, viral, and non-viral cancers. Damage to cellular barriers against infection facilitates cancer-associated infection takeover of the cell. Smoking not only is known to impair the immune system but changes the microbiome as well.[58–60] Mutagens in tobacco smoke inactivate or deregulate immunity, damaging cell architecture and barriers to infection and cancer. Infections that can cause cancer may escape surveillance even in this prototype "non-viral" cancer.

There is unequivocal proof that at least seven viruses each cause one or more human cancers. Known viral cancers include leukemias, tropical spastic parapesis, cervical cancer, head and neck cancer, anogenital cancer, Kaposi's sarcoma, Burkitt's lymphoma, hepatocellular carcinoma, Merkel cell polyoma, and B-cell lymphomas. Multiple viruses have been found in breast cancers[1] and up to 12 different HPV viruses have been found in HeLa cells.[61] These and many additional viruses are under active investigation for their potential roles in human cancers of the brain, bone, mesothelium, prostate,[62] breast, lung,[63] ovary,[64,65] colon-rectum,[66] etc.

For the past decade, cancer treatment and research has been based on the idea that cancer is caused by mutations in only a few significant driver genes. Driver genes made up <0.1% of mutations and controlled cell fate, survival or genome maintenance but did not have any coherent effects on cell function. Finding hundreds of identical genes with damage shows that hundreds more genes are involved in cancer. In the hundreds of cancers studied, gene damage is not uniformly distributed and cannot occur by chance. Damage to genes encoding immune responses and cellular barriers can represent a substantial percentage of coding genes mutated.

Damage to architectural barriers may also facilitate cancer invasion. Finding hemorrhages within biopsies of breast cancers and some ductal carcinoma in situ (DCIS) can be taken as evidence that extracellular barriers and barriers created by controlled cell attachments have broken down. Mutations in breast duct basement membranes and in breast duct cells cause damage to immune defenses and to internal host cell barrier skeletons. Mutations that deregulate MMPs can aggravate damage to basement membranes. Myoepithelial cells represent another barrier inside the basement membrane. Genes encoding myoepithelial cell markers are mutated in some of the breast cancers, suggesting the loss of this barrier. In various breast cancers, markers with mutations include MYH10, MYH14, CNN2, P63, CDH3/Pcadherin, MME/CD10, MYH11 (smooth muscle myosin heavy chains), CNN1, and CNNM2. The total effects of these mutations could easily activate or disperse infections and cancer cells (Figs. 2 and 3), adversely affecting prognosis.

A great deal of generally accepted data places tumor viruses in the breast duct in normal women. For example, high-risk viral sequences from multiple tumor viruses are present in breast milk in normal women[1] and transmission of many viruses (including HIV-1) occurs through breast milk. Specifically, HPV is present in the circulation of cancer patients[67] and could also enter the ductal system via the blood stream (Fig. 2). However, the structures in Figure 2 are essential to prevent other infections as well.

As a practical consequence, identifying the structures that have sustained damage can provide evidence whether a DCIS lesion is likely to become invasive. Loss of four cell adhesion genes has been reported in DCIS: SCRIB, MIR661, NTM, OPCML, and JAM3.[68] The loss of cell barriers further predicts that cancer cells are easier to infect than normal cells. Understanding the cell structural barriers that have been damaged has practical value in therapy and the greater ease of infecting cancer with infections that can exploit individual sets of mutations may be useful in designing therapy. Breast cancer cells should be more susceptible to such infections than normal surrounding cells.

Some mutations affect genes with no obvious direct effect on breast cancer or immunity. In some cases, these genes may be connected to viral infection because they were known sites of viral integration. The neurexins are integration sites for HBV and perhaps for other viruses as well. Neurexin isoforms are mutated in four different breast cancer exomes and in two cervical cancers. Other mutations can be explained by translocations involving genes essential for immunity or barrier functions. For example, the *TAL2* gene participates in translocations involving the T-cell antigen receptor.

Summary

Damage to immune defenses and normal barriers to infection may make breast cancer cells highly susceptible to some infections.

Damage occurs in the same protective functions in breast, viral, and non-viral cancers. Identical genes can be damaged in viral cancers even though they occur in different organs.

The large numbers of mutated genes concerned with protection from infections contradict the widely held belief that only a few driver genes cause cancer.

Breast cancer cells may be either already infected or more susceptible to infections that can exploit existing individual sets of gene mutations. Either possibility may provide a basis for therapy.

Author Contributions

Conceived and designed the experiments: BF. Analyzed the data: BF. Wrote the first draft of the manuscript: BF. Made critical revisions: BF. The author reviewed and approved of the final manuscript.

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