SciMedCentral

Journal of Cancer Biology & Research

Review Article

The Pathobiology of Mammographic Density

Kara Britt[1,5]*, Wendy Ingman[2], Cecilia Huo[3], Grace Chew[3] and Erik Thompson[3,4]

[1]Peter MacCallum Cancer Centre, Australia
[2]School of Medicine, University of Adelaide, Australia
[3]Department of Surgery, University of Melbourne, Australia
[4]St. Vincent's Institute, Australia
[5]The Sir Peter MacCallum Department of Oncology, University of Melbourne, Australia

Abstract

High mammographic density confers a significantly increased risk of breast cancer. As it is relatively common in the normal population the risk of cancer attributable to increased mammographic density could potentially account for an important percentage of total BCa cases. The underlying cause for high mammographic density and its association with increased BCa risk and progression is unknown. In this review we describe the work that has been done to define the histopathological characteristics of mammographic density. Mammograms define breast tissues with areas of high density due to an increased amount of radio-opaque tissue (stromal and epithelial cells) and also less areas of radiolucent fat. Histological work however can define the roles played by each cell type. We review the work that has been performed assessing changes in epithelial cells, stromal cells, the extracellular matrix, and immune infiltrate. To determine how these changes may be increasing breast cancer risk we also discuss the roles of each of the cell types in breast cancer initiation and progression.

Special Issue on
Breast Cancer Therapeutics

*Corresponding author

Kara Britt, Peter MacCallum Cancer Centre, 7 St Andrews Place East Melbourne, Victoria, 3002 Australia, Tel: +61396561285; Email: kara.britt@petermac.org

Submitted: 16 January 2014

Accepted: 28 February 2014

Published: 15 March 2014

Keywords

- Mammographic density
- Extracellular matrix
- Stromal fibroblasts
- Immune cells

ABBREVIATIONS

DCIS: Ductal Carcinoma In Situ; IDC: Invasive Ductal Carcinoma; MD: Mammographic Density; HMD: High Mammographic Density; LMD: Low Mammographic Density, BCa: Breast Cancer; BRCA1: Breast Cancer Susceptibility gene 1; ECM: Extracellular Matrix; CAF: Cancer Associated Fibroblast

INTRODUCTION

An Australian woman's lifetime risk of breast cancer (BCa) is 1 in 8, with approximately 14,000 new diagnoses and 2,200 deaths annually [1]. This mirrors risk estimates in other Western countries such as the UK and USA. Risk factors for BCa include increasing age, a family history of BCa, increased number of menstrual cycles and high mammographic density (MD). Women with high MD have a 4-6 times increased risk of BCa [2,3], a relative risk that is substantially larger than any other risk factor for BCa, independent of BRCA1/2 carrier status. For comparsion 55-65% of high risk BRCA1 mutation carriers will develop breast cancer by age 70, yet these cancers are thought to account for only 2-5% or less of all BCa cases [4,5]. High Mammographic Density (HMD), on the other hand, is relatively common in the normal population. Fifty percent of women between the age of 40 and 49 years, and 30% of women aged 70–79 years, have breasts that are at least 50% dense [6]. Due to the moderate risk and high prevalence of MD, the risk of cancer attributable to increased MD could potentially account for a significant proportion of total BCa cases. The underlying cause for MD and its association with increased BCa risk and progression is not known.

On a mammogram, fat appears radiolucent or dark, whereas stromal and epithelial tissue appears radio-opaque or white. Therefore, not surprisingly, HMD has been associated with more glandular tissue, increased collagen-rich breast stroma, and decreased fat content [7]. In this review we will discuss the studies that have attempted to correlate the histological structure of the breast with increased mammographic density and also define why changes in each of these might lead to an increased breast cancer risk. We will also review the technological advances in cell separation and sequencing which may help to define how these cell specific changes can affect lifetime breast cancer risk in future studies.

High mammographic density increases the risk of developing breast cancer

Four to six times higher BCa risk than women with little or Low Mammographic Density. Limited work exists showing the stage of tumourigenesis at which HMD exerts its protumourigenic effects. Ursin and colleagues retrospectively assessed mammograms from patients taken at the time of the Ductal Carcinoma In-Situ (DCIS) diagnosis, and where available, from the most recent mammogram prior to the mammogram at diagnosis. They found that DCIS occurred preferentially in the areas that were mammographically dense [3]. Similarly, many other studies have

associated increased breast density with DCIS risk, including (but not limited to) the works of Yaghjyan, Gill and Reinier [8-10]. One of the most well cited of these is that from Boyd and colleagues who showed that women with 50-75% breast density or >75% breast density had a 7.33 or 12.2 times greater risk of developing atypical ductal hyperplasia respectively, compared to women with low mammographic density [11]. Another indirect suggestion that HMD increases breast cancer initiation comes from work of Habel and colleagues. They assessed MD in the ipsilateral breasts of DCIS patients and found that the risk of contralateral BCa in patients with HMD was twice that of women with Low Mammographic Density (LMD) [12].

Emerging data indicates that high density can also increase breast cancer progression. HMD is associated with larger tumours and a higher risk of invasive cancers [10,13]. It is not clear whether MD also increases the risk of metastasis. Two studies have shown that HMD is associated with an increased rate of local recurrence after breast conserving surgery or lumpectomy and radiotherapy [14,15], but not distant recurrence [15]. We have indirect evidence that HMD may be associated with increased metastasis. Dense matrices generated from prophylactic ductal carcinoma tissues showed elevated ROCK1 activity and increased cell migration [16].

Determining which cell type confers increased mammographic density

As both epithelial and stroma cells appear radio-dense on a mammogram, it is difficult to define which cell type is responsible for the increased density. There have been several studies over the last 3 decades that have attempted to correlate the mammographic density measured on the mammogram with the histological appearance of the breast at the microscopic level. We have divided the review of this literature into 3 major cell types; epithelial cells, stromal cells and adipose tissue (Table 1). We have also included a section on immune cells considering the emerging links between inflammation and cancer. We have also explained why we believe each cell type may be implicated in increasing breast cancer risk.

Epithelial cells: The majority of adult human cancers (90%) carcinomas, are cancers that arise from epithelial tissues. These tissues consist of layers of epithelial cells within certain organs (including the breast, prostate and intestine), which continuously renew throughout life under tight control. In breast cancer, carcinomas (cancers that arise from epithelial cells of the breast), comprise the vast majority of all breast cancers, with sarcomas (cancers arising from stromal cells) comprising only 1% of all breast cancers. Assessing changes in epithelial cells of high and low density may reveal how density affects breast cancer risk.

Changes in epithelial proliferation with HMD: As mentioned, epithelial cells and stromal cells appear radio opaque (or dense) on mammograms. Several studies have assessed the two cell types in isolation to determine whether the increased density is due to changes in one or both cell types. There are 7 studies that have assessed the proportion of epithelial cells within HMD and LMD human breast tissue to ascertain if the epithelial cells are responsible for the increased density. Five of the studies have reported that HMD is associated with the presence of ductal epithelial hyperplasia [17], or an increased percentage/proportion of epithelial cells [11,18-20]. This was evident despite the use of alternate methods of density categorization and epithelial cell counting protocols. There are however 2 studies which have not shown an association with HMD and epithelial cell content. Alowami and colleagues studied breast tissue from 62 women with breast cancer as part of the Manitoba Provincial Screening Program. The breast tissue for the MD analysis was taken from breast tissue containing the lesion, albeit, at a site distant from the lesion. They found no difference in epithelial density between HMD and LMD regions [21]. Similarly, Lin and colleagues in our own laboratory [22] assessed BRCA1/2 mutation carriers and found no difference in the epithelial cell density between HMD and LMD regions of the same breast. It is possible that the discrepancies between studies is due to the type of breast tissue used for the analysis, however until direct histological comparisons between the HMD and LMD regions of normal breast tissues obtained from reduction mammoplasty and high risk prophylactic mastectomy samples can be made, we cannot be sure. It is also possible that there are distinct changes in the maturation status of the breast epithelial cells in HMD tissue. Cell surface markers, originally identified in mouse mammary gland, can now be used to isolate human breast stem cells, luminal progenitor cells and mature cells [23]. It would be interesting to determine whether the HMD has an increased number of luminal progenitor cells. The number of luminal progenitors cells has been found to be disproportionately abundant in the mammary tissue of high-risk BRCA1 mutation carriers [23]. The luminal progenitor cells in these women have also been shown to have a less differentiated (more stem like) phenotype [24]. As the basal breast cancers arising in BRCA1 mutation carriers also show high gene expression correlation with this epithelial cell type, the luminal progenitor cells have been suggested to represent the cancer cell of origin in the cancers that arise in these women [25].

Cancer arises in cells that have mutations in genes normally serving to restrict or control cell proliferation and/or in those factors that control cell death and survival. Thus, many investigators have assessed whether the levels of proliferation are higher in the ductal cells of the HMD, at risk breast. Collectively they show only minimal evidence that proliferation is increased in regions of high density. Ghosh and colleagues [18] assessed 59 women (mean age of 51) for changes in epithelium and stroma in high and low MD regions of healthy women. Whilst changes were observed in the cell compartment (epithelial cells, stroma and fat, which are all discussed separately) there was no significant difference in Ki67 staining. Verheus, looked in the benign tissue of 159 women with breast cancer and also found no association of MD with proliferation [26]. There are another 4 studies which have assessed proliferation in benign tissue of breast cancer cases, mastecomy specimens, high risk women or reduction mammoplasty specimens [6,19,27,28]. Three of these showed no association of HMD with increased proliferation, whilst one [27] showed a positive association with density and Ki67 in ducts. As this is the only study thus far to show an increase in proliferation, it would have been encouraging to see representative images of the increased Ki67 in HMD tissue. The lack of consistent data linking epithelial proliferation and HMD may be influenced by the acknowledged variance in Ki67 quantification and reporting [29]

Table 1: Histological studies correlating cell changes with high mammographic density.

Reference	Study	Number of women	Analysis information	Cell type altered in high MD
Ghosh [18]	Mayo Clinic, Minnesota	N=59 women Mean age 51 years	Ultrasound guided biopsy in dese and non dense regions. Histological assessment on H&E sections. Ki67 for proliferation.	HMD had greater epithelial cell content and stroma. HMD had less fat. No difference in Ki67
Bartow [17]	New Mexico office of medical investigation	N=515 women. Age 15-98 years.	Whole breast mammograms. Wolfe system of radiological patterns.	Dense parenchymal pattern associated with prevalence of marked cystsic change and ductal hyperplasia in young women.
Guo [55]	Women's College Hospital, Toronto, Canada	N=92 Mean age 48.5 N=46 age matched pairs	Tissue sections from blocks of tissues surrounding benign lesions. 50% from breasts with little/no MD and 50% from breasts with extensive densities	Percentage nuclear area and total collagen were increased in HMD breasts.
Li [20]	New Mexico Autopsy study	N=236 Mean age 43 years	Mammograms by faxitron. Breasts classified using Wolfe system. Epithelial and non epithelial areas counted and collagen assessed.	HMD was associated with greater total nuclear area, greater nuclear epithelial and non-epithelial areas and greater collagen as well as greater glandular area.
Hawes [19]	University of Southern California	N=12 Median age 33years Large breasts	Density determined by a high degree of connective tissue in H&E. Assessed number of epithelial cells and also proliferation (MIB1)	Breast epithelial cells were concentration in areas of high connective tissue. Proliferation was in areas of high and medium density.
Alowami [21]	Manitoba Provincial Screening Program.	N=62 women Patients were >50 years of age	Breast tissue taken distant from lesion which was benign or pre-invasive. Percent of collagenous stroma, fibrosis and number of ducts were determined	HMD was associated with more extensive fibrosis. No difference in epithelial density
Lin [22]	Peter MacCallum Cancer Centre	N=12 Mean age=39 years	Stereotactially guided vaccum assisted biopsy. Percentage components measured using JMicrovision	No difference in glandular areas. HMD had less fat and more stromal cells.
Bright [54]	Boston	N=320	Mammograms and histologic slides of women with breast symptoms and no cancer. Max degree of epithelial hyperplasia and degree of fibrosis measured	In premenopausal - those with marked fibrosis had large nodular densities on a mammogram. In post menopausal – epithelial hyperplasia or atypia was related to having a Wolfe classification of P2
Boyd [11]	Canadian National Breast Screening Study	N=945 women. Mean age 44 years. 70% premenopausal.	Mammograms classified into 6 categories of density. Histological slides assessed	More extensive MD was associated with greater proportion of collagen, less fat and more epithelium.

Abbreviations: MD: Mammographic Density; HMD: High Mammographic Density; LMD: Low Mammographic Density

and underscores the importance of developing a standardized approach for assessing proliferation. Further studies on Ki67 in epithelial compartments are required and should use a large number of women and also a large number of tissue sections per women, correcting for the number of epithelial cells counted. Further studies could also assess if the increased breast cancer risk in HMD tissue is related to other epithelial cell features apart from proliferation index, such as general cell to cell contact and polarity, which are often lost in the early stages of breast cancer initiation, prior to changes in proliferation.

Stromal cells: The stromal cells lie within a rich extracellular matrix and supply physical support to adjacent epithelial cells within glandular tissues. The stroma includes the vasculature, adipocytes, resident immune cells, and fibroblasts, as well as the many cellular products that these cells generate, including the Extracellular Matrix (ECM). It is primarily the fibroblasts within the stroma, which secrete the macromolecules that make up the ECM, however collagen production by the epithelial cells, and cooperatively between the epithelial and stromal cells has been documented in testicular systems [30,31]. The ECM was originally thought to serve as a relatively inert scaffold to stabilize the physical structure of tissues, but it now known to actively regulate the behavior of the cells that contact it, influencing their survival, development, migration, proliferation, shape, and function [32,33]. The ECM is predominantly composed of proteoglycans and structural/adhesive proteins such as collagens, elastins, fibronectins and laminins.

Altered stromal architecture and ECM composition has been documented in both benign and malignant breast pathologies. Fibrosis is often present in low-risk benign proliferative lesions and the stromal reaction that accompanies DCIS and invasive carcinoma. The majority of breast cancers are associated with a strong desmoplastic stroma and inflammatory response similar to the stromal response observed during chronic would healing

[34-36]. This includes ECM remodeling, growth factor secretion, inflammation, cell migration and angiogenesis. The desmoplastic stroma associated with breast tumours is significantly stiffer than that of the disease free breast [37]. The importance of the stromal fibroblasts in breast cancer initiation is shown by gene expression studies that showed that changes in the stroma occur very early in tumor formation often preceding the onset of invasion, comprising 90% of the alterations that occur during the normal to DCIS stages [38].

In cancer, the stroma modulates tumorigenicity in adjacent nonmalignant or non-tumorigenic epithelia. However, in both breast and prostate cancer, the influence of the Cancer Associated Fibroblasts (CAFs) was not thought to extend to cancer initiation, as tumour growth could not be stimulated in normal prostate or breast epithelial cells [39, 40]. However two recent examples highlight the tumorigenic influence of the stroma when there are no underlying changes present in the associated epithelium. Firstly, stromal specific inactivation of TGFbRII (a TGFβ receptor) causes PIN lesions and prostate cancer in mice [41], secondly stromal loss of PTEN in mammary epithelial causes malignancy [42]. These findings open the door to the possibility that the activation of stromal fibroblasts may play a role in increasing breast cancer risk.

Considering the close relationship between stroma and collagen in previous studies, we wanted to briefly review the relationship between collagen and breast cancer risk. The breast epithelial cell matrix is required for proper differentiation, proliferation, polarity and maintenance [43-45]. The matrix actively participates in controlling most of the stages of breast cancer progression [46]. Breast cancers often re-express ECM proteins which were restricted to embryonic or prenatal development. This includes type 1 trimer collagen, ectodomain D of fibronectin and oncofetal fibronectin, elastin, tenascin [47-50]. Increased deposition of stromal matrix (predominantly collagen) is associated with increased mammographic density and increased breast cancer risk. Cancers are known to originate in the dense regions of the breast rather than less dense regions [3,7]. The strongest indication that ECM changes drive mammographic density and breast cancer risk comes from a mouse model that has increased collagen deposition due to a disruption in the collagenase 1 gene which controls collagen degradation [51]. When *Col1a1* transgenic mice were crossed with MMTV PyMT transgenic mice, the mice developed more tumors and increased metastasis. The alignment of the collagen fibers in a perpendicular orientation rather than parallel to the tumour has also been shown to stimulate cancer progression [52]. The deposition of additional ECM, its crosslinking by lysyl oxidase and its alignment can create a more stiff ECM [37,52,53], which in turn can lead to Rho mediated cellular contractility that activates integrin-mediated signaling pathways and increases epithelial cell proliferation and survival. Evidence for this is supplied in studies showing that a dense or stiff matrix activates pERK in normal mammary epithelial cells, whilst a non-stiff (compliant) matrix does not [37,51].

Studies associating an increase in stromal cells or ECM with HMD: Many of the studies mentioned above that assessed the percentage of epithelial cells in high mammographically dense tissue also assessed the percentage of stromal fibroblasts. Ghosh and colleagues from the Mayo Clinic in Minnesota assessed 69 women with a mean age of 51 and showed that HMD tissue had 46% more stroma than LMD tissue [18]. Similarly Lin and colleagues in our own laboratory assessed 12 high risk women and showed that HMD was associated with higher stromal cell numbers [22]. Many of the other studies have assessed the stroma in terms of either the area of nuclear non-epithelial cells (presumed stroma), total collagen content, or percentage of fibrosis [11,20,21,54]. Further exploration of the contribution of stromal cells to MD should utilize an immunohistochemical approach to quantify the abundance of stromal cells with a specific marker such as vimentin.

There are 4 studies that have assessed the proportion of collagen in breast tissue from LMD and HMD. They have all shown an increase in collagen in HMD regardless of whether they assessed collagen though histological appearance in H&E stained sections [11,21] or with Masons Trichrome collagen staining [20,55]. These studies have not assessed however the structure of the collagen to determine the degree of fibrillar/linearized collagen. A recent study on parous mice has provided indirect evidence that the orientation of the collagen may be altered in HMD tissue [56]. Parous women (have borne children) are protected against breast cancer with women who bear children having an up to 70% reduced risk of breast cancer compared to nulliparous women [57]. Whilst not identified as a cause for the protection that exists in these women, parous women are known to have decreased MD compared to nulliparous women; each birth reducing MD by approximately 2% [58]. Maller and colleagues assessed the collagen content and collagen fiber linearization in the breast tissue of parous rats using Second Harmonic Generation (SHG) imaging. They showed that parous rats had less linearized collagen and decreased stromal stiffness compared to nulliparous rats [56]. To define whether the stromal cell number, and or collagen abundance and orientation are involved in increasing the risk of breast cancer in high dense areas, more thorough analysis of stromal cell numbers and collagen fiber accumulation and orientation should be performed.

TGF-β is thought to be an early tumor suppressor and late tumor promoter during disease progression, however it is not clear exactly when the switch occurs, or what regulates it [59]. Despite this, it appears that in the HMD breast there may be a loss of TGFβ signaling that leads to a pro-tumorigenic environment. Work in normal breast biopsies of tumour bearing breasts has shown that TGFβ signaling is decreased in HMD regions whilst COX2 expression is increased [60,61]. These results are consistent with a pro-inflammatory environment in HMD tissue. Fibroblasts that lack TGFβ signaling are known increase the expression of CXCL1, CXCL5, CXCLl2 leading to progression of various cancer types including breast [62-64]. The increased levels of these cytokines would result in an influx of immune cells suggesting that the immune cells may also play a role in mediating the increased BCa risk in HMD tissue. Both TGFβ and Cox2 have accepted roles in the regulation of T cell maturation [65,66].

Immune cells and breast cancer

Many investigators have proposed that a natural function of the immune system is to seek out and eradicate aberrant

(dysplastic and neoplastic) cells and thus inhibit tumor formation. The concept of immunosurveillance was proposed several decades ago by Burnet and Thomas, and suggests that lymphocytes constantly patrol the body to identify transformed tumor cells [67-69]. Only recently however has the theory been rigorously examined. With the advent of mouse knockout technologies, immunosurveillance has now been demonstrated in mice. IFN deficient mice, STAT1 deficient mice, RAG2 mutant mice and perforin knockout mice all exhibit increased tumorigenesis due to deficiencies in immune cell compartments [70-73]. In humans indirect evidence is also present. Patients with immune deficiencies due to either genetic disorders such as infantile X-linked agamaglobulimia, severe combined immunodeficiency, Wiskott Aldrich or Ataxia telangiectasia [74] or medical immunosuppression during organ transplant [75] demonstrate a high risk for cancer development. On the other hand, aberrant inflammation can also lead to increased risk of cancer. Cancers frequently arise in areas of chronic inflammation, including colon carcinoma associated with inflammatory bowel disease, stomach cancer in H. pylori infection, and hepatocellular carcinomas in hepatitis C infection [76].

The relationship between the immune system and breast cancer specifically has been directly assessed using breast cancer tissue samples. Immunohistochemical staining was performed to assess the changes in density of mononuclear inflammatory cells that accompanies the progression from normal breast, through to invasive ductal carcinoma [77]. The density of CD20+ B cells, CD68+ (monocytes/macrophages), CD3+ T cells and granzyme B+ cytotoxic T cells were determined in 53 mastectomy specimens from normal breast, benign proliferative disease, DCIS and infiltrating ductal carcinoma. Hussein and Hassan determined the density of CD20+ B cells, CD68+ (monocytes/macrophages), CD3+ T cells and granzyme B+ cytotoxic T cells. The most significant finding was a 30-fold increase in CD3+ T cells in the normal to benign proliferative disease, which then remained high with cancer development and progression. There was also a less substantial increase in CD20+ B cells and also macrophages. Together these data suggest that both the innate and adaptive immune system may play an important role in the initiation of breast cancer. Many studies have assessed how the influx of immune cells into primary breast cancers can affect prognosis. The level of tumour associated macrophages, CD4+ T effector cells and CD8+ Cytotoxic T cells has been shown to predict breast cancer prognosis. Tumours that showed a $CD68^{hi}$, $CD4^{hi}$ and $CD8^{low}$ (rather than $CD68^{low}$ $CD4^{low}$ and $CD8^{high}$) had decreased overall survival and relapse free survival [78] in 2 independent tissue microarrays cohorts [79, 80], which agrees with early work in the ratio of CD4+ T cells to CD8+ T cells [81]. This signature was an independent predictor or decreased overall survival and relapse free survival after controlling for grade, nodal staus, tumour size, ER, PR, Her2 and Ki67 [78]. The role of T cells in mediating tumour progression has been strengthened by recent gene expression arrays defining a stromal gene signature that can predict breast cancer prognosis [82]. Finak and colleagues showed that the good outcome patients over expressed a distinct set of immune related genes, which together indicated a Th1 type immune response. In contrast, those individuals with poor outcome had an increase in hypoxic and angiogenic response and a decrease in chemokines that normally act to stimulate NK cell migration and T cells. With emerging roles in breast cancer initiation and progression it is plausible the immune cell complement may also be altered in HMD tissue.

The involvement of the immune cells in mediating HMD: There have been no studies to date that have assessed the abundance or activation status of immune cell subsets in HMD versus LMD. However, there has been a study assessing Single Nucleotide Polymorphisms (SNPs) in cytokines and growth factors, an assessment of serum levels of 3 important cytokines and also a clinical study showing the changes in MD that occur with the use of Non-Steroidal Anti-Inflammatory Medications (NSAIDS). Samples from the Norwegian Breast Cancer Screening Program were used to investigate the association between 89 single nucleotide polymorphisms (SNPs) in 7 cytokine/growth-factor genes (*FGFR2, IGFBP1, IGFBP3, TGFB1, TNF*, *VEGF*, *IL6*) with the percent MD in 301 premenopausal women (aged 50 to 55 years) [83]. Ozhand and colleagues found a significant association in 9 tagging SNPs in the gene encoding interleukin-6 (IL6). They also replicated the association of one of the IL6 SNPs (rs10242595) in an independent study of Singapore Chinese women [83]. They found both positive and inverse associations with different IL6 SNPs, which makes the data hard to interpret. Future work will need to assess how the IL-6 tagging SNPs modify IL-6 protein levels in serum and also locally within the breast. IL6 is a promising target to follow up, as it is a growth factor for B cells, and regulator of CD4 T cell differentiation [84,85]. In a separate study the inflammatory markers, IL-6, tumor necrosis factor-α (TNF-α), and C - Reactive Protein (CRP), were assessed in serum samples of postmenopausal women and correlated with mammographic density in a large sample of 542 women. Whilst some association was observed, when the results were adjusted for age, BMI and additional covariates any association with MD was lost suggesting that serum levels of these factors do not correlate with MD [86]. An alternate study was performed on 3286 women (Australian Mammographic Density Twins and Sisters Study and the Genes Behind Endometriosis Study) to determine whether the breast cancer preventative effects of NSAID drugs such as aspirin occur through changes in MD [87]. No association between either dense area or percent dense area was found with any of the NSAIDs examined [87]. To define whether the immune cells are at increased levels in HMD versus LMD and whether they participate in increasing the breast cancer risk in these women, we will need to directly assess the immune cell subsets in HMD and LMD specimen taken from large cohorts of women.

Fat composition and breast cancer risk: Mammary gland stromal fibroblasts have been shown to undergo reversible differentiation into either mature adipocytes, when cultured in adipogenic medium, or into capillary structures that are sensitive to anti-angiogenic drug treatment [88]. It is believed that this may play a role in mediating the rapid changes in mammary gland composition (particularly of the fat and vascular cells) that occurs during pregnancy, lactation and involution. Similarly it may contribute to the pathological changes that occur during mammary carcinogenesis, with the loss of mature adipocytes and an increase in tumor vasculature. The adipocyte differentiation potential of CAFs isolated from breast cancer patients was 6-fold

less than that in stromal fibroblasts isolated from normal breasts, indicating that the loss of adipocyte differentiation may favor a more pro-tumorigenic stromal fibroblast phenotype [89]. Despite this work, it is not clear what regulatory factors determine the relative abundance of fat and stromal tissue during breast development.

HMD and decreased fat

LMD-associated fibroblasts and HMD-associated fibroblasts were purified from disease free breast biopsies of women with either 25-50% MD or >75% MD and then assessed for proliferation and adipocyte differentiation. Both LMD and HMD-associated fibroblasts accumulated fat under differentiation culture conditions; however the HMD fibroblasts accumulated significantly less (3-fold less). This would indicate a pro-tumorigenic phenotype since the CAFs also have a reduced adipocyte differentiation potential. It is not known what regulates the adipocyte differentiation in these cells, however estrogen may play a role, as mice with non-functional estrogen receptor alpha exhibit increased adiposity [90]. The fact that obesity is related to increased breast cancer risk [91], but a favorable mammographic density pattern [92-94] may imply that the number of adipoyctes in the breast is not the most dominant factor regulating risk in women with high mammographic density.

CONCLUSION

High mammographic density is clearly correlated with an increase in both epithelial cells and stromal cells with a consistent reduction in fat. The percentage of collagen is one of the strongest correlates with breast density, however work remains to identify whether the collagen present in HMD and LMD exhibit similar orientation, which is emerging as a key indicator for the pro-tumorigenic ability of the collagenous stroma. It is not clear at present whether the epithelial cells present in HMD are proliferating at a higher rate, as most studies have shown no difference according to density. Further studies, possibly with automated counting systems (stereological assessment, Imaris cell quantification) may determine if proliferation is increased. Using advances is epithelial cell isolation we now have the power to assess if the maturation status of the breast changes with density and also can determine whether the gene expression and function of these cells is altered.

ACKNOWLEDGEMENT

KB is supported by an Australian National Breast Cancer Foundation (NBCF) Early Career fellowship, WI is supported by an NBCF Early Career fellowship and is the THRF A/Prof of Breast Cancer Research, Erik Thompson is supported in part by the NBCF, Cecilia Huo is supported by the Australian Postgraduate Awards scholarship by the University of Melbourne, Grace Chew is supported by a National Health and Medical Research Council scholarship. This study benefitted from support by the Victorian Government's Operational Infrastructure Support Program to St Vincent's Institute.

REFERENCES

1. AIHW. Australian Institute of Health and Welfare and Cancer Australia 2012. Breast Cancer in Australia: an overview. Canberra, Australia. 2012.
2. Boyd NF, Byng JW, Jong RA, Fishell EK, Little LE, Miller AB, et al. Quantitative classification of mammographic densities and breast cancer risk: results from the Canadian National Breast Screening Study. Journal of the National Cancer Institute. 1995; 87: 670-675.
3. Ursin G, Hovanessian-Larsen L, Parisky YR, Pike MC, Wu AH. Greatly increased occurrence of breast cancers in areas of mammographically dense tissue. Breast Cancer Res. 2005; 7: R605-608.
4. Group ABCS. Prevalence and penetrance of BRCA1 and BRCA2 mutations in a population-based series of breast cancer cases. Anglian Breast Cancer Study Group. British journal of cancer. 2000; 83: 1301-1308.
5. Peto J, Collins N, Barfoot R, Seal S, Warren W, Rahman N, Easton DF. Prevalence of BRCA1 and BRCA2 gene mutations in patients with early-onset breast cancer. J Natl Cancer Inst. 1999; 91: 943-949.
6. Stomper PC, D'Souza DJ, DiNitto PA, Arredondo MA. Analysis of parenchymal density on mammograms in 1353 women 25-79 years old. AJR Am J Roentgenol. 1996; 167: 1261-1265.
7. Martin LJ, Boyd NF. Mammographic density. Potential mechanisms of breast cancer risk associated with mammographic density: hypotheses based on epidemiological evidence. Breast cancer Res. 2008; 10: 201.
8. Gill JK, Maskarinec G, Pagano I, Kolonel LN. The association of mammographic density with ductal carcinoma in situ of the breast: the Multiethnic Cohort. Breast Cancer Res. 2006; 8: R30.
9. Reinier KS, Vacek PM, Geller BM. Risk factors for breast carcinoma in situ versus invasive breast cancer in a prospective study of pre- and post-menopausal women. Breast Cancer Res Treat. 2007; 103: 343-348.
10. Yaghjyan L, Colditz GA, Collins LC, Schnitt SJ, Rosner B, Vachon C, et al. Mammographic breast density and subsequent risk of breast cancer in postmenopausal women according to tumor characteristics. J Natl Cancer Inst. 2011; 103: 1179-1189.
11. Boyd NF, Jensen HM, Cooke G, Han HL. Relationship between mammographic and histological risk factors for breast cancer. J Natl Cancer Inst. 1992; 84: 1170-1179.
12. Habel LA, Capra AM, Achacoso NS, Janga A, Acton L, Puligandla B, et al. Mammographic density and risk of second breast cancer after ductal carcinoma in situ. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2010; 19: 2488-2495.
13. Kavanagh AM, Byrnes GB, Nickson C, Cawson JN, Giles GG, Hopper JL, et al. Using mammographic density to improve breast cancer screening outcomes. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2008; 17: 2818-2824.
14. Cil T, Fishell E, Hanna W, Sun P, Rawlinson E, Narod SA, et al. Mammographic density and the risk of breast cancer recurrence after breast-conserving surgery. Cancer. 2009; 115: 5780-5787.
15. Park CC, Rembert J, Chew K, Moore D, Kerlikowske K. High mammographic breast density is independent predictor of local but not distant recurrence after lumpectomy and radiotherapy for invasive breast cancer. International journal of radiation oncology, biology, physics. 2009; 73: 75-79.
16. Raviraj V, Fok S, Zhao J, Chien HY, Lyons JG, Thompson EW, et al. Regulation of ROCK1 via Notch1 during breast cancer cell migration into dense matrices. BMC Cell Biol. 2012; 13: 12.

17. Bartow SA, Pathak DR, Mettler FA, Key CR, Pike MC. Breast mammographic pattern: a concatenation of confounding and breast cancer risk factors. Am J Epidemiol. 1995; 142: 813-819.

18. Ghosh K, Brandt KR, Reynolds C, Scott CG, Pankratz VS, Riehle DL, et al. Tissue composition of mammographically dense and non-dense breast tissue. Breast Cancer Res Treat. 2012; 131: 267-275.

19. Hawes D, Downey S, Pearce CL, Bartow S, Wan P, Pike MC, et al. Dense breast stromal tissue shows greatly increased concentration of breast epithelium but no increase in its proliferative activity. Breast Cancer Res. 2006; 8: R24.

20. Li T, Sun L, Miller N, Nicklee T, Woo J, Hulse-Smith L, et al. The association of measured breast tissue characteristics with mammographic density and other risk factors for breast cancer. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2005; 14: 343-349.

21. Alowami S, Troup S, Al-Haddad S, Kirkpatrick I, Watson PH. Mammographic density is related to stroma and stromal proteoglycan expression. Breast Cancer Res. 2003; 5: R129-135.

22. Lin SJ, Cawson J, Hill P, Haviv I, Jenkins M, Hopper JL, et al. Image-guided sampling reveals increased stroma and lower glandular complexity in mammographically dense breast tissue. Breast cancer research and treatment. 2011; 128: 505-516.

23. Lim E, Vaillant F, Wu D, Forrest NC, Pal B, Hart AH, et al. Aberrant luminal progenitors as the candidate target population for basal tumor development in BRCA1 mutation carriers. Nat Med. 2009; 15: 907-913.

24. Proia TA, Keller PJ, Gupta PB, Klebba I, Jones AD, Sedic M, et al. Genetic predisposition directs breast cancer phenotype by dictating progenitor cell fate. Cell Stem Cell. 2011; 8: 149-163.

25. Visvader JE, Lindeman GJ. Cancer stem cells: current status and evolving complexities. Cell stem cell. 2012; 10: 717-728.

26. Verheus M, Maskarinec G, Erber E, Steude JS, Killeen J, Hernandez BY, et al. Mammographic density and epithelial histopathologic markers. BMC Cancer. 2009; 9: 182.

27. Harvey JA, Santen RJ, Petroni GR, Bovbjerg VE, Smolkin ME, Sheriff FS, et al. Histologic changes in the breast with menopausal hormone therapy use: correlation with breast density, estrogen receptor, progesterone receptor, and proliferation indices. Menopause. 2008; 15: 67-73.

28. Khan QJ, Kimler BF, O'Dea AP, Zalles CM, Sharma P, Fabian CJ. Mammographic density does not correlate with Ki-67 expression or cytomorphology in benign breast cells obtained by random periareolar fine needle aspiration from women at high risk for breast cancer. Breast Cancer Res. 2007; 9: R35.

29. Pathmanathan N, Balleine RL. Ki67 and proliferation in breast cancer. J Clin Pathol. 2013; 66: 512-516.

30. Raychoudhury SS, Irving MG, Thompson EW, Blackshaw AW. Collagen biosynthesis in cultured rat testicular Sertoli and peritubular myoid cells. Life Sci. 1992; 51: 1585-1596.

31. Skinner MK, Tung PS, Fritz IB. Cooperativity between Sertoli cells and testicular peritubular cells in the production and deposition of extracellular matrix components. J Cell Biol. 1985; 100: 1941-1947.

32. Spencer VA, Xu R, Bissell MJ. Extracellular matrix, nuclear and chromatin structure, and gene expression in normal tissues and malignant tumors: a work in progress. Adv Cancer Res. 2007; 97: 275-294.

33. Spencer VA, Xu R, Bissell MJ. Gene expression in the third dimension: the ECM-nucleus connection. J Mammary Gland Biol Neoplasia. 2010; 15: 65-71.

34. Bissell MJ, Radisky D. Putting tumours in context. Nat Rev Cancer. 2001; 1: 46-54.

35. Dvorak HF. Tumors: wounds that do not heal. Similarities between tumor stroma generation and wound healing. N Engl J Med. 1986; 315: 1650-1659.

36. Tlsty TD, Coussens LM. Tumor stroma and regulation of cancer development. Annu Rev Pathol. 2006; 1: 119-150.

37. Paszek MJ, Zahir N, Johnson KR, Lakins JN, Rozenberg GI, Gefen A, et al. Tensional homeostasis and the malignant phenotype. Cancer Cell. 2005; 8: 241-254.

38. Ma XJ, Dahiya S, Richardson E, Erlander M, Sgroi DC. Gene expression profiling of the tumor microenvironment during breast cancer progression. Breast Cancer Res. 2009; 11: R7.

39. Olumi AF, Grossfeld GD, Hayward SW, Carroll PR, Tlsty TD, Cunha GR. Carcinoma-associated fibroblasts direct tumor progression of initiated human prostatic epithelium. Cancer research. 1999; 59: 5002-5011.

40. Orimo A, Gupta PB, Sgroi DC, Arenzana-Seisdedos F, Delaunay T, Naeem R, et al. Stromal fibroblasts present in invasive human breast carcinomas promote tumor growth and angiogenesis through elevated SDF-1/CXCL12 secretion. Cell. 2005; 121: 335-348.

41. Li X, Placencio V, Iturregui JM, Uwamariya C, Sharif-Afshar AR, Koyama T, et al. Prostate tumor progression is mediated by a paracrine TGF-beta/Wnt3a signaling axis. Oncogene. 2008; 27: 7118-7130.

42. Trimboli AJ, Cantemir-Stone CZ, Li F, Wallace JA, Merchant A, Creasap N, et al. Pten in stromal fibroblasts suppresses mammary epithelial tumours. Nature. 2009; 461: 1084-1091.

43. Berdichevsky F, Gilbert C, Shearer M, Taylor-Papadimitriou J. Collagen-induced rapid morphogenesis of human mammary epithelial cells: the role of the alpha 2 beta 1 integrin. J Cell Sci. 1992; 102 : 437-446.

44. Li ML, Aggeler J, Farson DA, Hatier C, Hassell J, Bissell MJ. Influence of a reconstituted basement membrane and its components on casein gene expression and secretion in mouse mammary epithelial cells. Proceedings of the National Academy of Sciences of the United States of America. 1987; 84: 136-140.

45. Persson I, Thurfjell E, Holmberg L. Effect of estrogen and estrogen-progestin replacement regimens on mammographic breast parenchymal density. Journal of clinical oncology: official journal of the American Society of Clinical Oncology. 1997; 15: 3201-3207.

46. Lochter A, Bissell MJ. Involvement of extracellular matrix constituents in breast cancer. Semin Cancer Biol. 1995; 6: 165-173.

47. Inaguma Y, Kusakabe M, Mackie EJ, Pearson CA, Chiquet-Ehrismann R, Sakakura T. Epithelial induction of stromal tenascin in the mouse mammary gland: from embryogenesis to carcinogenesis. Dev Biol. 1988; 128: 245-255.

48. Jones PL, Boudreau N, Myers CA, Erickson HP, Bissell MJ. Tenascin-C inhibits extracellular matrix-dependent gene expression in mammary epithelial cells. Localization of active regions using recombinant tenascin fragments. Journal of cell science. 1995; 108: 519-527.

49. Koukoulis GK, Howeedy AA, Korhonen M, Virtanen I, Gould VE. Distribution of tenascin, cellular fibronectins and integrins in the normal, hyperplastic and neoplastic breast. J Submicrosc Cytol Pathol. 1993; 25: 285-295.

50. Pechoux C, Clezardin P, Dante R, Serre CM, Clerget M, Bertin N, et al. Localization of thrombospondin, CD36 and CD51 during prenatal

development of the human mammary gland. Differentiation; research in biological diversity. 1994; 57: 133-141.

51. Provenzano PP, Inman DR, Eliceiri KW, Knittel JG, Yan L, Rueden CT, et al. Collagen density promotes mammary tumor initiation and progression. BMC Med. 2008; 6: 11.

52. Provenzano PP, Vanderby R Jr. Collagen fibril morphology and organization: implications for force transmission in ligament and tendon. Matrix Biol. 2006; 25: 71-84.

53. Goetz JG, Minguet S, Navarro-Lérida I, Lazcano JJ, Samaniego R, Calvo E, et al. Biomechanical remodeling of the microenvironment by stromal caveolin-1 favors tumor invasion and metastasis. Cell. 2011; 146: 148-163.

54. Bright RA, Morrison AS, Brisson J, Burstein NA, Sadowsky NS, Kopans DB, et al. Relationship between mammographic and histologic features of breast tissue in women with benign biopsies. Cancer. 1988; 61: 266-271.

55. Guo YP, Martin LJ, Hanna W, Banerjee D, Miller N, Fishell E, et al. Growth factors and stromal matrix proteins associated with mammographic densities. Cancer epidemiology, biomarkers & prevention: a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2001; 10: 243-248.

56. Maller O, Hansen KC, Lyons TR, Acerbi I, Weaver VM, Prekeris R, et al. Collagen architecture in pregnancy-induced protection from breast cancer. J Cell Sci. 2013; 126: 4108-4110.

57. MacMahon B, Cole P, Lin TM, Lowe CR, Mirra AP, Ravnihar B, et al. Age at first birth and breast cancer risk. Bull World Health Organ. 1970; 43: 209-221.

58. Boyd NF, Guo H, Martin LJ, Sun L, Stone J, Fishell E, et al. Mammographic density and the risk and detection of breast cancer. N Engl J Med. 2007; 356: 227-236.

59. Pickup MW, Laklai H, Acerbi I, Owens P, Gorska AE, Chytil A, et al. Stromally derived lysyl oxidase promotes metastasis of transforming growth factor-beta-deficient mouse mammary carcinomas. Cancer research. 2013; 73: 5336-5346.

60. Sun X, Gierach GL, Sandhu R, Williams T, Midkiff BR, Lissowska J, et al. Relationship of mammographic density and gene expression: analysis of normal breast tissue surrounding breast cancer. Clinical cancer research : an official journal of the American Association for Cancer Research. 2013; 19: 4972-4982.

61. Yang WT, Lewis MT, Hess K, Wong H, Tsimelzon A, Karadag N, et al. Decreased TGFbeta signaling and increased COX2 expression in high risk women with increased mammographic breast density. Breast Cancer Res Treat. 2010; 119: 305-314.

62. Cheng N, Bhowmick NA, Chytil A, Gorksa AE, Brown KA, Muraoka R, et al. Loss of TGF-beta type II receptor in fibroblasts promotes mammary carcinoma growth and invasion through upregulation of TGF-alpha-, MSP- and HGF-mediated signaling networks. Oncogene. 2005; 24: 5053-5068.

63. Franco OE, Jiang M, Strand DW, Peacock J, Fernandez S, Jackson RS, et al. Altered TGF-β signaling in a subpopulation of human stromal cells promotes prostatic carcinogenesis. Cancer Res. 2011; 71: 1272-1281.

64. Meng W, Xia Q, Wu L, Chen S, He X, Zhang L, et al. Downregulation of TGF-beta receptor types II and III in oral squamous cell carcinoma and oral carcinoma-associated fibroblasts. BMC Cancer. 2011; 11: 88.

65. Baratelli F, Lin Y, Zhu L, Yang SC, Heuzé-Vourc'h N, Zeng G, et al. Prostaglandin E2 induces FOXP3 gene expression and T regulatory cell function in human CD4+ T cells. J Immunol. 2005; 175: 1483-1490.

66. Oh SA, Li MO. TGF-β: guardian of T cell function. J Immunol. 2013; 191: 3973-3979.

67. Burnet FM. The concept of immunological surveillance. Prog Exp Tumor Res. 1970; 13: 1-27.

68. Burnet M. Cancer: a biological approach. III. Viruses associated with neoplastic conditions. IV. Practical applications. Br Med J. 1957; 1: 841-847.

69. Thomas L. Cellular and Humoral Aspects of the Hypersensitive States. New York: Hoeber-Harper; 1959 3149043]. Available from: http://www.ncbi.nlm.nih.gov/pubmed/21795664.

70. Chan SR, Vermi W, Luo J, Lucini L, Rickert C, Fowler AM, et al. STAT1-deficient mice spontaneously develop estrogen receptor alpha-positive luminal mammary carcinomas. Breast Cancer Res. 2012; 14: R16.

71. Shankaran V, Ikeda H, Bruce AT, White JM, Swanson PE, Old LJ, et al. IFNgamma and lymphocytes prevent primary tumour development and shape tumour immunogenicity. Nature. 2001; 410: 1107-1111.

72. Smyth MJ, Thia KY, Street SE, Cretney E, Trapani JA, Taniguchi M, et al. Differential tumor surveillance by natural killer (NK) and NKT cells. J Exp Med. 2000; 191: 661-668.

73. van den Broek ME, Kägi D, Ossendorp F, Toes R, Vamvakas S, Lutz WK, et al. Decreased tumor surveillance in perforin-deficient mice. J Exp Med. 1996; 184: 1781-1790.

74. Gatti RA, Good RA. Occurrence of malignancy in immunodeficiency diseases. A literature review. Cancer. 1971; 28: 89-98.

75. Vial T, Descotes J. Immunosuppressive drugs and cancer. Toxicology. 2003; 185: 229-240.

76. Mantovani A, Allavena P, Sica A, Balkwill F. Cancer-related inflammation. Nature. 2008; 454: 436-444.

77. Hussein MR, Hassan HI. Analysis of the mononuclear inflammatory cell infiltrate in the normal breast, benign proliferative breast disease, in situ and infiltrating ductal breast carcinomas: preliminary observations. Journal of clinical pathology. 2006; 59: 972-977.

78. DeNardo DG, Brennan DJ, Rexhepaj E, Ruffell B, Shiao SL, Madden SF, et al. Leukocyte complexity predicts breast cancer survival and functionally regulates response to chemotherapy. Cancer Discov. 2011; 1: 54-67.

79. Brennan DJ, Rexhepaj E, O'Brien SL, McSherry E, O'Connor DP, Fagan A, et al. Altered cytoplasmic-to-nuclear ratio of survivin is a prognostic indicator in breast cancer. Clinical Cancer Res. 2008; 14: 2681-2689.

80. Paulsson J, Sjoblom T, Micke P, Ponten F, Landberg G, Heldin CH, et al. Prognostic significance of stromal platelet-derived growth factor beta-receptor expression in human breast cancer. The American journal of pathology. 2009; 175: 334-341.

81. Kohrt HE, Nouri N, Nowels K, Johnson D, Holmes S, Lee PP. Profile of immune cells in axillary lymph nodes predicts disease-free survival in breast cancer. PLoS Med. 2005; 2: e284.

82. Finak G, Bertos N, Pepin F, Sadekova S, Souleimanova M, Zhao H, et al. Stromal gene expression predicts clinical outcome in breast cancer. Nat Med. 2008; 14: 518-527.

83. Ozhand A, Lee E, Wu AH, Ellingjord-Dale M, Akslen LA, McKean-Cowdin R, et al. Variation in inflammatory cytokine/growth-factor genes and mammographic density in premenopausal women aged 50-55. PLoS One. 2013; 8: e65313.

84. Dienz O, Rincon M. The effects of IL-6 on CD4 T cell responses. Clin Immunol. 2009; 130: 27-33.

85. Lin ZY, Chuang YH, Chuang WL. Cancer-associated fibroblasts up-

regulate CCL2, CCL26, IL6 and LOXL2 genes related to promotion of cancer progression in hepatocellular carcinoma cells. Biomed pharmacother. 2012; 66: 525-529.

86. Reeves KW, Weissfeld JL, Modugno F, Diergaarde B. Circulating levels of inflammatory markers and mammographic density among postmenopausal women. Breast Cancer Res Treat. 2011; 127: 555-563.

87. Stone J, Willenberg L, Apicella C, Treloar S, Hopper J. The association between mammographic density measures and aspirin or other NSAID use. Breast Cancer Res Treat. 2012; 132: 259-266.

88. Zangani D, Darcy KM, Masso-Welch PA, Bellamy ES, Desole MS, Ip MM. Multiple differentiation pathways of rat mammary stromal cells in vitro: acquisition of a fibroblast, adipocyte or endothelial phenotype is dependent on hormonal and extracellular matrix stimulation. Differentiation; research in biological diversity.1999; 64: 91-101.

89. DeFilippis RA, Chang H, Dumont N, Rabban JT, Chen YY, Fontenay GV, et al. CD36 repression activates a multicellular stromal program shared by high mammographic density and tumor tissues. Cancer discovery. 2012; 2: 826-839.

90. Heine PA, Taylor JA, Iwamoto GA, Lubahn DB, Cooke PS. Increased adipose tissue in male and female estrogen receptor-alpha knockout mice. Proc Natl Acad Sci U S A. 2000; 97: 12729-12734.

91. van den Brandt PA, Spiegelman D, Yaun SS, Adami HO, Beeson L, Folsom AR, et al. Pooled analysis of prospective cohort studies on height, weight, and breast cancer risk. Am J Epidemiol. 2000; 152: 514-527.

92. Gram IT, Funkhouser E, Tabár L. The Tabár classification of mammographic parenchymal patterns. Eur J Radiol. 1997; 24: 131-136.

93. Lam PB, Vacek PM, Geller BM, Muss HB. The association of increased weight, body mass index, and tissue density with the risk of breast carcinoma in Vermont. Cancer. 2000; 89: 369-375.

94. Salminen TM, Saarenmaa IE, Heikkilä MM, Hakama M. Unfavourable change in mammographic patterns and the breast cancer risk factors. Breast Cancer Res Treat. 1999; 57: 165-173.

Cite this article

Britt K, Ingman W, Huo C, Chew G, Thompson E (2014) The Pathobiology of Mammographic Density. J Cancer Biol Res 2(1): 1021.