# Is Mammographic Density a Biomarker to Study the Molecular Causes of Breast Cancer?

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## 1. Introduction

Breast cancer is the most common cancer affecting females worldwide. It accounts for 31% of all new cancer cases diagnosed in females with 23% of cases diagnosed in women younger than 50 years old (Jemal et al., 2002; Smigal et al., 2006). Being the second cause of cancer deaths after lung cancer (Jemal et al., 2002), enormous scientific efforts have been done aiming to better understand, treat and prevent breast cancer. Despite these research efforts, breast cancer remains a major health problem.

Since their implementation, the mammographic screening programs have resulted in breast cancer detection at an earlier stage and consequently have contributed to a greater reduction in the breast cancer mortality rate (Lebovic et al., 2010). Routine screening mammograms usually target women aged from 50 to 69 years old (Bryant & Mai, 2011). However, many studies have identified 40 years old as an appropriate age to begin annual mammographic screening (Bryant & Mai, 2011; Lebovic et al., 2010). Besides being a screening and diagnostic device, the mammography is also used as a research tool.

Variations in the mammographic appearance of the breast referred to as "mammographic density", reveal histological changes in breast tissue composition. Nowadays, elevated mammographic density is proven to be one of the strongest risk factors for breast cancer. Whatever the method used to classify mammographic density, all have shown a substantial increased breast cancer risk associated with increased mammographic density (McCormack & dos Santos Silva, 2006). Mammographic density has been consistently associated with increased risk of hyperplasia, atypical hyperplasia and carcinoma *in situ* (Boyd et al., 2000). In addition, several recognized risk factors for breast cancer (such as number of full-term pregnancies, age at first full-term pregnancy, family history of breast cancer, personal history of breast biopsies and use of hormone replacement therapy) are also associated with mammographic density (Boyd et al., 2010; Heine & Malhotra, 2002). Mammographic density is related to levels of hormonal and growth factors involved in cellular proliferation, differentiation and apoptosis (Diorio et al., 2005b; Greendale et al., 2005; Howard & Gusterson, 2000) which have established roles in the etiology of breast cancer (Imagawa et al., 2002; Shekhar et al., 2003).

Mammographic density reflects the proportion of the breast occupied by epithelial and stromal tissues. Therefore, mammographic density is hypothesized to reflect the quantity of

non-adipose breast tissue (Boyd et al., 2010; Li et al., 2005), representing the population of breast cells at risk of carcinogenic transformation (Trichopoulos et al., 2005). Breast cancer generally arises from epithelial cells (Russo et al., 1990), and an increase in the overall epithelial cell number is believed to increase the risk of breast cancer (Preston-Martin et al., 1990). However, there is also growing evidence that stroma plays an important role, not only in mammary gland development, but also in breast carcinogenesis (Imagawa et al., 2002; Shekhar et al., 2003; Woodward et al., 1998). Stroma is a major component of the breast (Imagawa et al., 2002; Page & Winfield, 1986; Shekhar et al., 2003) and variations in mammographic density may be more sensitive to changes in the stroma than to those in the epithelium (Warren & Lakhani, 2003). Knowledge of molecular markers (such as proteins and genes expression) in normal breast epithelium and stroma that are associated with mammographic density may provide important clues regarding breast cancer etiology. Studying the influence of such genes/proteins on mammographic density used as an end point in epidemiological studies may reduce the long term follow-up period needed to detect their effects on breast cancer incidence, thus providing an easier and more economic way to study the pathogenesis of breast cancer. A better understanding of molecular markers influencing mammographic density may also help to identify preventive targets.

In this review, we intend to shed light on the possibility of using mammographic density as an intermediate biomarker to study the molecular causes of breast cancer. We hypothesize that if breast cancer biomarkers ultimately exert their influence on mammographic density in the same direction as they influence breast cancer risk, then mammographic density could be considered as an intermediate biomarker for breast cancer rather than just a risk factor. An overview with emphasis on changes in genes and proteins expression in normal breast tissue and their relationship with mammographic density is provided.

## 2. Mammographic density

The breast is composed mainly of fibroglandular tissue and adipose tissue at different proportions. These constituents differ in their X-ray attenuation characteristics. Fibroglandular tissue, mainly composed of epithelial cells and supporting stroma, attenuates more X-rays and thus appears white on mammograms, while adipose tissue, which is radiolucent, appears dark on mammograms. Hence, variations in the radiological appearance of the breast on a mammogram reflect variations in breast tissue composition. Mammographic density represents the proportion of the breast occupied by radiologically dense tissue. Results from Li's study show that elevated mammographic density is associated with a greater nuclear area of both epithelial and non-epithelial cells, as well as with a greater proportion of collagen and a greater area of glandular structures (Li et al., 2005). It is believed that elevated mammographic density reflects the effects of mitogens and mutagens which influence cellular proliferation and genetic damage of these cells (Martin & Boyd, 2008). This provides a potential mechanism by which elevated mammographic density could influence the risk of breast cancer. Moreover, it has been suggested that mammographic density is more related to stromal changes rather than epithelial changes (Heine & Malhotra, 2002; Warren & Lakhani, 2003). This is consistent with Lin and colleagues findings who reported a higher number of stromal cells but not epithelial cells in high mammographic density regions when compared to low mammographic density regions within the same woman ($p$ for trend < 0.01) (Lin et al., 2011).

## 3. Assessment and classifications of mammographic density

In 1976, Wolfe first described a method for classifying variations in the mammographic appearance of the breast into four categories based on the visual assessment of the extent of dense breast tissue on the mammogram as well as of characteristics of densities seen (prominent ducts and dysplasia). The four categories correlate with breast cancer risk, where breasts with the densest pattern were associated with the highest risk of developing breast cancer (Wolfe, 1976). Later, the Wolfe categories have been largely replaced by the American College of Radiology's Breast Imaging Reporting and Data System (BI-RADS). The BI-RADS, which is still frequently in use, also classifies mammographic density into four categories: 1 = almost entirely fatty breast, 2 = breast containing scattered densities, 3 = heterogeneously dense breast and 4 = extremely dense breast. Since then, several methods have been developed seeking to provide a quantitative and continuous measure of mammographic density. In 1995, Boyd proposed a six-category scale based on the visual estimation by a radiologist of the proportion of the breast occupied by radiologically dense tissue. The six categories are: 0%, < 10%, 10%-< 25%, 25%-< 50%, 50%-< 75%, ≥ 75% (Boyd et al., 1995). These previously mentioned methods depend on the visual assessment and are thus subjected to bias (Warren, 2004). Mammographic density is now often measured by a computer-assisted method on digitized images allowing the quantitative assessment of mammographic density. Results from such a method are expressed as absolute area of mammographic density in square centimeters or in percentage of mammographic density. The computer-assisted method is known to have a high degree of reliability and reproducibility for determining mammographic density (Boyd et al., 1995; Boyd et al., 2010). The limitation of this method is that it does not take into account the gradual transition from dense to non-dense breast tissue. Generally, the quantitative approaches have the advantage over the qualitative categorical approaches in providing a continuous measure of mammographic density. However, quantitative methods do not consider the breast volume; instead they take into account the breast area. Nonetheless, quantitative approaches give a more consistent estimate of the associated breast cancer risk and this risk persists after adjustment for other risk factors.

## 4. Mammographic density and breast cancer risk

The association between elevated mammographic density and increased breast cancer risk was investigated in many studies. In order to confirm this association, McCormak and dos Santos Silva conducted a systematic meta-analysis on 42 previously published studies (McCormack & dos Santos Silva, 2006). They included 14,000 women with breast cancer and 226,000 women without breast cancer. They concluded that elevated mammographic density, regardless of the type of assessment, was consistently positively associated with an increased risk of breast cancer. Women with the most dense breast (> 75%) have 3-5 fold greater risk of developing breast cancer compared to women with the least dense breast (< 5%). The authors also reported that the associations were stronger when mammographic density was measured in percent mammographic density rather than categorized according to Wolfe scale. Thus, it is well established that high mammographic density constitutes an independent risk factor for breast cancer and that it accounts for a large proportion of the disease incidence (Boyd et al., 2010). It has been recently shown that women who experienced a decrease in mammographic density of at least one BI-RADS category over a

period of six years had a 28% lower breast cancer risk compared to women whose mammographic density was unchanged (Tanne, 2010).

## 5. Breast cancer risk factors associated to mammographic density

Mammographic density is not constant throughout life. It declines with increasing age reflecting the age-related changes in breast tissue composition. These age-related changes, referred to as involution, reveal the reduction of the epithelium and stroma within the breast and the simultaneous increase in fat. Premenopausal women have consistently been found to have more elevated mammographic density than postmenopausal women. It might be slightly confusing knowing that mammographic density, which constitutes a breast cancer risk factor, decreases with age while breast cancer incidence increases with advanced age. This apparent paradox can be resolved by the breast cancer incidence model proposed by Pike (Pike et al., 2004). Pike based his model on the concept that the relevant measure to describe the age-specific incidence of breast cancer is the cumulative breast tissue exposure rather than the chronological age. The breast tissue exposure reflects the cumulative exposure of mammary cells to hormones and growth factors and the accumulation of genetic damage. According to Pike's model, breast tissue exposure is at its peak at menarche, and then it decreases gradually during pregnancy, until it reaches its lowest values during the postmenopausal period. Thus, the incidence of breast cancer and the cumulative exposure of breast tissue both increase with age (Henson & Tarone, 1994; Pike et al., 2004).
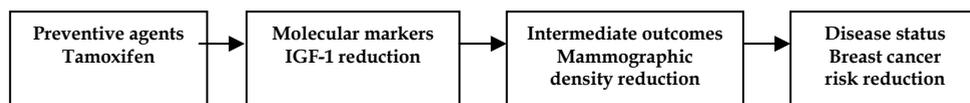
Several known risk factors for breast cancer; hormonal, reproductive and anthropometric variables, are also associated with variations in mammographic density. The strongest associations are observed with body weight. Mammographic density is inversely associated with body mass index (Brisson et al., 1984; Diorio et al., 2005a). This is consistent with the inverse association between body mass index or weight and breast cancer risk among premenopausal, but inconsistent with the positive association among postmenopausal women (Hunter & Willett, 1993; Trentham-Dietz et al., 1997). Height, which is proven to be positively associated with an increased risk of breast cancer among pre- and postmenopausal women, is also positively associated with increased mammographic density (Brisson et al., 1984; Trentham-Dietz et al., 1997).

Moreover, hormonal-related breast cancer risk factors such as nulliparity, low number of live births, late age at first birth and combined hormone replacement therapy are positively associated with increased percent mammographic density (Boyd et al., 2010; El-Bastawissi et al., 2000). However, the association of circulating estrogen levels (known to be strongly positively associated with breast cancer risk) with mammographic density has so far been inconsistent (Boyd et al., 2002c; Greendale et al., 2005). It is believed that the exposure to endogenous and exogenous hormones accompanying reproductive and hormonal changes increases mammographic density by affecting the proliferative activity and the quantity of stroma and epithelium in breast tissue (Boyd et al., 2010). Furthermore, menopause was found to be associated with a reduction in dense areas accompanied by an increase in non-dense areas. These changes in mammographic density due to menopausal status are not fully explained by the effect of age on density (Boyd et al., 2002a).

However, all these epidemiological risk factors explain only about 20%-40% of the variability in percent mammographic density (Vachon et al., 2000). It seems that genetic

factors (heritability) could be responsible for greater variations in mammographic density. Current evidence shows that mammographic density is highly heritable and is inherited as a quantitative trait (Boyd et al., 2002b; Ursin et al., 2009). Heritability accounts for 50%-60% of the variation in mammographic density. Heritability of mammographic density may explain, at least in part, the familial aggregation pattern of breast cancer. Women with elevated percent mammographic density are more likely to have a first-degree relative with a history of breast cancer (Ziv et al., 2003).

Recently, it has been demonstrated that tamoxifen, which reduces the extent of mammographic density, also decreases the burden of invasive breast cancer (Cuzick et al., 2011). In that trial, for the 46% of women in the tamoxifen arm whose density was reduced by 10% or more, the risk of breast cancer was reduced by 63% relative to the control group (odds ratio (OR) = 0.37, $p$ = 0.002) while for the 54% of women whose density was reduced by less than 10%, there was no reduction in breast cancer risk (OR = 1.13, $p$ = 0.60). The action of tamoxifen is mediated through its effects on several gene products including insulin-like growth factor-1 (IGF-1) (Pollak et al., 1990), a protein that is believed to play a role in mammographic density (Diorio et al., 2005b) and breast cancer development (Schernhammer et al., 2005). This action can therefore be schematized as follow:

| **Preventive agents** **Tamoxifen** | → | **Molecular markers** **IGF-1 reduction** | → | **Intermediate outcomes** **Mammographic density reduction** | → | **Disease status** **Breast cancer risk reduction** |
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The number of proteins / genes that can be measured in breast tissue rather than in the circulation is rapidly increasing because of new molecular technologies. Given that some preventive agents can decrease mammographic density and consequently breast cancer risk through their modifying effects on several proteins and/or genes, it seems important to study proteins/genes that could influence mammographic density. These proteins/genes may be further used as preventive targets in future breast cancer intervention studies.

## 6. Proteins expression in breast tissue associated to mammographic density

### 6.1 Insulin-like growth factors

Knowing that mammographic density declines gradually with age and that circulating levels of IGF-1 also decrease with age and that both are related to an increased risk of breast cancer, it might be of great importance to study the relationship between the two. A pioneer study investigated the association between IGF-1 expression in breast tissue and mammographic density among women aged 45-55 years old who underwent breast biopsies with a final diagnosis of benign lesions (Guo et al., 2001). They used 92 formalin-fixed paraffin blocks of breast tissues surrounding benign lesions obtained from women with little (< 25%, n = 46) or extensive (> 50%, n = 46) mammographic density matched according to age at time of biopsy. In this study, tissue levels of IGF-1 from nuclear areas were analysed by immunohistochemistry and quantified using quantitative microscopy. Guo and colleagues found that breast tissue from subjects with extensive mammographic density had a greater IGF-1 staining when compared to subjects with little mammographic density ($p$ = 0.02). After stratification by age, this association was significant only for subjects less than 50 years old. The IGF-1 staining was about three times greater in blocks obtained from women

with high mammographic density compared to blocks from women with low mammographic density among subjects less than 50 years of age ($p = 0.0009$). A main pitfall of Guo's study was the lack of information regarding the menopausal status of the participants. Nonetheless, data from laboratory and epidemiological studies seem to support these findings.

The IGF-1 is a member of a superfamily of multifunctional peptides. The majority of circulating IGFs are produced by the liver in response to growth hormone, but many other tissues including the breast tissue can also express IGFs. IGF-1 plays a crucial role in regulating many cellular functions such as cell proliferation, differentiation and apoptosis. It is well known that IGF-1 is essential for normal mammary cells development (Marshman & Streuli, 2002). Strange and colleagues have demonstrated that IGF-1 increases the proliferative activity of cultured mammary cells and hence the risk of breast cancer. They added that IGF-1 is able to stimulate the growth of cultured human breast epithelial and stromal cells in a dose-dependent manner (Strange et al., 2002; Strange et al., 2004). Several epidemiological studies have provided evidence that elevated circulating levels of IGF-1 are associated with increased breast cancer risk in pre- but not postmenopausal women (Hankinson et al., 1998; Krajcik et al., 2002; Schernhammer et al., 2005; Toniolo et al., 2000). This is in line with the positive association between increased circulating levels of IGF-1 and percent mammographic density among pre- ($p = 0.02$) but not postmenopausal women ($p = 0.37$) that was observed in our large study specifically designed to confirm what other studies of smaller sample size had observed (Diorio et al., 2005b). More recently, we showed that single nucleotide polymorphisms (SNPs) located on IGF-1 gene were also associated with premenopausal mammographic density, and this association remained after adjustment for IGF-1 levels (Diorio et al., 2008). Although circulating levels of IGF-1, mainly produced in the liver, have been linked to breast morphogenesis and breast cancer risk, our results support the idea that IGF-1 at tissue levels are important in this respect, at least among premenopausal women. This association only found among premenopausal women may reflect a cross-talk between IGF-1 and steroid hormones. In fact, a previous study has observed a strong evidence of a cross-talk between IGF-1 and estrogen in breast tissue (Martin & Stoica, 2002). Estrogen induces IGF-1 expression through binding to estrogen receptor-α (ERα), and IGF-1, by binding to its receptor, initiates a cascade of phosphorylation that ends by activating ERα. This estrogen-IGF-1 cross-talk may induce mammary epithelial cells proliferation and possibly tumor development.

## 6.2 Hormone receptors

Since mammographic density reflects the cumulative effect of steroid hormones on breast tissue and blood estradiol level is a risk factor for breast cancer, then mammographic density is expected to be associated to hormone receptors in breast tissue. So far, two studies assessed the association between the two subtypes of ER, ERα and ERβ, separately and mammographic density (Lundstrom et al., 2006; Verheus et al., 2009), while two others assessed the association between ER, with no mention of the subtype, and mammographic density (Harvey et al., 2008; Yang et al., 2010). Among these, three groups also assessed the association between the progesterone receptor (PgR) and mammographic density (Harvey et al., 2008; Verheus et al., 2009; Yang et al., 2010).

Lundstrom and colleagues obtained normal tissue specimens from 28 postmenopausal women aged ≥ 52 years old undergoing surgery for breast cancer as first treatment.

Radiological examination was performed on tissue blocks, and pair-wise samples of dense and non-dense tissue were selected. They analysed semi-quantitatively ERα and ERβ in glandular epithelium by immunohistochemical staining intensity. They observed a higher ERα staining in glandular epithelium in mammographically dense compared to non-dense breast tissue ($2.4 \pm 0.2$ versus $1.6 \pm 0.2$; $p < 0.01$). Conversely, there was no significant association between ERβ and mammographic density ($2.2 \pm 0.2$ versus $1.9 \pm 0.3$; $p > 0.05$ for dense and non-dense tissue respectively) (Lundstrom et al., 2006). The major strengths of Lunstrom's study were the measurement of the two subtypes of ER separately and the usage of paired samples of dense and non-dense normal breast tissue at a distance of at least 2 cm from the tumor from each patient. The small sample size, the lack of premenopausal women included in the study sample and the usage of normal breast tissue obtained from breast cancer patients which may not be completely representative of normal breast tissue were the main limitations of this study.

In the study conducted by Verheus and colleagues, they assessed the epithelial expression of ERα, ERβ, and PgR in relation to mammographic density among 159 pre- and postmenopausal women having breast cancer, 60 years old on average, and of different ethnicities. They prepared tissue microarrays (TMAs) from benign breast tissue samples obtained from tumor blocks (mean = 1.7 specimens per woman). The TMAs were then immunostained for ERα, ERβ and PgR, which were measured by quantitative microscopy and categorized as $< 10\%$ or $\geq 10\%$ of staining. Mammographic density from the craniocaudal views before breast cancer diagnosis was assessed using a computer-assisted method. None of the markers measured in breast tissue was significantly associated with percent or absolute mammographic density among the whole study population or among Japanese women. However, they observed a negative association between ERα expression and percent mammographic density ($p = 0.04$), a positive association between ERβ and percent mammographic density ($p = 0.05$) and a positive association between PgR and absolute mammographic density ($p = 0.03$) among Caucasian women (Verheus et al., 2009). Assessment of ERα and ERβ separately, the usage of TMA approach which allows the assessment of several markers expression in a large number of samples under the same staining conditions, the classification of mammographic density by a computer-assisted method and the adjustment for several potential confounders in the analysis were the main advantages of Verheus' study. However, the usage of benign breast tissue obtained from tumor blocks may not be fully representative of normal breast tissue and markers expression in benign tissue could have been influenced by potential paracrine effects of the adjacent tumors.

Harvey and colleagues included in their study 56 postmenopausal women aged from 45 to 85 years old having a surgical treatment for breast cancer. Mammographic density of the contralateral breast was assessed by a computer-assisted method. The ER and PgR content in ducts, lobules and fibrous stroma was measured by immunohistochemistry performed on tissue blocks free from cancer, and the intensity of staining, as negative or positive, was visually estimated. Contrary to what one might have expected, the authors did not find any significant association between ER or PgR and mammographic density after adjustment for hormonal replacement therapy use and age (Harvey et al., 2008). The small sample size, the lack of premenopausal women among the study population, the usage of tissue blocks from mastectomy specimens which might not be completely free from foci of carcinoma in addition to be sampled in densest areas of the breast and the lack of adjustment for potential

confounding factors may explain the absence of association. The major strength of this study was the use of a computer-assisted method providing a continuous and reproducible measure of mammographic density.

For their study, Yang and colleagues identified 27 premenopausal women and 39 postmenopausal women (29-88 years old) who underwent surgery for breast cancer as first treatment. These women had low (BI-RADS 1 or 2, n = 28) or high (BI-RADS 3 or 4, n = 38) mammographic density. They analysed ER and PgR expression immunohistochemically in stroma and epithelium separately, from normal frozen breast tissue located at least 5 cm away from the tumor. The Allred scoring system was used by a breast pathologist to measure each marker. In this study, the authors did not find any significant positive association between ER or PgR and mammographic density in univariate models (Yang et al., 2010). Unfortunately, their small sample size did not allow analysis stratified by menopausal status. As in the above studies, they used normal breast tissue obtained from breast cancer patients which may not truly represent normal breast tissue.

Therefore, out of the studies that analysed the association between specified subtypes of ER expression in breast tissue and mammographic density, one study observed a positive association between epithelial ERα expression and mammographic density among postmenopausal women (Lundstrom et al., 2006), while another study observed an inverse association among pre- and postmenopausal Caucasian women (Verheus et al., 2009). In this latter population, a positive association of ERβ and PgR expression in breast tissue with mammographic density was also observed (Verheus et al., 2009). However, none of the other two studies that assessed PgR found any significant association among pre- and/or postmenopausal women (Harvey et al., 2008; Yang et al., 2010).

It is well known that ovarian steroid hormones play a critical role in normal mammary cell development, proliferation and differentiation (Graham & Clarke, 1997; Nilsson et al., 2001). While most of the studies agree that estrogen increases the risk of breast cancer, the effect of progesterone on this risk remains a subject of controversy (Hankinson & Eliassen, 2007). In fact, PgR could be considered a marker of both estrogen and progesterone actions in the mammary gland, since PgR expression is regulated by estrogen (Shyamala et al., 2002). Estrogen exerts its biological activity on mammary cells particularly through nuclear ERs. ERs are further subdivided into two subtypes; ERα and ERβ, having different expression levels in normal and malignant breast tissues. ERα and ERβ, encoded by different genes, exert different biological actions on breast tissue cells. Activation of ERα increases the rate of breast cells proliferation by up-regulating several genes involved in cellular proliferation such as cyclin D (Liu et al., 2002b), while down-regulating other genes known to inhibit proliferation such as transforming growth factor-β (TGF-β) (Chang et al., 2006). In a recent study, Woolcott and colleagues observed that ERα levels were higher in nonneoplastic epithelium obtained from breast cancer cases when compared to control biopsies obtained from women free from breast cancer (OR = 2.6; 95% CI 1.1-6.2). However, there was no significant difference between PgR expression in nonneoplastic epithelium obtained from breast cancer cases and that obtained from control women (OR = 0.7; 95% CI 0.4-1.3) (Woolcott et al., 2008). The ERα-induced breast cells proliferation is consistent with Lundstrom's finding (Lundstrom et al., 2006) of increased epithelial ERα expression in dense breast tissue compared to non-dense tissue. These results suggest that breast tissue could be influenced by increased ERα expression, leading to increased mammographic

density and finally to increased breast cancer risk at least among postmenopausal women. On the other hand, the overexpression of ERβ decreases ERα transcriptional activity. ERβ has been found to modulate the expression of many ERα regulated genes involved in cell proliferation such as cyclin D and TGF-β genes (Chang et al., 2006; Liu et al., 2002b). Also, ERβ down-regulates the ERα protein level (Chang et al., 2006). Furthermore, ERβ staining was inversely correlated with the Ki67, marker of cellular proliferation, in normal breast tissue and preinvasive mammary tumor (Roger et al., 2001). This suggests that ERβ acts as a tumor suppressor (Bardin et al., 2004). Thus, it is evident that the ratio between both subtypes is the most important determinant of the overall response. The increased expression of ERα concomitant with decreased ERβ expression was found to take place in early breast cancer stages (Leygue et al., 1998a; Roger et al., 2001). Knowing that ER subtypes have opposite actions on breast tissue could provide further explanation regarding the opposite associations found in Verheus and colleagues study, who did not consider the ratio or the mutual adjustment of both subtypes in their analysis since both subtypes were associated to mammographic density.

## 6.3 Aromatase enzyme

A recent study assessed the association between breast tissue aromatase expression, a rate-limiting enzyme in the estrogen pathway, and mammographic density (Vachon et al., 2011). They looked for this association among 49 healthy Caucasian women aged from 40-82 years old. Mammographic density was visually assessed using the mammographic films and identifying areas of low and high mammographic density. They applied a fine-needle guided ultrasound to obtain tissue biopsies from dense and from non-dense breast areas for each woman. Biopsies were analysed by immunohistochemistry, and immunostaining for aromatase was scored visually in terms of extent and intensity of staining for each cell type (stroma, epithelium and adipocytes) using a modified H-score (Santen et al., 1994). They observed greater aromatase staining in dense breast tissue when compared to non-dense tissue ($p < 0.0003$). This result was consistent for pre- and postmenopausal women when assessed separately. The aromatase staining was higher in both stromal and epithelial cells from dense tissue compared to those from non-dense tissue (both $p < 0.01$). Conversely, it was lower in adipocytes from dense tissue versus non-dense tissue ($p < 0.01$). Concerning dense breast tissue, aromatase staining in stromal cells was threefold higher than that in epithelial cells. The major strengths of Vachon's study were the paired samples of dense and non-dense normal breast tissue taken from healthy subjects and the scoring of aromatase using both the extent and the intensity of staining. To our knowledge, this is the only study assessing the association between breast tissue aromatase level and mammographic density and therefore further larger studies are recommended to confirm these promising findings.

The aromatase enzyme catalyzes the conversion of androgens, androstenedione and testosterone to estrogen, estrone and estradiol. Thus, it is largely responsible for estrogen levels in breast tissue, especially after menopause, when the *in situ* production of estrogen in mammary adipose tissue takes the upper hand following the cessation of ovarian estrogen synthesis. Increased aromatase expression would result in higher estrogen levels and subsequently greater breast tissue exposure to estrogen. Irahara and colleagues observed an elevated level of aromatase mRNA in breast cancer cells when compared to normal breast tissue (Irahara et al., 2006). Furthermore, aromatase has been shown to stimulate the growth

of breast cancer cells *in vitro* (Macaulay et al., 1994). The aromatase is the product of the CYP19 gene which is a member of the cytochrome P450 enzymes family. A comprehensive haplotype analysis of CYP19 and breast cancer risk reveals that a common specific long-range haplotype is associated with an increased risk of breast cancer (OR = 1.31; 95% CI 1.11-1.54) (Haiman et al., 2003). In contrast, no association has been observed in a recent comprehensive examination of CYP19 variants and mammographic density (Olson et al., 2007). However, the common long-range haplotype associated with breast cancer risk has not been assessed by Olson and colleagues. The identification of causal variants associated with the CYP19 long-range haplotype should be investigated.

## 6.4 Epidermal growth factors

Among the complete family of epidermal growth factor receptors and their ligands expressed in breast tissue, human epidermal growth factor receptor-2 (HER-2) and transforming growth factor-α (TGF-α) have been evaluated for their association with mammographic density.

In the study conducted by Verheus and colleagues described earlier (Verheus et al., 2009), the authors also measured the expression of HER-2 in breast tissue. They observed no association between HER-2 staining and mammographic density among the whole study population ($p = 0.82$ and $p = 0.74$ for percent and absolute mammographic density respectively), among Caucasian women ($p = 0.38$ and $p = 0.99$ for percent and absolute mammographic density respectively) or among Japanese women ($p = 0.57$ and $p = 0.73$ for percent and absolute mammographic density respectively). HER-2 belongs to the human epidermal growth factor receptor family which is involved in the regulation of normal breast growth and development. In fact, HER-2 is overexpressed in 20-30% of cases of breast cancer (Cho et al., 2008). When HER-2 is overexpressed, it sends signals to the nucleus to proliferate, resulting in increased cellular multiplication and consequently malignant growth (Yarden, 2001). A recent meta-analysis found that the functional SNP Ile655Val of HER-2 is associated to breast cancer risk among Asian women but not among Caucasian nor European women (Tao et al., 2009). Moreover, no association has been observed between HER-2 Ile655Val SNP and mammographic density among Australian women (Stone et al., 2007). The Ile655Val is believed to destabilize the active HER-2 reducing its activation (Fleishman et al., 2002).

In the study previously considered, Guo and colleagues also quantified TGF-α staining, and found no association with mammographic density before or after stratification for age (all $p > 0.30$) (Guo et al., 2001). The influence of TGF-α, a member of the epidermal growth factor family of peptides, on breast cancer incidence is not fully explored. However, a previous laboratory study has demonstrated that TGF-α enhances the growth of normal mouse mammary epithelial cell line (Casey et al., 2007). Furthermore, TGF-α can act as a mitogen and differentiation factor in mammary epithelium inducing multifocal hyperplastic and malignant lesions in an animal model (Smith et al., 1995).

Additional research will be needed to clarify the role of epidermal growth factor family in breast morphogenesis and breast cancer risk.

## 6.5 Matrix metalloproteinases

In a recent study, Steude and colleagues attempted to assess the association between matrix metalloproteinases (MMPs) 1, 3, 9, 12 and their inhibitor (TIMP-3) in breast tissue and

mammographic density (Steude et al., 2009). Their study population was composed of 75 premenopausal and 202 postmenopausal breast cancer patients with mainly Caucasian and Japanese ancestry. To achieve their goal, they prepared TMAs with up to 4 cores from areas representing benign breast tissues (mean = 2.9 cores per woman). The expression of MMPs and TIMP-3 was immunohistochemically measured and visually classified in the stroma as no versus any stain (available for 169 women) and in the epithelium as no, weak or strong stain intensity (available for 259 women) separately. A computer-assisted method was applied for the evaluation of mammographic density from prediagnostic digitized mammograms from the craniocaudal views. Because the expression of MMP-3 and MMP-9 was observed in less than 20% of epithelial and less than 5% of stromal breast tissue, the authors did not examine their associations with percent mammographic density. Contrary to what was expected, MMP-1, MMP-12 and TIMP-3 analysed in stromal and in epithelial tissue were not significantly related to percent mammographic density after adjustment for confounders among the whole study population or across ethnic groups.

Although Guo and colleagues did not measure MMPs, they assayed TIMP-3 in addition to IGF-1 and TGF-α reported earlier (Guo et al., 2001). They found that TIMP-3 expression was higher in tissue from subjects with extensive mammographic density compared to age-matched subjects with little mammographic density ($p$ = 0.08). Moreover, this association was statistically significant for women less than 50 years old ($p$ = 0.004), but not for women 50 years old or more ($p$ = 0.48). Guo and colleagues hypothesized that TIMP-3 may influence epithelial and stromal proliferation contributing to higher mammographic density and subsequently increased breast cancer risk. The discrepancy between Steude and Guo's studies could be due to different means used in classifying mammographic density, different immunostaining techniques applied, and different study population regarding their breast diseases, ethnicity and mean age. In particular, participants in Guo's study were younger (ranging from 45-55 years old) than those recruited in Steude's study (mean age = 60.2 ± 8.7 years old) suggesting that the association between TIMP-3 and breast density could be limited to young women. A further larger study, stratifying analyses according to menopausal status, will be needed to clarify these observations.

The MMPs are proteases responsible for cleaving proteins of the extracellular matrix. MMPs regulate a wide range of biological functions such as cell death, proliferation, differentiation, tumor associated angiogenesis and malignant conversion (Coussens et al., 2002). They can also induce tumor invasion and metastasis (Balduyck et al., 2000). MMPs have been found to be greatly expressed in malignant breast tissue when compared to the surrounding normal breast tissue (Bartsch et al., 2003; Garbett et al., 2000). However, their roles in breast cancer development are unclear. According to Shin and colleagues, none of the two common SNPs in the MMP-12 gene (A-82G in the promoter region and A1082G in the exon) was associated with breast cancer risk among pre- and postmenopausal Chinese women (Shin et al., 2005). A recently published meta-analysis, looked for the association between MMP-1 (rs1799750), MMP-2 (rs243865), MMP-3 (rs3025058) and MMP-9 (rs3918242) SNPs and breast cancer risk (Zhou et al., 2011). They selected 9 case-control studies including 2,597 cases and 2,618 controls, and found only an association between MMP-2 SNP and breast cancer risk. However, MMP-2 expression in breast tissue has not yet been examined in association with mammographic density.

TIMP-3 was found able to induce cellular apoptosis (Baker et al., 1999; Jiang et al., 2002). However, an increased TIMP-3 expression was observed in breast cancer (Byrne et al., 1995;

Uria et al., 1994). This is in line with Guo's observation who suggested that TIMP-3 might increase the risk of premenopausal breast cancer through its effect on epithelial and stromal cells proliferation. According to Peterson, who evaluated the association between 19 SNPs of TIMP-3 and breast cancer risk among 1,062 Chinese women with breast cancer and 1,069 without, women with the rs9609643 AA genotype had 60% lower risk of developing breast cancer when compared to women with GG genotype (OR = 0.4, 95% CI 0.2-1.0). Whereas, women with the rs8136803 TT genotype were 5 times more at risk of developing breast cancer when compared to women with the GG genotype (OR = 5.1, 95% CI 1.1-24.3) (Peterson et al., 2009). However, it is still unknown if these SNPs have a functional relevance. Lei and colleagues observed a moderately increased breast cancer risk for the C allele carriers of the TIMP-3 rs9619311 SNP (OR = 1.25, 95%CI 1.05-1.50) among Swedish women (Lei et al., 2007). A similar non-significant association was also observed in Peterson's study (OR = 1.2, 95%CI 0.9-1.5). Since TIMP-3 rs9619311 SNP is located in the promoter region, it is possible that it may affect transcription binding sites. Further studies will be needed to confirm if TIMP-3 is associated with mammographic density and breast cancer risk.

## 6.6 Proteoglycans

Two groups examined the association between the expression of some proteoglycans in breast tissue and mammographic density (Alowami et al., 2003; Lundstrom et al., 2006). In the study conducted by Alowami and colleagues, they assessed the association between the expression of small leucine-rich proteoglycans, lumican and decorin in breast tissue and percent mammographic density. In their study, they included 62 women aged 54-75 years old diagnosed with benign or preinvasive breast diseases. Immunohistochemical analysis of lumican and decorin was performed on tissue blocks obtained from resection margins distant from the lesion, and immunostainings were scored visually in terms of extent and intensity of staining. Following the localization of biopsies on previous screening mammograms, the authors assessed mammographic density of the surrounding breast tissue distant from the lesion using the six-category Boyd scale. For this study, they excluded all women whose mammographic density score ranged from 25%-50% and subdivided all remaining participants into low (< 25%, n = 35) and high (> 50%, n = 27) mammographic density subgroups. They observed that the expression of lumican and decorin was restricted to the stroma surrounding the epithelium. The median value of the expression of both lumican and decorin was 2-3 times higher in specimens from women with high mammographic density compared to those with low mammographic density (both with $p < 0.0001$). No adjustment for age has been made, but women in the categories of low and high mammographic density had similar range of age (54-74 and 54-75 years old, respectively). In their study, Alowami and colleagues included mainly postmenopausal women thus reducing the proportion of cases with high mammographic density and limiting the generalizability of their results.

The study considered earlier in the ERα-related section also compared the expression of syndecan-1, a cell surface heparan proteoglycan, in pair-wise samples of dense and non-dense normal breast tissue from 28 postmenopausal women undergoing surgery for breast cancer (Lundstrom et al., 2006). They performed an immunohistochemical staining to assess syndecan-1 intensity semi-quantitatively in stroma, stromal cells, ductal and lobular

epithelium. They found that syndecan-1 expression was significantly higher in dense than non-dense normal breast tissue ($p$ = 0.004, $p$ = 0.02, $p$ = 0.008 and $p$ = 0.02 for stroma, stromal cells, ductal and lobular epithelium respectively). Moreover, syndecan-1 expression was higher in stroma than in epithelial tissue ($p$ < 0.01). They concluded that the redistribution of syndecan-1 from epithelium to stroma maybe a key step in increased mammographic density.

The main proteoglycan synthesis inducer in breast tissue remains unknown. In addition, the role of proteoglycans in tumorigenesis has not been sufficiently explored. Previous studies have demonstrated an increased lumican expression in stroma from invasive breast carcinoma compared to stroma from adjacent normal breast tissue (Leygue et al., 1998b; Leygue et al., 2000). However, the exact mechanism by which lumican can induce cellular proliferation is not known. Contrary to lumican, decorin is believed to suppress cellular growth through epidermal growth factor receptor activation and p21 cell-cycle inhibitor induction (Moscatello et al., 1998; Santra et al., 1997). This is in line with Leygue's findings, who reported a decreased decorin expression in stroma from neoplastic breast tissue compared to stroma from normal adjacent breast tissue (Leygue et al., 2000). Kelemen and colleagues investigated the association between 14 common SNPs in the lumican (LUM) and decorin (DCN) genes among 1641 Caucasian women (798 breast cancer cases and 843 controls). Then, SNPs showing the strongest associations (one SNP per gene) were assessed among 4,470 breast cancer cases and 4,560 controls from England. The authors identified three SNPs in LUM (rs2268578, rs10859110 and rs17018765) and three SNPs in DCN (rs7441, rs516115 and rs3138165) which were each associated with increased breast cancer risk among their first study population. However, only LUM rs2268578 SNP was associated with increased breast cancer risk among pooled studies, but did not reach statistical significance among English women only (Kelemen et al., 2008). Since this SNP is located in an intron, more research will be needed to identify a functional SNP in LUM gene with an effect on mammographic density and breast cancer risk.

Proteoglycans are present either free in the extracellular compartment or attached to the cell surface as syndecans and glypicans (Liu et al., 2002a). The heparin sulphate proteoglycans (HSPG) play a crucial role in normal developmental processes, such as embryogenesis, as well as pathological conditions such as tumorigenesis (Liu et al., 2002a; Perrimon & Bernfield, 2000). In particular, syndecan-1 plays a critical role in the regulation of cellular adhesion, migration and proliferation. Syndecan-1 is expressed in stroma of breast cancer tissue as well as in normal epithelium but less in normal breast tissue stroma (Maeda et al., 2004). This suggests that syndecan-1 is redistributed in cancerous tissue (Lofgren et al., 2007). Syndecan-1 expression in stromal cells seems to promote growth of breast carcinoma cells in direct coculture by inducing cell proliferation rather than reducing its apoptosis. It is thought that the expression of syndecan-1 in stroma of tumors is a sort of oncofetal reaction of a regulatory pathway. Syndecan-1 exerts its biological action on the cell, through binding to various extracellular proteins, growth factors and cytokines via the heparan sulphate chain (Lofgren et al., 2007; Maeda et al., 2004). Syndecan-1 forms a complex with fibroblast growth factor (FGF-2), FGF-2/HSPG/FGF receptor-1, determining breast cancer cells response to FGFs *in vitro* (Mundhenke et al., 2002). Knowing that syndecan-1 is more abundantly expressed in stroma of malignant breast tissue and that it can promote cellular proliferation, one may hypothesize that syndecan-1 can affect mammographic density by promoting cellular proliferation and subsequently increasing the risk of breast cancer.

Recently, Menashe and colleagues conducted a pathway analysis of breast cancer genome-wide association study (Menashe et al., 2010). In their study, they included 69,525 SNPs representing 421 pathways and 3,962 genes, and performed the analysis on 1,145 postmenopausal women of European ancestry with invasive breast cancer and 1,142 controls. They observed that genetic alterations associated with three pathways, including the top ranked "syndecan-1 signaling", may contribute to breast cancer susceptibility. The pathway related to "syndecan-1 signaling" namely "FGF signaling" was ranked 10th in this study. The "syndecan-1 signaling" pathway contains 13 genes involved in different cellular processes mediated by syndecan-1. In this study, syndecan-1 rs7563245 SNP was moderately associated with increased breast cancer risk ($p$ for trend = 0.019).

### 6.7 Cyclooxygenase-2

One study investigated the cyclooxygenase-2 (COX-2) enzyme expression in breast tissue from 66 women aged 29-88 years old with dense or non-dense breasts according to BI-RADS categories (Yang et al., 2010). In addition to hormone receptors previously described, they analysed COX-2 expression immunohistochemically in stroma and epithelium of normal breast tissue obtained from mastectomy specimens, and quantified immunostainings using the Allred scoring system. In their study, Yang and colleagues observed higher COX-2 expression in stroma ($p < 0.001$) and epithelium ($p < 0.02$) from dense breasts compared to non-dense breasts. However, after inclusion of all immunohistochemical factors in the model, only COX-2 expression in the stroma was statistically significant ($p < 0.01$).

The COX enzymes influence cellular proliferation by catalyzing the formation of prostaglandins (Soslow et al., 2000). Growing evidence supports the role of COX-2 in promoting tumorigenesis by catalyzing the synthesis of several prostaglandins, mainly prostaglandin E2, which in turn stimulates angiogenesis and inhibits the immune surveillance (Ben-Av et al., 1995; Soslow et al., 2000). By inducing prostaglandin E2 synthesis, COX-2 can also promote increased aromatase activity and mRNA production in the mammary gland and thereby augment estrogen production (Subbaramaiah et al., 2008; Zhao et al., 1996). Nevertheless, COX-2 has been shown to inhibit TGF-β which is known to reduce mammary epithelial cell proliferation (Studer & Chu, 2005; Yang et al., 2010). In support of this, COX-2 has been found to be up-regulated in 41% and 80% of invasive breast tumors and ductal carcinoma *in situ* respectively, which is significantly different from the negligible expression in nonneoplastic epithelium ($p < 0.0001$) (Soslow et al., 2000). In an effort to better assess the association between COX-2 and breast cancer risk, Yu and colleagues conducted a meta-analysis on the relationship between common SNPs in the COX-2 gene and breast cancer risk (Yu et al., 2010). In their meta-analysis, they found no clear association of rs5275, rs5277 or rs20417 SNPs with breast cancer risk in any model (codominant, dominant or recessive model). However, a novel SNP located in exon 2 (169 C > G) of the COX-2 gene has been recently associated to breast cancer risk (OR (GG vs. CC) = 1.76, 95% CI 1.20-3.05) (Li et al., 2009). Because of its location in an exon, the role of this SNP on the COX-2 expression should be investigated.

## 7. Genes expression in breast tissue associated to mammographic density

The hypothesis that specific genetic pathways could influence mammographic density was tested by two research groups (Haakensen et al., 2010; Yang et al., 2010). The first study,

described above (Yang et al., 2010), used RNA isolated from frozen biopsy specimens from normal breast tissue containing at least 60% epithelial content and obtained at a distance of more than 5 cm from the primary tumor. The authors compared about 34,000 different genes expression in tissues from women with high (n = 28) and low (n = 38) mammographic densities. They identified 73 genes differentially expressed by ≥ 1.5 folds with a $p < 0.001$ (false discovery rate (FDR) < 0.10). Of those, 26 genes were up-regulated in dense breast tissue versus non-dense tissue, and 47 genes were down-regulated in dense tissue when compared to non-dense tissue. Consistent with the link between stroma and mammographic density, the biological functions analysis revealed that the differentially expressed genes were involved in tissue morphology, connective tissue development, function and disorders, developmental, skeletal and muscular disorders and tumor morphology. It was also found from both network and canonical pathways analysis that decreased TGF-β signaling was related to mammographic density, including the identification of the TGF-β receptor II (TGFBR2) as being lowly expressed in breast tissue of women with high compared to low mammographic density.

In addition to increased COX-2 enzyme expression (an inhibitor of TGF-β), Yang and colleagues observed a decreased signaling of TGF-β in dense versus non-dense breast tissue (Yang et al., 2010). Growing evidence suggests a dual action of TGF-β, shifting from tumor suppressor in early tumor initiation steps to tumor growth promoter in advanced malignant stages. The dual action of TGF-β is mediated through two opposing receptors, the antiangiogenic receptor ALK5 (TGFBR1) and the proangiogenic receptor ALK1. The balance between the two pathways determines the net result. TGF-β exerts its protective effect against breast cancer by inducing cell cycle arrest and promoting apoptosis of normal mammary epithelial cells (Casey et al., 2007). Data from 6,703 cases and 6,840 controls have been assessed in a recent meta-analysis performed by Scollen and colleagues in attempt to better understand the association between TGF-β and breast cancer risk (Scollen et al., 2011). The authors identified the most common variants in 17 genes comprising both arms of TGF-β signaling pathways. The minor G allele of SNP rs10512263 of the TGFBR1 gene and the G allele of SNP rs4522809 of the TGFBR2 gene had a protective effect (OR (G vs. A) = 0.87, 95% CI 0.81-0.95, $p = 0.001$ and OR (G vs. A) = 0.95, 95% CI 0.91-0.99, $p = 0.02$, respectively). The TGFBR1 rs10512263 SNP, located in intron 1, is either a causal variant of unknown function or marking the causal SNP, while rs4522809 SNP located in intron 2 of the TGFBR2 gene is most likely marking a putative causal variant. Conversely, for rs1982073 SNP located in TGFB1 gene, there was a dose-dependent association between the proline-encoding allele and increased breast cancer risk (OR (Pro vs. Leu) = 1.05, 95% CI 1.02-1.09, $p = 0.002$). The Pro allele induces TGFB1 secretion *in vitro* compared to the Leu allele. The authors observed no detectable effect of SNPs on genes that regulate the proangiogenic pathway.

The second study that examined genes differentially expressed in breast tissue according to mammographic density included 79 healthy women (Haakensen et al., 2010). They assessed percent mammographic density using a computer-assisted method from digitized craniocaudal mammograms of both breasts. In this study, they used RNA isolated from biopsies that were taken from areas with no visible pathology, but with some mammographic density in order to obtain sufficient RNA amount. They tested 9,767 different probes expression for their associations with percent mammographic density, and identified 25 probes representing 24 genes of decreased expression associated with

increased mammographic density (FDR < 0.25). No particular biological function or pathway was found to be associated to this set of genes. Among identified genes, Haakensen and colleagues found that three uridine 5'-diphospho-glucuronosyltransferase genes (UGT2B7, UGT2B10 and UGT2B11) and the ERα gene were down-regulated in tissues with high mammographic density compared to those with low mammographic density. When the expressions of these genes were in models taking into account age and body mass index, only UGT2B10 expression among women younger than 50 years old and ERα expression among women 50 years or older remained statistically significant. Inclusion of healthy women was the major strength of this study.

The UGT genes encode enzymes responsible for the inactivation of several compounds, including sex hormones. They catalyse the glucuronidation of sex hormones to less active compounds (Guillemette et al., 2004). Like UGT2B7, UGT2B10 is capable of estrone conjugation. A study has demonstrated that the mRNA and activity of both UGT were down-regulated in cancerous breast tissue when compared to normal breast tissue (Starlard-Davenport et al., 2008). The mRNA expression of UGT2B7 and UGT2B10 were decreased by almost four folds and more than eight folds respectively in cancerous breast tissue biopsies compared to normal breast tissue ($p = 0.01$ and $p = 0.04$, respectively). Moreover, estrogen glucuronidation, which is considered as an index of UGT activity, was reduced by two folds in cancerous breast tissue compared to normal tissue. These results suggest that UGT2B7 and UGT2B10 have a protective role for breast tissue against estrogen metabolites and that decreased UGT2B7 and UGT2B10 expressions can promote breast carcinogenesis. This finding is consistent with that of Haakenssen and colleagues who observed decreased UGT2B7 and UGT2B10 expressions in mammographically dense breast tissue. The association between UGT variants or levels and breast cancer risk is not yet reported.

Contrary to what one would expect, Haakensen and colleagues found that the expression of ERα gene was significantly down-regulated in breasts with higher mammographic density among women 50 years or older. According to the authors, this decrease in ERα expression in dense breast tissue could be explained, at least in part, by the increased estrogen levels in postmenopausal breast tissue (Borras et al., 1994; Saceda et al., 1988). However, as stated previously, measurement of both ERα and ERβ expressions according to menopausal status may be required to evaluate the link between these receptors and mammographic density.

There was no overlap in lists of genes up-regulated or down-regulated according to mammographic density between both studies. The divergence between Yang and Haakensen results could be attributed to different means used to evaluate mammographic density (BI-RADS vs. computer-assisted method) and genes expression (Affymetrix U133Plus 2 Gene chip vs. Agilent Human Whole genome Oligo Microarrays G4110A platform), as well as the selection of normal breast tissue (≥ 60% epithelial content from breast cancer patient vs. mammographically dense tissue from healthy women). The main pitfall of both studies was the use of breast tissue specimens containing various proportions of types of cells (epithelial, stromal, adipocyte, etc.) We believe that it is important to distinguish tissue types, like epithelium from stroma, because it has been clearly determined that these two tissues exhibit distinct expression profiles (Finak et al., 2006). This is also in line with results from our previous sections.

## 8. Conclusion

In summary, some candidate proteins and genes in normal breast tissue such as IGF-1, TIMP3, TGF-β, UGT, stromal COX-2 and stromal and epithelial aromatase among pre- and postmenopausal women and epithelial ERα, stromal lumican and decorin, and epithelial and stromal syndecan-1 among postmenopausal women have been found to be associated with mammographic density. Among these, IGF-1, ERα, aromatase, TIMP3, lumican, syndecan-1, COX-2, TGF-β and UGT have also been related to breast cancer risk in the same direction of their association with mammographic density. However, the small number of studies that examined each protein/gene limited the conclusions that could be drawn.

Nonetheless, these data provide some support to the hypothesis that mammographic density could be used as an intermediate biomarker of breast cancer. Mammographic density promises a relatively quick exploration of the effect of several mitogens on breast tissue eliminating the long period of time elapsing between exposure to mitogen and the detection of their effect on breast tissue. Future investigations incorporating both pre- and postmenopausal women and examining the association of various molecular markers (proteins and genes expression) on epithelial and stromal breast tissue separately with mammographic density are therefore required to confirm the above findings and to identify new promising ones. The identification of molecular markers influencing mammographic density may provide important clues regarding the molecular causes of breast cancer.

## 9. Acknowledgments

## 10. References

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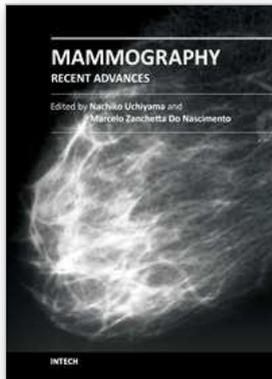
**Mammography - Recent Advances**

Edited by Dr. Nachiko Uchiyama

In this volume, the topics are constructed from a variety of contents: the bases of mammography systems, optimization of screening mammography with reference to evidence-based research, new technologies of image acquisition and its surrounding systems, and case reports with reference to up-to-date multimodality images of breast cancer. Mammography has been lagged in the transition to digital imaging systems because of the necessity of high resolution for diagnosis. However, in the past ten years, technical improvement has resolved the difficulties and boosted new diagnostic systems. We hope that the reader will learn the essentials of mammography and will be forward-looking for the new technologies. We want to express our sincere gratitude and appreciation?to all the co-authors who have contributed their work to this volume.

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