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BREAST CANCER: PROGNOSTIC EVALUATION PERSPECTIVES BY TRANSCRIPTION FACTORS USING HISTOCHEMICAL IMMUNOLOCALIZERS AND FUNCTIONAL GENOMICS.

CÂNCER DE MAMA: PESPECTIVAS DE AVALIAÇÃO PROGNÓSTICA POR FATORES DE TRANSCRIÇÃO USANDO IMUNOLOCALIZADORES HISTOQUÍMICOS E GENÔMICA FUNCIONAL.

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Abstract

The molecular characteristics of breast carcinomas have provided information on their behavior and have allowed the establishment of more effective strategies for their treatment. Transcription factors have been the target of study in neoplasms because they act in the modulation of genes that encode oncogenic proteins or tumor suppressors. In this broader context, the SOX transcription factors have been appreciated as potential prognostic and survival markers, as they are included in the list of emerging tumor biomarkers for breast cancer. Studies involving genes and proteins of the SOX family in breast cancer may provide important clues for the pathophysiology, diagnosis, and treatment of the disease.

Keywords: SOX; HER-2; Breast cancer; IHQ; Invasive Ductal Carcinoma; Prognosis.

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Resumo

As características moleculares dos carcinomas mamários têm disponibilizado informações sobre seu comportamento e permitido estabelecer estratégias mais eficazes para seu tratamento. Fatores de transcrição tem sido alvos de estudo nas neoplasias por atuarem na modulação de genes que codificam proteínas oncogênicas ou supressores tumorais. Nesse amplo contexto, os fatores de transcrição SOX têm sido apreciados como potenciais marcadores de prognóstico e sobrevida, por estarem incluídos no rol de biomarcadores tumorais emergentes para câncer de mama. Estudos envolvendo genes e proteínas da família SOX no câncer de mama poderão fornecer pistas importantes para a fisiopatologia, diagnóstico e tratamento da doença.

Palavras-chave: SOX; HER-2; Câncer de Mama; IHQ; Carcinoma Ductal Invasor; Prognóstico.

INTRODUCTION

Breast cancer is a multifactorial disease, triggered, fundamentally, by the loss of balance between the activity of oncogenes and tumor suppressor genes, which results in altering the control of cell proliferation (VAN'T VEER, 2002; VIALE, 2012). In addition, it is increasingly understood as a heterogeneous disease, because neoplasms of similar morphology may have different molecular profiles, not detectable by conventional histopathological examination. It is speculated that each tumor is unique and that its DNA contents are individually distinct (BARROS; LEITE, 2015; PEROU et al., 2000).

Characteristics such as age, ethnicity, lifestyle, eating habits, changes in reproductive factors, family history, presence of mutations in the *BRCA1* and *BRCA2* genes (breast cancer genes-tumor suppressors) and use of hormone replacement are factors that interfere in the diagnosis and development of breast cancer (FENG et al., 2018).

Despite advances in research, breast cancer is still the malignant neoplasm that most affects women, except for non-melanoma skin cancer. On the other hand, the breast cancer mortality rate is quite different between developed and developing countries. In developed countries there has been a significant reduction in mortality in recent years, however it remains the second leading cause of death in women aged 45 to 55 years in the United States (APURI, 2017). In developing countries, on the other hand, there was stability or even a continuous increase in mortality from this cancer (TORRES et al., 2017). This discrepancy can be attributed to the differences in policies for early detection of the disease, since breast cancer is the tumor that most presents scientific evidence on the impact of screening on reducing mortality (HARRIS, 2013).

The neoplasm, if diagnosed early and treated appropriately, has a good prognosis, with an average five-year survival of 83% to 92%, reaching 98% in cases of localized disease. Thus, it is necessary to develop new studies aimed not only at the diagnosis of breast cancer in the early stages and effective treatment, but also at the quality of life and the reduction of post-treatment morbidity (ASSI et al. 2013; IARC 2015; TAO et al., 2015).

Breast cancer is less frequent in women under the age of 40, however the mortality rate is higher in this group of patients, when compared to postmenopausal women. It is not

uncommon for the disease to present itself in women under 40 with a higher histological grade, greater lymph vascular invasion, negative estrogen (ER) and progesterone (PR) receptors and increased expression of type 2 human epidermal growth factor receptor (HER2 or CERBB2), these women have a greater chance of local and systemic recurrence, and a lower overall survival rate. It was demonstrated, through genomic analyzes, that tumors in young patients represent a distinct biological entity, characterized by unique molecular patterns and a worse prognosis (CANCELLO et al., 2010).

Thus, treatment for breast cancer has required a multidisciplinary approach (with surgery, chemotherapy, radiotherapy, targeted therapy, and hormone therapy), and has been experiencing a period of rapid evolution due to advances in the genomic field, tumor biology and immunology. These areas have provided valuable information on the heterogeneity between breast tumors, key to the oncogenic pathway and the role of the immune system in the natural history of the disease. Although the diagnosis of the neoplasm is mainly histological, auxiliary tests support its diagnosis, classification, prognosis, and prediction of response to therapy. The elementary morphological classification widely used in the classification of breast cancer has become insufficient to describe these tumors.
The identification and differentiation of molecular phenotypes in breast cancer through the analysis of the immunohistochemical profile is important in relation to the prognosis of the disease. The expression of these markers is causally related to the appropriate treatment for each type of cancer (CHEN et al., 2014; CHENG et al, 2013).

Tumor markers are tissue, plasma, and genetic components capable of defining different characteristics of a pathology (SATO et al. 2014). Through the study of tumor markers, it is possible to have a better understanding of the molecular and cellular bases of cancer initiation and progression (GOBBI, 2012). Studies show that the positivity of the markers ER, PR, HER-2, Cytokeratin 5/6 (Ck5/6), monoclonal antibody Ki-67 (Ki67) and epithelial growth factor receptor (EGFR) have a prognostic and predictive factor, related to the disease. In contrast, cancers negative for such tumor markers give patients a worse prognosis, as they have clinical characteristics linked to the larger tumor size, high degree staging and high risk of developing distant metastasis (BRUFSKY et al., 2014; O-CHAROENRAT, 2015).

Molecular methods undoubtedly provide prognostic and predictive information and may help identify new therapeutic targets, and the interest in molecular classifiers and their potential application is perfectly understandable, one important carcinogenesis route that has been studied in relation to breast cancer is the expression of members of the *SOX* (*SRY*-related HMG-box genes) family pathway. *SOX* genes encode proteins that act as transcription factors with an important role in embryonic development and carcinogenesis. The *SOX* family represents 20 genes responsible for regulating the gene expression patterns of cell lines and tissue, controlling various developmental processes, including cell differentiation, sexual differentiation, and organogenesis. As is the case with many genes involved in the regulation of development, *SOX* genes are often deregulated in cancer (THU et al., 2014). Members of the *SOX* family can act as oncogenes, tumor suppressor genes, or both, depending on the cellular context, and can be activated or inactivated through a variety of genetic and epigenetic mechanisms, including changes in the number of DNA copies, changes in methylation of DNA and aberrant miRNA expression (CASTILLO; SANCHEZ-CESPEDES, 2012; GRIMM et al., 2019).

Several studies have demonstrated the functions performed by members of the SOX family, even in very early embryonic stages, playing a critical role in the biology of stem cells, in organogenesis and in animal development (GUTH; WEGNER, 2008; LEFEBVRE et al., 2007; LOVELL-BADGE, 2010). As examples of the oncogenic roles of SOX family proteins, it was possible to observe the expression of SOX2 in 43% of breast carcinomas of the Basal-like type and strongly correlated with Ck5/6, EGFR and vimentin immunoreactivity, suggesting that SOX2 should play a role in the development of a less differentiated phenotype of these tumors (DEY et al., 2019; RODRIGUEZ -PINILLA et al., 2007).

Therefore, it can be inferred that breast cancer is a heterogeneous disease, which is reflected in its molecular classification, morphology, clinical course and response to treatment (SEONG et al., 2015; VAN SCHOONEVELD et al., 2015). Nevertheless, despite the range of discoveries surrounding breast cancer, the clinical outcome of affected patients remains unsatisfactory. This is mainly due to the incomplete understanding of the molecular mechanisms involved in the development and behavior of this carcinoma, making it urgent to explore them more deeply. Research on the molecular characteristics of breast carcinomas has provided a lot of information on the behavior of these tumors and, with this, has allowed to establish more effective treatment strategies (GRALOW et al., 2008).

In this context, it is imperative that one should investigate in breast tumors, the transcription factors of the *SOX* family, including the *SOX2*, along with other members of the *SRY* genes. The present review focus on the importance correlating genomics and proteomics with other tumor characteristics of prognosis and predictors of response to treatment that have already been validated in the literature, and thus encourage thoughts on the development of new putative target genes and therapeutic strategies.

BREAST CANCER IN BRAZIL AND THE WORLD

The World Health Organization (WHO) classifies cancer in the group of non-communicable diseases, along with heart disease, infarction, chronic respiratory diseases and diabetes, with this group being the main cause of death in the world (WHO, 2019). According to the International Agency for Research on Cancer, in 2018 2.1 million new cases of breast cancer were diagnosed worldwide, representing almost one in four cancer cases among women. With an index of 630,000 deaths from the same disease, which is still the main cause of cancer-related deaths in women in Europe and worldwide (BRAY et al., 2018).

For Brazil, approximately 60,000 new cases of breast cancer are estimated for each year of the 2018-2019 biennium, with an estimated risk of ~ 56 cases per 100,000 women (Table 1). Without considering non-melanoma skin tumors, this type of cancer is also the first most frequent in women in the South (~ 73/100 thousand), Southeast (~ 70/100 thousand), Midwest (~ 52/100 thousand) and Northeast (~ 40/100 thousand). In the Northern Region, it is the second most incident tumor (~ 19/100 thousand). In 2018 16,724 women died in Brazil due to breast cancer, corresponding to 16.1% of all cases of death in women affected by neoplasms, almost 5% more cases of death in relation to the runner-up (INCA, 2018).

The detection of breast cancer has increased considerably since the introduction of mammographic screening and continues to grow with an aging population. The most important risk factors include genetic predisposition, exposure to estrogens (endogenous and exogenous, including long-term hormone replacement therapy), ionizing radiation, low parity, high breast density and a history of atypical hyperplasia.Western-style diet, obesity and

alcohol consumption also contribute to the increasing incidence of breast cancer. As in all epithelial neoplasms, the incidence of breast cancer increases rapidly according to age, gradually increasing until menopause and decreasing after menopause (ARAGÓN et al., 2014; CAPLAN, 2014). Due to the increasing worldwide incidence, high mortality rates and high cost of treatment, breast cancer has been considered a public health problem in several countries.

Table 1 - Proportional distribution of the ten most common types of cancer estimated for 2018 by sex, except non-melanoma skin.

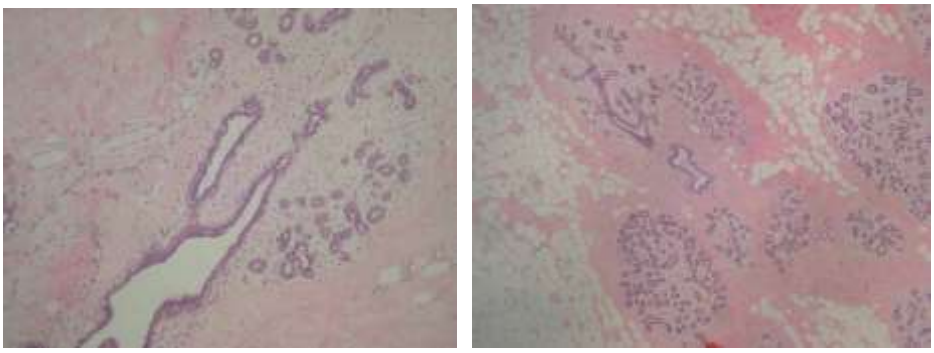
Primary location	**Cases**	**%**			**Primary location**	**Cases**	**%**
Prostate	68.220	31,7%			Female Breast	59.700	29,5%
trachea Bronchus, Lung	18.740	8,7%			Colon/Rectum	18.980	9,4%
Colon/Rectum	17.380	8,1%			Cervix	16.370	8,1%
Stomach	13.540	6,3%	MALE	FEMALE	trachea Bronchus, Lung	12.530	6,2%
Oral cavity	11.200	5,2%			Thyroid gland	8.040	4,0%
Esophagus	8.240	3,8%			Stomach	7.750	3,8%
Bladder	6.690	3,1%			Uterus	6.600	3,3%
Larynx	6.390	3,0%			Ovary	6.150	3,0%
Leukemias	5.940	2,8%			Central Nervous System	5.510	2,7%
Central Nervous System	5.810	2,7%			Leukemias	4.860	2,4%

Source: INCA (2018)

THE HETEROGENEITY OF BREAST CANCER

The human breast is composed of a branching of duct networks containing luminal epithelial cells, and myoepithelial cells (**Figure 1**) that are enclosed in small ductal structures called terminal lobular ductal units (TDLU). The ductal epithelial cells form the ducts, the alveolar epithelial cells are the milk-producing cells and the myoepithelial cells, are contractile cells that line the ducts and alveoli (KAKARALA; WICHA, 2008). The mammary stroma is formed by adipose and connective tissue involving TDLU, blood vessels and lymphatic vessels (AMERICAN CANCER SOCIETY, 2012).

Figure 1 - Terminal duct-lobular unit of normal breast tissue.



Representative slide of mammary tissue, prepared by conventional technique of clinical pathology, stained with eosin-hematoxylin. Optical microscopy (400µm). **Source**: R.C. Rodrigues, 2019 (personal archive).

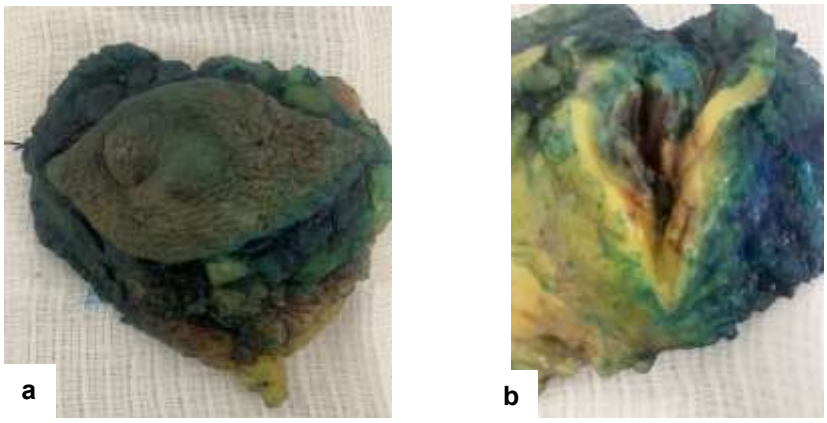
Nevertheless, the mammary gland derived from the epidermis has its development dependent on the stroma-epithelium interactions that modulate the normal development of the breast and also participate in the malignant transformation of the tissue, regulating the growth, survival, migration and differentiation of the breast epithelium (KASS et al., 2007). The tumor formation process is complex and involves multiple factors that facilitate mutations in cells, which determines the expression of oncogenes and the suppression of genes that prevent its development. The different mutations confer several selective advantages for tumor cells, allowing their growth (HANAHAN; WEINBERG, 2011).

The natural history of breast cancer is still not well understood, as its evolutionary behavior does not reproduce uniformly in all women. We seek to explain this behavioral divergence of some tumors that have the same clinical characteristics, with the knowledge acquired through the prognostic factors that involve the general context of breast cancer. Thus, in addition to the diagnosis of the disease itself, there are aspects of clinical and biological findings that are associated with differences in disease-free time and overall survival.

PATHOLOGICAL CLASSIFICATION OF THE TUMOR-NODE-METASTASIS SYSTEM (TNM - AJCC)

Prognostic factors in breast cancer include clinical characteristics of patients and pathological and biological aspects of tumors that determine the clinical evolution of the disease, that is, the probability, at the time of diagnosis or surgical treatment, of recurrence of the neoplasia and the patient's overall survival without adjuvant treatment. Predictive factors, in contrast, are clinical, pathological, and biological characteristics (**Figure 2**), used to estimate the likelihood of response to a specific type of adjuvant therapy. Prognostic and predictive markers must be technically validated, have statistical significance proven by clinical tests and influence clinical decision (GOLDHIRSCH et al., 2009; SOERJOMATARAM et al., 2008).

Figure 2 - Surgical Management of the Breast



Lumpectomy (a), macroscopic image of the tumor, surgical piece stained with Patent Blue V, a lymphatic marker used to identify the sentinel lymph node in oncological surgeries (b). **Source:** R.C. Rodrigues, 2019 (personal archive).

Clinical-pathological variables have a profound impact on survival and are responsible for most of the differences in clinical outcome between patients with breast cancer and are best illustrated by the clinical stage of the disease based on physical examination and imaging findings. The TNM staging system by the American Joint Committee of Cancer (AJCC) / International Cancer Control Union (UICC), incorporates tumor size, regional lymph node status and distant metastases (Table 2) (HORTOBAGYI, 2017).

Table 2 - Pathological classification of the tumor-node-metastasis system.

Brest			
Tis	**Carcinoma *in situ***		
T1	**≤ 2cm**		
T1mic	**≤ 0,1cm**		
T1a	**> 0,1cm-0,5cm**		
T1b	**> 0,5cm-1cm**		
T1c	**> 1cm-2cm**		
T2	**> 2cm- 5cm**		
T3	**> 5cm**		
T4	Chest wall / skin		
T4a	Chest wall		
T4b	Edema / ulcer, satellite skin nodules		
T4c	**T4a + T4b**		

T4d	Inflammatory Carcinoma		
N1	Mobile axillary lymph nodes (ALN)	**pN1mi**	**Micromestasis >0,2mm ≤2mm**
		pN1a	**1-3 ALN**
		pN1b	**Microscopic metastasis in LN of internal mammary**
		PN1c	**1-3 axillary lymph nodes + internal mammary LN with micro metastasis**
N2a	**Fixed ALN**	**pN2a**	**4-9 ALN+**
N2b	**Internal breast LN clinically apparent (MI)**	**pN2b**	**LNMI + ALN -**
N3a	**LN infra-clavicular**	**pN3a**	**≥ 10 LNA + ou infra-clavicular +**
N3b	**ALN + e LNMI +**	**pN3b**	
N3c	**LN supraclavicular**	**pN3c**	**LN supra-clavicular**
M	**Distant metastasis**		**Most common sites: bones, liver, lung**

Source: https://cancerstaging.org/. < access in oct.09.2020.

Morphological heterogeneity is one of the characteristics of malignancy and forms the basis for the histopathological classification of breast cancer. Since infiltrative ductal and lobular carcinomas, either in their pure form or in combination with other types of tumor, are the most common forms of breast cancer. Invasive ductal carcinoma (ICD) of a non-special or otherwise unspecified type (NOS) is the most common histological type (40-75%) of invasive breast cancer. Although common, the WHO classification defines ICD NOS by exclusion, as "the heterogeneous group of tumors that do not have sufficient characteristics to achieve classification as a specific histological type".

In addition to the CDI NOS, the WHO classification includes 21 subtypes with distinct morphological characteristics, of which invasive lobular carcinoma (CLI) is the most frequent (5–15%) (LAKHANI et al., 2012). The other special subtypes of breast carcinoma are rare and differ significantly in prognosis and response to adjuvant treatment. Tubular, mucinous and papillary carcinomas usually have excellent clinical results compared to ICD and CLI and are not always treated with chemotherapy. Mildly differentiated metaplastic carcinomas and ICD NOS have significantly worse results and are routinely treated with systemic chemotherapy (COLLEONI, 2011). Patients with infiltrative ductal tumors generally have a higher incidence of positive axillary lymph nodes and worse clinical prognosis than patients with less common types of infiltrative tumor (ALBAIN; ALLRED et al., 1994).

The histological grade also highlights the tumor heterogeneity in breast cancer. The grade is assessed according to a three-rank system (low grade, intermediate grade, high grade), based on the assessment of three parameters: percentage of tumor arranged in the tubular structures of the gland, the degree of nuclear pleomorphism and the mitotic rate (ELSTON; ELLIS, 1991). The degree of breast carcinoma is a strong prognostic factor and is one of the tools incorporated in decision making, as well as the Nottingham Prognosis Index and Adjuvant! Online (PETIT, 2012).

Breast cancers of different degrees also show different profiles by proteomic, genomic, and transcriptomic analysis (SOTIRIOU et al., 2011). In multivariate models that include gene signatures, the degree remains an independent prognostic factor for positive ER tumors. Grade 1 and 3 breast carcinomas probably represent two quite different diseases, and molecular data indicate that progression from low to high grade carcinoma is exceedingly rare (NATRAJAN et al., 2009).

Consequently, the standard treatment of breast cancer has been based on the characteristics of the tumor, including tumor stage, histopathological characteristics and profile of biomarkers, and is affected by the patient's age, menopausal status and the patient's general health (HARRIS, 2013).

MOLECULAR CLASSIFICATION IN BREAST CANCER AND ITS RELEVANCE

Breast cancer is such a complex genetic disease that, it is characterized by the accumulation of multiple molecular changes. It constitutes a heterogeneous group of neoplasms, consisting of several histological types, which differ in clinical manifestations, evolution and therapeutic response. Until recently, the classification of breast carcinomas was essentially based on their morphological aspects (ELSTON; ELLIS, 1991). However, tumors classified under the same descriptive term can have varied molecular aspects and biological evolution. The molecular heterogeneity of breast cancer, which is not morphologically evaluable, represents a major challenge to the study and treatment of this disease (GOLDHIRSCH et al., 2011). Although heterogeneity at the cellular level has been recognized in breast cancer since the 19th century, its clinical relevance was first established about 30 years ago, with the introduction of estrogen receptor testing.

Gene expression profile studies have suggested that molecular tests could perform better than traditional histopathology, being used as a "gold standard" in terms of prognosis and prediction of response to treatment (PEPPERCORN; PEROU; CAREY, 2008).

Due to the costs, time and technical knowledge required for molecular assays, alternative methods have been developed for indirect assessment of the molecular subtypes that can be used in most laboratories. The immunohistochemistry staining panel comprising ER, PR, HER2, Ki-67, EGFR and Ck5/6 can identify the molecular subtypes of breast cancer with satisfactory and reproducible precision.

There are currently three molecular prognostic / predictive factors validated for routine clinical use in the treatment of patients with breast cancer. They are the hormonal receptors for estrogen (ER) and progesterone (PR) and the epidermal growth factor type 2 (HER) receptor. -2). The expression of ER, PR and HER2 are routinely evaluated in all invasive breast carcinomas by immunohistochemistry (IHC), according to the recommendations of the American Society of Clinical Oncology / College of American Pathologist (ASCO / CAP). Such biomarkers are already well-established prognostic and predictive factors and their

expression in breast carcinomas is essential to guide treatment. In addition to these, other markers have been used adding information to the molecular profile of breast cancer, such as Ki-67, p53, vascular markers, p63, CK5 and P-cadherin (FASCHING et al., 2011).

ER and PR are expressed in approximately 80% and 60-70% of breast carcinomas, respectively. Although ER positive tumors co-express PR (ER + / PR +) in 70-80% of cases, some breast carcinomas are ER + / PR- or, rarely, ER- / PR + (Figure 2). The response to hormonal treatment also varies, showing a better response (approximately 60% rate) in ER + / PR + tumors and lower rates in ER + / PR− and ER− / PR + tumors.

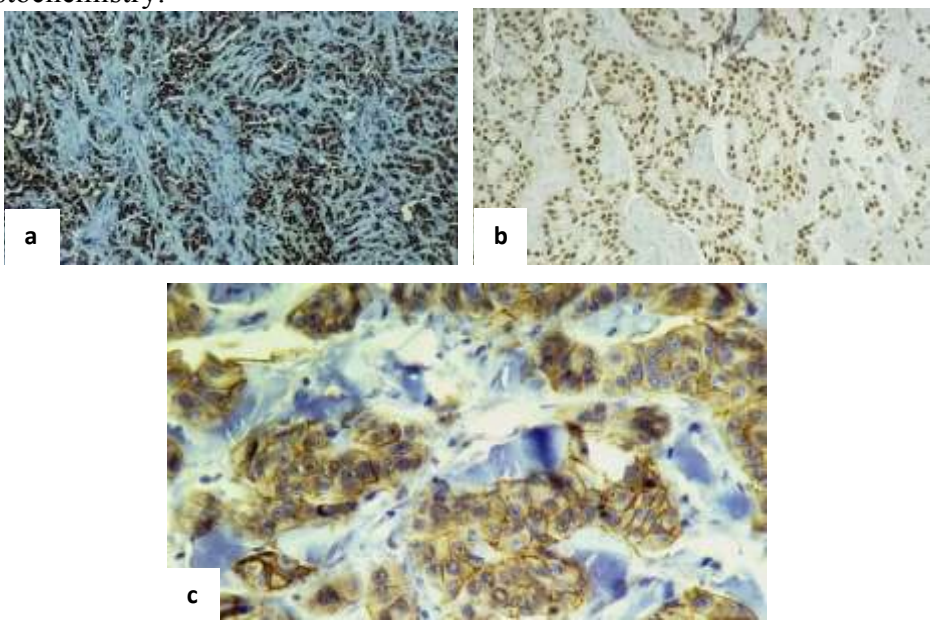
HER2 is a proto-oncogene that encodes a transmembrane receptor protein with tyrosine kinase activity involved in cell growth, differentiation, apoptosis and metastasis (YARDEN, SLIWKOWSKI, 2001). Overexpression of the HER-2 protein is found in approximately 18-20% of breast cancers, with gene amplification being the main mechanism of gene overexpression (Figure 2). This overexpression is associated with high-grade tumors, with impairment of the lymphatic chain (BURSTEIN et al., 2011), and a high rate of recurrence and mortality (WOLFF et al., 2013). Although HER2-positive breast carcinomas have a more unfavorable prognosis, they have shown a high rate of response to targeted anti-HER2 therapy, the so-called monoclonal antibodies, as documented by the complete response to post-neoadjuvant treatment in about 50 to 60% of patients with HER2 positive tumors (COTAZAR et al., 2014).

With regard to hormone receptors, we know that they act in the growth and differentiation of the normal mammary epithelium. The estrogen receptor gene encodes a nuclear transcription factor activated by estrogen and the progesterone receptor is a gene regulated by estrogen. They are used as an indicator of hormonal therapy, improving patient survival. The nuclear protein Ki-67, encoded by the *MKI67* gene (antigen identified by monoclonal antibody), is associated with cell proliferation, being expressed in all phases of the cell cycle, except G0.

Breast carcinomas that do not express ER, PR and HER2, are called "triple-negative" and constitute an extremely heterogeneous group of tumors both histologically and genetically and in relation to the prognosis for response to treatment.

Perou et al. (2000) demonstrated that the phenotypic diversity of breast tumors was associated with the corresponding diversity of gene expression. To reach this conclusion, the authors analyzed 65 tissue samples and selected a subset of 456 genes, called "intrinsic" gene subset, and consisted of genes with significantly greater expression variation between different tumors than between paired samples of the same tumor. Using this subset, the authors were able to identify 4 different molecular subtypes of breast cancer: positive for estrogen receptors (ER) / luminal type A, basal breast, positive and normal HER2. Subsequent data expanded the classification to distinguish between luminal A and luminal B. These 5 molecular subtypes have been confirmed in independent data sets and, mainly, the gene expression subtype appears consistent between primary tumors and metastatic lesions that occur years later.

Figure 3 – Overexpression of ER, PR and HER2 in invasive breast carcinomas by immunohistochemistry.



(a) ER expressed positive; (b) PR expressed positive; (c) HER-2 positive. Source: R.C. Rodrigues, 2019 (personal archive). Optical microscopy (a, b = 400µm; c = 1 nm).

In addition, subtypes are associated with differences in clinical outcome. In a subsequent study by the same group of authors, they examined a subset of 49 patients with locally advanced breast cancer who were treated with doxorubicin and had an average follow-up of 66 months, found that disease-free survival and overall survival differed significantly between subtypes of breast cancer, luminal tumors A had longer survival times compared to the basal and HER2 subtypes having these shorter survival times, whereas luminal tumors B had intermediate survival times (SORLIE et al., 2001).

Therefore, the analysis of gene expression classifies breast cancer into four large intrinsic molecular subtypes with prognostic and therapeutic implications: luminal A, luminal B, enriched with HER2 and basal. Subtypes A and B luminal exemplify tumor heterogeneity of ER-positive breast carcinomas and have better survival than subtypes enriched with HER2 or basal. Both luminal subtypes express ER, but luminal B tumors are characterized by increased expression associated with gene proliferation and have a worse prognosis than luminal A tumor (WIRAPATI et al., 2008).

The basal subtype is enriched for genes expressed in basal epithelial cells and is triple negative in 70% of cases. Additional subtypes include tumors with a low level of claudin with signature of the stem cell type and RA-positive apocrine molecular tumors (PRAT; PEROU, 2011). The study of gene expression suggests that the prognostic impact of different signatures is related to the genes associated with proliferation. Although gene expression profiles can predict the response to chemotherapy and risk of recurrence, classification of breast carcinoma based on gene expression is hampered by clinical factors and molecular heterogeneity.
Patients with breast carcinoma of the same molecular subtype and who received identical treatments have different clinical outcomes and / or acquire resistance to therapy (EBCTCG,

2011). More recent studies have produced other molecular subgroups, including a molecular classification based on integrated transcriptomic genomics of 2,000 breast tumors, producing 10 new breast cancer subtypes with distinct clinical outcomes (ALI et al., 2014).
However, it is important to understand the limitations and above all to critically assess the role of molecular classification in improving the prognosis of breast cancer above and beyond traditional variables in a practical and economical way. Bearing in mind that there are numerous lines of evidence to suggest that these molecular tests complement and do not replace traditional pathological variables, such as the Nottingham Graduation System and thus define the ideal therapy for breast cancer patients (WEIGELT; GEYER; REIS- FILHO, 2010).

SOX PROTEINS AND THEIR RELATIONSHIPS WITH NEOPLASMS

The first *SOX* gene to be successfully cloned was the *SRY* gene, and it remains the defining member of the family (GUBBAY et al., 1990). However, as human *SOX* genes have been cloned, they have become potential candidates in the genesis of the most varied diseases and mutation analyzes have helped to correlate structural domains with their biological functions (PRIOR; WALTER, 1996).

Until recently, investigations of the functions of SOX proteins have focused on embryonic development and information on their physiological functions in adult tissues. However, since the beginning of the last decade, several correlations have been found between the transcription factors of the SOX family and cancer, albeit with considerable uncertainty as to how these proteins exert their oncogenic or tumor suppressive potential (KUMAR; MISTRI, 2019).

The role of the *SOX* gene family in carcinogenesis has been attributed to its properties involved in the regulation of cell differentiation, proliferation and survival in multiple essential processes (CHOU et al., 2013). Different members of the *SOX* family can play the most varied roles in many malignant tumors. Some of them demonstrate oncogenic potential to promote the development of cancers while others behave as tumor suppressor genes, acting to block the growth of carcinomas (SONG et al., 2016). *SOX* genes are potent modulators involved in embryonic development and cell fate, organogenesis, stem cell maintenance and carcinogenesis in multiple processes. The role of *SOX* genes in carcinogenesis has been attributed to their properties involved in the regulation of cell diffusion, proliferation and survival (KAMACHI, 2000).

Increasing evidence shows that SOX proteins play essential roles in various cellular processes that mediate or contribute to oncogenic transformation and tumor progression. In the context of breast cancer, *SOX* function as oncogenes and tumor suppressor genes, demonstrating to be associated with the degree and stage of the tumor as well as a more reserved prognosis. It has been noticed that a subset of SOX proteins regulates critical aspects of the biology of breast cancer, including cancer stenosis and other various signaling pathways, leading to altered cell proliferation, epithelial-mesenchymal transition, cell migration with consequent tumor development and metastasis.

SOX 2 PROTEIN AND ITS INTERACTION WITH BREAST CANCER

SOX2 is known as *SRY* (sex determining region Y) -box 2, an embryonic transcription factor gene located on chromosome 3q26.3-q27, belonging to the SRY-SOX family, members essential to the development and maintenance of stem cells, as well as cell proliferation and

differentiation. (LENGERKE et al., 2011). SOX2 protein has 3 main domains: N-terminal domain, a high mobility group domain and a transactivation domain. An increasing number of studies have shown that SOX2 is related to a variety of tumors. Silencing *SOX2* can induce the transcription of p21Cip1 and p27Kip1, leading to cell cycle arrest and: inhibition of cell growth (KELBERMAN et al., 2006).

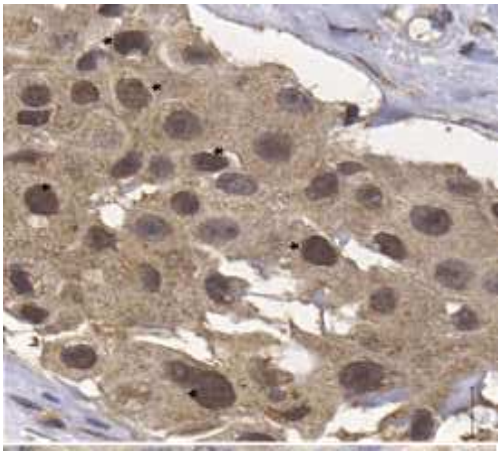
Studies carried out in recent years have shown that the SOX2 protein plays an important role in inhibiting apoptotic cell and promoting cell proliferation, mediates the aggressiveness and migration of phenotypes by activating MMP3, MMP2 and PI3K / AKT / mTOR, indicating that SOX2 also plays a role in tumor invasion and metastasis. Therefore, it promotes cell proliferation in breast, prostate and cervical cancers, and involves the escape of apoptotic signs in prostate, gastric and non-small cell lung cancer (Figure 2). Thus, the SOX2 protein appears to be overexpressed in a variety of different breast cancer phenotypes and has been found sporadically in basal molecular carcinomas and more frequently in the most common molecular types in women in recent post-menopause (FENG, LU , 2017).

Lengerke et al. (2011), evaluated the expression of SOX2 in 95 patients with primary post-menopausal breast carcinomas and reported its association with various types of early post-menopausal breast cancer and lymph node metastases. Like Rodriguez-Pinilla (2007), based on statistics, demonstrated high expression of SOX2 in basal breast cancers. When these researchers investigated the association of SOX2 with the basal type in 226 sporadic invasive breast cancers with negative lymph nodes, 43.3% showed immunoreactivity to SOX2, while the expression SOX2 was found only in 13.3% of the HER2-positive and 10.6% of luminal tumors by immunohistochemical analysis. They reported that the expression SOX2 is directly related to the size of the tumor, Ck5/6, epidermal growth factor receptor and expression of vimentin and that when the expression of SOX2 is increased, there is a decrease in the expression of ER and PR.

It appears that SOX2 expression is closely related to tumor size, histological grade, lymph node metastasis and triple negative invasive phenotype, when analyzing the relationship between SOX2, tumor pathology and clinical parameters of breast cancer patients (NOVAK, 2019; ZHANG et al., 2012). They believed that detecting the expression SOX2 could be of great value in determining the diagnosis and prognosis of patients with breast cancer.

The SOX2 protein has different importance in the prognosis of the 5 molecular subtypes of breast cancer: basal, luminal A, luminal B, HER2 + and normal breast, however, the mechanisms by which high levels of SOX2 regulate the progression and metastasis of breast cancers, remain largely unexplored. The determination of the presence and quantification of the SOX2 protein in breast cancer (**Figure 4**) with its likely suppressive or activating oncogenic potential, may create early diagnosis pathways, improve response to treatment, leading to increased survival, disease-free time and impact positive about all its comorbidities. In this regard, recently our laboratory has demonstrated the association between SOX2 transcription factor and tumors with worse TNM staging, as well as its overexpression in positive HER-2 tumors. (RODRIGUES, 2020).

Figure 4 – Slide representative of SOX2 protein overexpression in BC tumor cells.



(*) SOX2 immunostaining of nuclei of tumor cells in dark brown. Source: R.C. Rodrigues, 2019 (personal archive). Optical microscopy (1200µm).

FINAL CONSIDERATIONS AND DISCUSSION

Breast cancer represents one of the main causes of death in women. Statistics indicate an increase in the frequency of this neoplasia, both in developed and developing countries. According to data in the literature, the probability of developing breast cancer increases with age, and although it has a higher prevalence in postmenopausal women, the disease occurs in practically all age groups from reproductive age (REGINELLI et al., 2014). In a study being developed in our laboratory (RODRIGUES, 2020), the mean age at diagnosis of breast cancer and positivity for SOX2 agreed with the world literature.

The natural history of breast cancer is still not well understood, as its evolutionary behavior does not reproduce uniformly in all women. We seek to explain the behavioral divergence of some tumors that have the same clinical characteristics, with the knowledge acquired through the prognostic factors that involve the general context of breast cancer (ELOMRANI et al., 2015).

A growing body of evidence is building up support for the hypothesis that cancer stem cells, or tumor-initiating cells, direct and maintain many types of human malignancy (DIEHN et al., 2009). Normal and cancerous stem cells share phenotypes that may reflect the activity of common signaling pathways, with high expression of SOX2 among others (SIMOES et al., 2011). The role of SOX2 in the organogenesis or function of the breast is still not well understood, however in healthy breast tissue there is no significant expression of SOX2. Already in breast cancer cells it was overexpressed both in quantitative analysis of mRNAs in

RT-PCR and by Western blotting (CHEN et al., 2014).
In agreement with these findings, we have verified recently (RODRIGUES, 2020) that all samples of invasive ductal carcinoma show strong nuclear marking for SOX2 by immunohistochemical analysis, overexpressed in more than 40% of the samples. An active role for SOX2 during breast tumorigenesis is further supported by data collected in breast cancer cell lines, where SOX2 promotes cell proliferation and tumorigenesis in vivo, partially

facilitating the G1 / S transition and regulating, together with β -catenin, the expression of effector genes, such as CCND1 (CHEN et al., 2008).

High levels of SOX2 appear to be closely correlated with various processes during tumor development, including initiation, maintenance, invasion and metastasis. Therefore, SOX2 seems to play a role in tumor invasion and metastasis, since the expression of the transcription factor SOX2 is closely related to the increase in tumor size and grade, lymph node metastasis and high invasiveness of neoplastic cells (FENG; LU, 2017).

Consistently, our work showed an intimate relationship between vascular invasion, lymphatic embolization and lymph node metastasis and more importantly, we were able to identify the positive relationship of SOX2 with these markers of worse prognosis and in patients with worse TNM staging in the initial diagnosis of the disease. This is mainly due to lymph node involvement, since in this study the transcription factor SOX2 is overexpressed in patients with lymph node metastasis (RODRIGUES, 2020).

Approximately 70-75% of breast cancer cases express the estrogen alpha receptor (ERa), and are related to a better prognosis, however, it has been reported that breast stem cells do not have ER or express it at very high levels. (CLAYTON et al., 2004). In a previous study it was shown that cancer stem cells, which express high levels of SOX2, lack or express very low levels of ER and therefore will be more resistant to tamoxifen, giving a less differentiated phenotype of the cancerous disease (PIVA et al, 2014). In our recent study, we were unable to demonstrate a significant difference between hormone receptor expression and SOX2 expression. On the other hand, results of our lab were encouraging when it came to the HER2 oncogene, which consistently identified the overexpression of SOX2 in HER2 positive tumors (RODRIGUES, 2020).

It is already known that 15 to 20% of breast cancers express HER2 and this oncogene is linked to the prognosis and the therapeutic response, having a substantial role in global and disease-free survival. As previously explained, in our study the presence of HER2 in cancerous samples was accompanied by the positivity of the transcription factor SOX2. Differing in part from another study that demonstrated a significantly higher expression of SOX2 in basal or triple negative tumors (RODRIGUES-PILLA et al., 2007). Probably, this discrepancy may be related to the sample used by the author, which involved a greater number of pre-menopausal patients with BRCA1 genetic alterations.

Breast cancer is classified into three main subtypes based on the presence or absence of molecular markers for estrogen or progesterone receptors and human epidermal growth factor 2 (HER2). Triple negative breast cancer has higher recurrence rates than the other two subtypes, with 85% survival in 5 years for stage I versus 94% to 99% for positive hormone receptors and HER2 positive, whereas the mean global cancer survival of metastatic tri-negative breast is approximately 1 year versus approximately 5 years for the other 2 subtypes. Here our team identified SOX2 overexpressed in the HER2 molecular subtype and negative in the subtypes where hormone receptors are present, collaborating with previous findings that list SOX2 to tumors with a worse therapeutic response, indicating that the levels of SOX2 are higher in patients after endocrine therapeutic failure and also in tumors of these patients compared to respondents (WUEBBEN; RIZZINO, 2017).

Finally, we can see that the transcription factor SOX2 has been seen as a potential marker of prognosis and survival, and should be included in the list of emerging tumor biomarkers for breast cancer in the future, therefore more studies like ours involving the SOX2 protein and breast cancer can provide important clues for the diagnosis and treatment of the disease.

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