Article

Patients with Invasive Lobular Carcinoma Show a Significant Increase in IRS-4 Expression Compared to Infiltrative Ductal Carcinoma—A Histopathological Study

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Abstract: *Background and Objectives:* Breast cancer (BC) is the first diagnosed type of cancer and the second leading cause of cancer-related mortality in women. In addition, despite the improvement in treatment and survival in these patients, the global prevalence and incidence of this cancer are rising, and its mortality may be different according to the histological subtype. Invasive lobular carcinoma (ILC) is less common but entails a poorer prognosis than infiltrative ductal carcinoma (IDC), exhibiting a different clinical and histopathological profile. Deepening study on the molecular profile of both types of cancer may be of great aid to understand the carcinogenesis and progression of BC. In this sense, the aim of the present study was to explore the histological expression of Insulin receptor substrate 4 (IRS-4), cyclooxygenase 2 (COX-2), Cyclin D1 and retinoblastoma protein 1 (Rb1) in patients with ILC and IDC. *Patients and Methods:* Thus, breast tissue samples from 45 patients with ILC and from 45 subjects with IDC were analyzed in our study. *Results:* Interestingly, we observed that IRS-4, COX-2, Rb1 and Cyclin D1 were overexpressed in patients with ILC in comparison to IDC. *Conclusions:* These results may indicate a differential molecular profile between both types of tumors, which may explain the clinical differences among ILC and IDC. Further studies are warranted in order to shed light onto the molecular and translational implications of these components, also aiding to develop a possible targeted therapy to improve the clinical management of these patients.

Keywords: breast cancer (BC); invasive lobular carcinoma (ILC); insulin receptor substrate 4 (IRS-4); cyclooxygenase 2 (COX-2); cyclin D1; retinoblastoma protein 1 (Rb1)

1. Introduction

Breast cancer (BC) is the most common type of diagnosed cancer in women and the second leading cause of cancer-related mortality in this group [1,2]. Following the American Cancer Society, 1 in 8 women will suffer from BC in their lives, and it has been projected that the global incidence of these tumors will reach 3.2 million new cases per year by 2050 [3]. Moreover, men can also suffer from BC, accounting for less than 1% of all cancers in men and less than 1% of all breast cancers for them [4]. Lifestyle habits like smoking, sedentarism, alcohol consumption and diet are some of the most important risk factors for suffering from BC, along with obesity, aging, race, hormonal/reproductive factors and history of familiar BC [5]. An early diagnosis and regular screening are crucial for a good prognosis and survival rate of BC patients. Indeed, thanks to these approaches, the 5-year relative survival rate can be over 80% in some developed countries [6]. However, the overall survival of these patients will depend mainly on two factors: the tumoral stage (invasiveness, metastasis), and the tumoral subtype [7]. According to its histological classification, the most common type of invasive BC is the infiltrative ductal carcinoma (IDC) of no special subtype [8]. Approximately 1 in 4 tumors are defined as histological 'special types', including at least 17 discrete pathological entities such as invasive lobular carcinoma (ILC) [9]. Furthermore, ILC is the second most common subtype of BC, and differs from IDC in a set of clinical and histopathological features. For instance, ILCs present more difficulties in their detection, often exhibit a poorer prognosis, more advanced stages, frequent late recurrences and lower responses to therapy [10]. In addition, there are a plethora of biological differences between these types of tumors, including in their molecular profiles, immune response, metastasis, metabolism and other hallmarks of cancer [11,12]. Thus, it is necessary to deepen examinations on the molecular and biological basis of ILC in order to develop further approaches and strategies to aid in the clinical management of this relevant malignancy.

Insulin receptor substrate 4 (IRS-4) is a relevant molecule altered in different types of malignancies like BC. It seems that this molecule promotes tumoral proliferation and therapy resistance due to the alteration of different molecular pathways [13–15]. Furthermore, previous studies have found that an overexpression of this component is frequently related to a poorer prognosis [16]. A similar role has been found with cyclooxygenase 2 (COX-2) in patients with BC, being frequently related to a poorer prognosis and a set of carcinogenic mechanisms [17]. In the same line, Cyclin D1 levels are often disrupted in different types of tumors, especially in BC, where approximately 50% of mammary carcinomas present an overexpression of this component [18]. Likewise, the retinoblastoma protein 1 (Rb1) is also involved in the tumorigenesis and impaired cell features, having been proposed as a promising therapeutic target of different types of tumors, including BC [18,19]. Despite the relevance and demonstrated role of these markers in the development and progression of BC, there is little evidence collected regarding the differential expression of each component in ILC vs. IDC.

Hence, the aim of the present review is to explore the histopathological detection of IRS-4, COX-2, Rb1 and Cyclin D1 in 45 patients with ILC in comparison to 45 IDC in order to establish potential biological differences between both types of tumors. With that purpose, we have conducted immunohistochemical studies of n patients with ILC and compared these with n patients with IDC.

2. Patients and Methods

2.1. Collection of Samples

For our study, we used paraffin-embedded sections of breast tissue from 45 patients diagnosed with invasive lobular carcinoma (ILC) and 45 patients diagnosed with infiltrative ductal carcinoma (IDC). The diagnosis followed the principles of Lakhani et al. [20]. The present study was designed as an observational, analytical, retrospective cohort study with longitudinal follow-up. Paraffin blocks and all details with extensive clinical information on patients and follow-up data were retrospectively reviewed.

The study was carried out in accordance with the basic ethical principles of autonomy, beneficence, non-maleficence and distributive justice, and its development followed the rules of Good Clinical Practice, the principles contained in the most recent Declaration of Helsinki (2013) and the Convention of Oviedo (1997). The data and information collected complied with current legislation on data protection (Organic Law 3/2018 of 5 December on the Protection of Personal Data and Guarantee of Digital Rights and Regulation (EU) 2016/679).

2.2. Histopathological and Immunohistochemical Studies

Immunohistochemical studies were performed on paraffin-embedded breast tissue samples. The antibody recovery step was described in the protocol specifications (Table 1). Antigen/antibody reactions were detected by the avidin-biotin complex (ABC) method, with avidin-peroxidase, following the protocols of Ortega et al. [21]. After incubation with the primary antibody (1 h and 30 min), samples were incubated with 3% BSA blocker (catalog #37525; Thermo Fisher Scientific, Inc., Waltham, MA, USA) and PBS overnight at 4 °C. The samples were then incubated with biotin-conjugated secondary antibody and diluted in PBS for 90 min at room temperature (RT; Rabbit IgG (RG-96, 1:1000, Sigma-Aldrich/Mouse IgG (F2012/045K6072) 1:300, Sigma-Aldrich, St. Louis, MI, USA). Avidin-peroxidase conjugate ExtrAvidin®-Peroxidase (Sigma-Aldrich; Merck KGaA, Darmstadt, Germany) was used for 60 min at RT (1:200 dilution with PBS), and then the level of protein expression was determined using a Chromogenic Diaminobenzidine (DAB) Substrate Kit (cat. no. SK-4100; Maravai LifeSciences, San Diego, CA, USA), which was prepared immediately prior to exposure (5 mL distilled water, two drops of buffer, four drops of DAB and two drops of hydrogen peroxide drops). The signal was developed with the chromogenic peroxidase substrate for 15 min at RT; this technique allows the detection of a brown staining. For the detection of each protein, sections of the same tissue were assigned as negative controls, substituting the incubation with the primary antibody for a blocking solution (PBS). In all tissues, contrast was performed with Carazzi hematoxylin for 15 min at RT.

2.3. Histopathological Evaluation

Tissue sections were viewed using a Zeiss Axiophot light microscope (Carl Zeiss, Oberkochen, Germany) equipped with an AxioCam HRc digital camera (Carl Zeiss, Oberkochen, Germany). Given the important role of the proteins studied, the evaluation of the histological results was carried out according to the intensity of expression for the immunohistochemical staining with Score. Therefore, the histological samples of patients diagnosed with breast cancer were classified as negative (0) or low/medium (1) and high (3) expression using the IRS-Score method [22]; the samples were evaluated by two independent pathologists (MAO, MAS), and in case of discrepancies, a third pathologist intervened (SC). For each established subject group, seven randomly selected microscopy fields were examined in each of the five sections. Subjects were classified as positive when the mean proportion of the labeled sample was greater than or equal to 5% of the total sample. This was completed by calculating the total percentage of the labeled tissue in each microscopy field to obtain a mean for the study sample as described [23]. The observation and quantification of the samples were carried out independently by two researchers.

2.4. Statistic Analysis

For the statistical analysis, the statistical package GraphPad Prism® 5.1 was used for the Mann–Whitney U test as appropriate. The data are provided as the mean ± standard deviation (SD). The error bars in the figures indicate the SD. Different levels of significance are distinguished as * $p < 0.05$, ** $p < 0.005$ and *** $p < 0.001$.

Table 1. Primary antibodies used, together with the dilutions and protocol specifications.

Antigen	Dilution	Provider	Protocol Specifications
IRS-4	1:250	Thermo Fisher Scientific—PA5-117329	Preincubation with Tris-EDTA buffer pH 9 and incubation with 0.1% TTX (Triton $\times$100 in TBS) for 5 min.
COX-2	1:750	Vitro, MAD-000335QD-3/V	——————
Rb1	1:500	Vitro, MAD-000900QD-3/V	——————
Cyclin D1	1:500	Vitro, MAD-000630QD-3/V	Preincubation with Tris-EDTA buffer pH 9 and incubation with 0.1% TTX (Triton $\times$100 in TBS) for 5 min.

3. Results

3.1. Clinical and Sociodemographic Characteristics of the Study Population

The present study was designed as an observational, analytical, retrospective cohort study with longitudinal follow-up. A total of 45 patients with ILC and 45 patients with IDC were analyzed, with a median age of 69.167 ± 13.663 years for ILC and 67.571 ± 10.717 years for IDC. All patients had a score greater than pT1. The percentage of expression of estrogen receptors was $70.000 \pm 22.887\%$ for ILC and $66.538 \pm 28.091\%$ for IDC. The percentage of expression of progesterone receptors was $54.333 \pm 26.245\%$ for ILC and $55.833 \pm 33.086\%$ for IDC. The Ki67 expression percentage was $12.609 \pm 6.373\%$ for ILC and $16.190 \pm 7.731\%$ for IDC.

3.2. Patients with Invasive Lobular Carcinoma Show a Significant Increase in the Expression of IRS-4

Our results demonstrate how patients with ILC show an increased expression of IRS-4 in the tissue compared to IDC patients. We observed how the IRS-Score expression score was 2.522 ± 0.574 in ILC and 1.322 ± 0.479 in IDC, *** $p < 0.001$ (Figure 1A–C).

3.3. Patients with Invasive Lobular Carcinoma Show a Significant Increase in the Expression of C0X-2, Rb1 and Cyclin D1

In addition, we have observed how patients diagnosed with ILC show an increased expression of COX-2, Rb1 and Cyclin D1. In the case of COX-2, we observed how the IRS-Score expression score was 1.806 ± 0.605 in ILC and 1.533 ± 0.537 in IDC, * $p = 0.0267$ (Figure 2A–C).

For Rb1, we observed how the IRS-Score expression score was 2.000 ± 0.674 in ILC and 1.544 ± 0.620 in IDC, ** $p = 0.0022$ (Figure 3A–C). In the case of Cyclin D1, we observed how the IRS-Score expression score was 2.117 ± 0.527 in ILC and 1.639 ± 0.793 in IDC, ** $p = 0.0018$ (Figure 4A–C).

Figure 1. (**A**) IRS-Score for IRS-4 in patients diagnosed with invasive lobular carcinoma (ILC) and infiltrative ductal carcinoma (IDC). (**B,C**) Histological images of IRS-4 expression using immunohistochemical techniques in the breast tissue of patients diagnosed with ILC (**B**) and IDC (**C**). *** $p < 0.001$.

Figure 2. (**A**) IRS-Score for COX-2 in patients diagnosed with invasive lobular carcinoma (ILC) and infiltrative ductal carcinoma (IDC). (**B,C**) Histological images of COX-2 expression using immunohistochemical techniques in the breast tissue of patients diagnosed with ILC (**B**) and IDC (**C**). * $p < 0.05$.
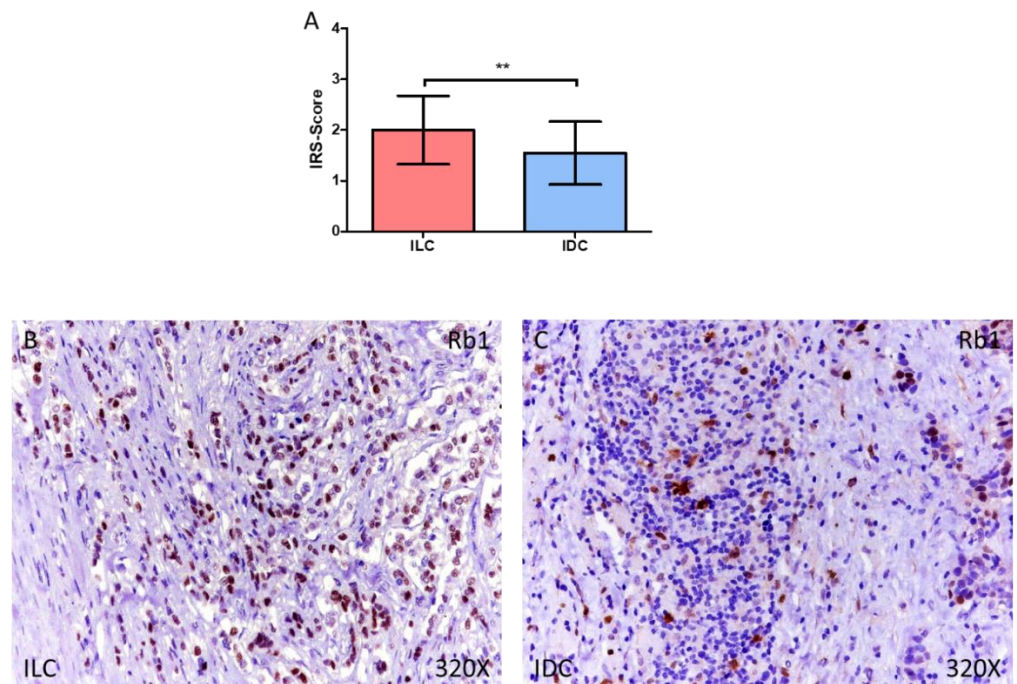
Figure 3. (**A**) IRS-Score for Rb1 in patients diagnosed with invasive lobular carcinoma (ILC) and infiltrative ductal carcinoma (IDC). (**B**,**C**) Histological images of Rb1 expression using immunohistochemical techniques in the breast tissue of patients diagnosed with ILC (**B**) and IDC (**C**). ** $p < 0.005$.
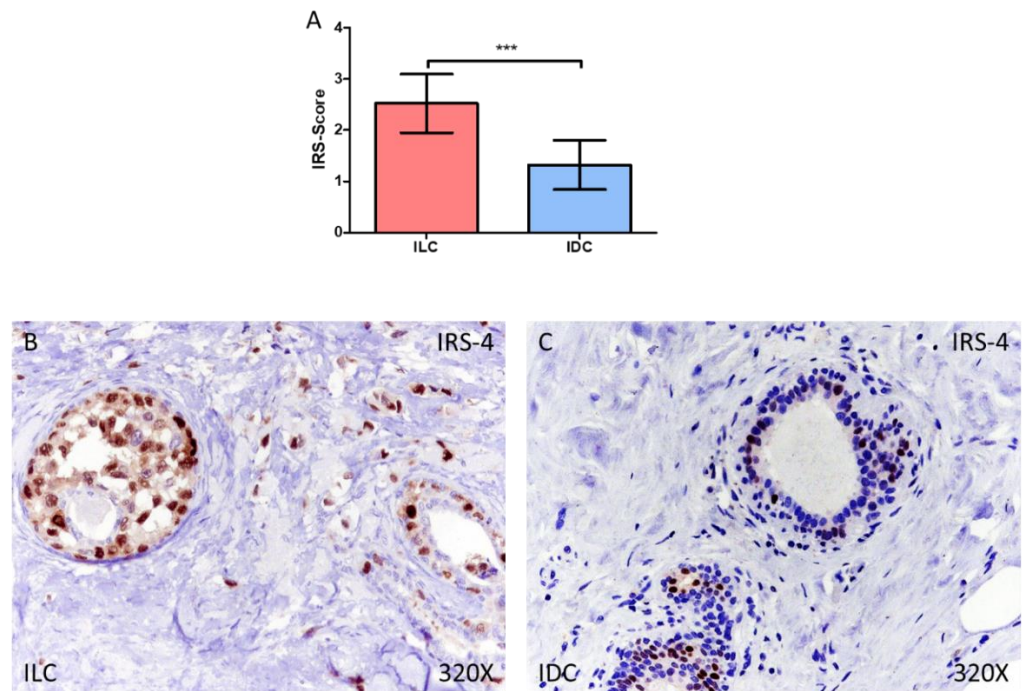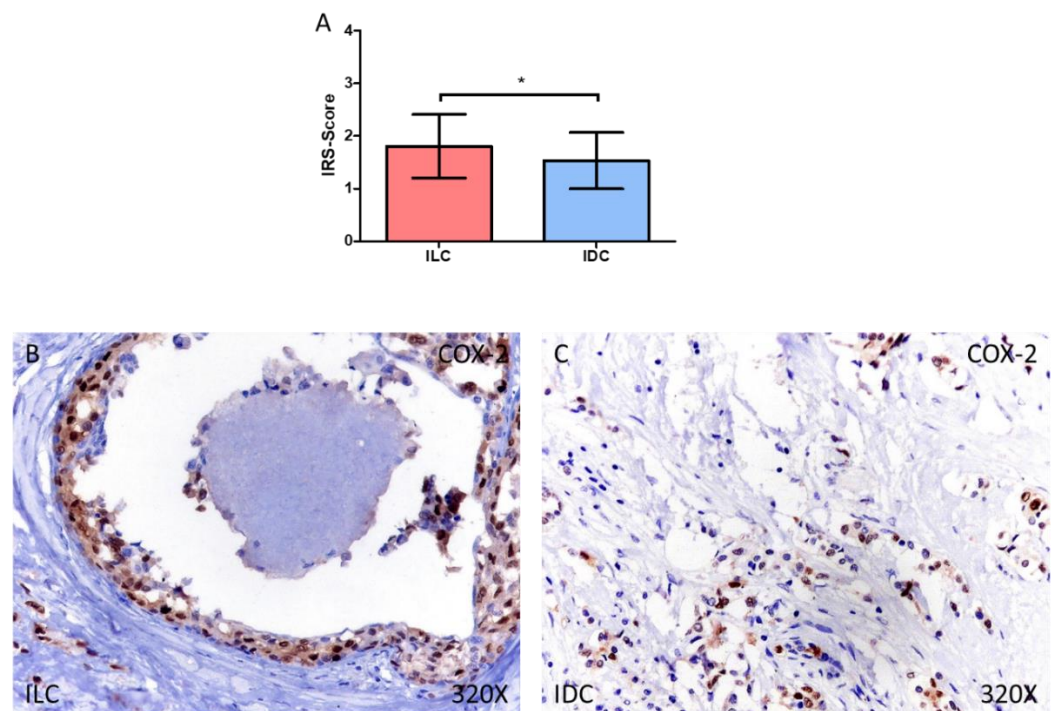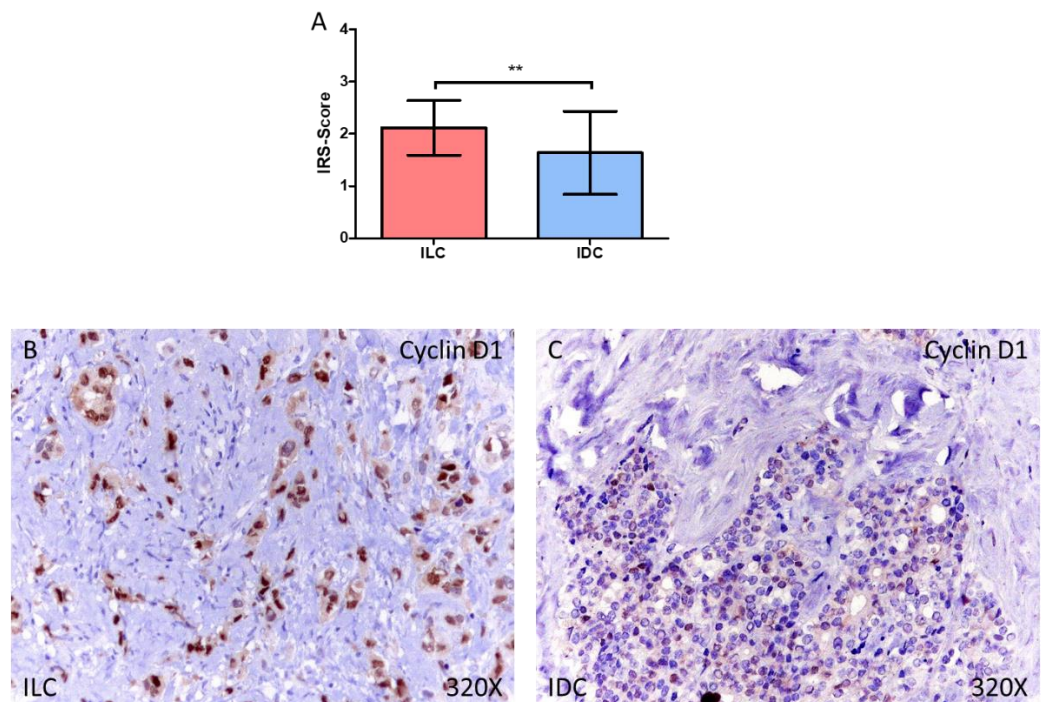

Figure 4. (**A**) IRS-Score for Cyclin D1 in patients diagnosed with invasive lobular carcinoma (ILC) and infiltrative ductal carcinoma (IDC). (**B**,**C**) Histological images of Cyclin D1 expression using immunohistochemical techniques in the breast tissue of patients diagnosed with ILC (**B**) and IDC (**C**). ** $p < 0.005$.

4. Discussion

The annual incidence and prevalence of BC are rising worldwide in the younger and elderly populations [24]. Despite the improvements achieved in early diagnosis, screening and survival, more knowledge is required to understand the biology of this cancer and

their specific types in order to develop better approaches for the clinical management of these patients. In this sense, our study gains further insights into the biology of ILC in comparison to IDC, aiding to explain some of the differences in the clinical presentation and histopathological features of these types of tumors.

ILCs are frequently associated with a poorer prognosis than IDCs, and the biological differences between both types of tumors have received significant attention. For instance, differences in the expression of genes and specific proteins/receptors have been reported among both groups [25]. IRS-4 was a pivotal marker overexpressed in ILC in comparison to IDC. To our knowledge, this is the first study demonstrating a possible pathophysiological role of this marker in these tumors. IRS-4 is an adaptor protein acting as a constitutive activator of critical cell transduction pathways in cancer, leading to the activation of the PI3K/Akt pathway collaborating with the actions of the human epidermal growth factor receptor 2 (HER2) [13]. HER2 is a central receptor involved in BC development and stratification. According to the absence or presence of HER2, estrogen receptor (ER) and progesterone receptor (PR), the molecular classification of breast tumors can be luminal A, luminal B, HER2+ enriched cells and triple negative BC. Luminal subtypes are ER/PR+, being luminal A subtype negative for HER2 and with low levels of ki67, whereas luminal B presents the HER2+ receptor and high levels of ki67. HER2+ enriched cells only present this marker, and triple negative BC lacks all of these receptors [26,27]. It is of note, that patients with ILC were HER2+, whereas IDC subjects did not show HER2+ expression. HER2+ ILC has distinct clinical characteristics and immune landscapes compared to IDC, and a poorer prognosis [28]. Hence, the augmentation of both IRS-4 and HER2+ may act synergically with the progression of ILC. Moreover, compelling evidence has established the central role of PI3K in the development of BC, being closely related to cell growth, proliferation, survival, motility, metastasis, metabolism and immune modulation [29]. In addition, previous studies have also found an association between the activation of PI3K/Akt and other pathways such as Ras-MAPK by IRS-4 with therapeutic resistance and tumor progression in lung cancer [13]. Thus, IRS-4 could mediate many of these processes in ILCs due to its hyperactivation of PI3K/Akt and other signaling pathways, aiding to explain the worse prognosis of these patients in comparison to IDC.

Likewise, the expression of IRS-4 was also related to the activation of Cyclin D1 and Rb1 in colorectal cancer, two markers also altered in our study [30]. Because of the increased expression of these components, it is likely that IRS-4 may collaborate with the overexpression of Cyclin D1 and Rb1. Cyclin D1 and Rb1 are major regulators of the cell cycle. In the case of Cyclin D1, prior research has detected a substantial dysregulation of this marker in several types of cancer [31]. In the field of BC, the overexpression of Cyclin D1 is associated with abnormalities in the cell cycle and a set of carcinogenic mechanisms in the breast, also mediating the effects of estrogen in this tissue [32]. Soslow et al. [33] claimed that 82% (23 out of 28) of ILCs exhibited a high expression of Cyclin D1 in comparison to the 54% of IDCs (18 out of 34). In agreement with previous studies, we have observed an increased expression of Cyclin D1 in ILCs in comparison to IDCs. In the case of Rb1, it may act as either a tumor suppressor or as promoting tumor growth [34]. It seems that the high expression of Rb1 is associated with a high proliferation of different invasive breast tumors [35]. Interestingly, the levels of Rb1 appear to be correlated with those of glucose transporter 1 (GLUT-1) in BC, suggesting a promising therapeutic approach using GLUT-1 inhibitors in patients with high Rb1 expression [36]. However, more studies are required to better understand the role of Cyclin D1 and Rb1 in lobular carcinoma before drawing any conclusions.

Last but not least, COX-2 is an enzyme ubiquitously involved in the mammary carcinogenesis. Its expression appears to be directly correlated with the stage, cancer progression, angiogenesis and metastasis [37]. Furthermore, COX-2-driven prostaglandin E2 (PGE-2) biosynthesis is related to a plethora of aggressive carcinogenic mechanisms in BC, having been proposed as a promising therapeutic target and also being associated with a poorer prognosis [17,38]. COX-2 can be released by cancer-associated fibroblasts, M2 macrophages

and cancer cells to the tumor microenvironment, inducing cancer stem cell-like activity and cell proliferation, angiogenesis, invasion, inflammation, metastasis and apoptotic resistance [39]. In consonance with our results, Holmes et al. [40] found that higher COX-2 expression was more observed in ILC than IDC, aiding to explain its worse prognosis. Other studies, however, found that COX-2 was highly expressed in both ILC and IDC, and the proportion of total COX-2 positive tumors range between studies [37,41]. These differences could be attributed to the different scoring systems and cut-off of immunohistochemistry, as these do not allow for the extraction of quantitative results, which may be a limitation of our study [42]. Similarly, COX-2 was also highly detected in lobular and ductal in situ carcinoma, being associated with an increased risk for developing subsequent invasive carcinomas [42,43], hence supporting the role of COX-2 in the early carcinogenesis as well.

5. Conclusions

Overall, we have found a differential molecular profile between ILC and IDC, showing an increased expression of IRS-4, COX-2, Cyclin D1 and Rb1 in favor of the former. Further studies are warranted in order to deepen exploration on the molecular and translational implications of these components, as well as to analyze a possible targeted therapy to improve the clinical management of these patients.

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Data Availability Statement: The datasets used and/or analyzed during the present study are available from the corresponding author on reasonable request.

Conflicts of Interest: The authors declare no conflict of interest.

References

1. Simon, A.; Robb, K. Breast Cancer. In *Cambridge Handbook of Psychology, Health and Medicine*, 2nd ed.; Cambridge University Press: Cambridge, UK, 2021; pp. 577–580. [CrossRef]
2. Bober, P.; Tomková, Z.; Alexovič, M.; Ropovik, I.; Sabo, J. The unfolded protein response controls endoplasmic reticulum stress-induced apoptosis of MCF-7 cells via a high dose of vitamin C treatment. *Mol. Biol. Rep.* **2019**, *46*, 1275–1284. [CrossRef] [PubMed]
3. Tao, Z.Q.; Shi, A.; Lu, C.; Song, T.; Zhang, Z.; Zhao, J. Breast Cancer: Epidemiology and Etiology. *Cell Biochem. Biophys.* **2015**, *72*, 333–338. [CrossRef] [PubMed]
4. Yousef, A.J.A. Male Breast Cancer: Epidemiology and Risk Factors. *Semin. Oncol.* **2017**, *44*, 267–272. [CrossRef] [PubMed]
5. Ataollahi, M.R.; Sharifi, J.; Paknahad, M.R.; Paknahad, A. Breast cancer and associated factors: A review. *J. Med. Life* **2015**, *8*, 6.
6. Sun, Y.S.; Zhao, Z.; Yang, Z.N.; Xu, F.; Lu, H.J.; Zhu, Z.Y.; Shi, W.; Jiang, J.; Yao, P.P.; Zhu, H.P. Risk Factors and Preventions of Breast Cancer. *Int. J. Biol. Sci.* **2017**, *13*, 1387. [CrossRef]
7. Łukasiewicz, S.; Czeczelewski, M.; Forma, A.; Baj, J.; Sitarz, R.; Stanisławek, A. Breast Cancer-Epidemiology, Risk Factors, Classification, Prognostic Markers, and Current Treatment Strategies-An Updated Review. *Cancers* **2021**, *13*, 4287. [CrossRef]
8. Oluogun, W.A.; Adedokun, K.A.; Oyenike, M.A.; Adeyeba, O.A. Histological Classification, Grading, Staging, and Prognostic Indexing of Female Breast Cancer in an African Population: A 10-Year Retrospective Study. *Int. J. Health Sci.* **2019**, *13*, 3.

9. Weigelt, B.; Horlings, H.M.; Kreike, B.; Hayes, M.M.; Hauptmann, M.; Wessels, L.F.A.; de Jong, D.; van de Vijver, M.J.; Van't Veer, L.J.; Peterse, J.L. Refinement of Breast Cancer Classification by Molecular Characterization of Histological Special Types. *J. Pathol.* **2008**, *216*, 141–150. [CrossRef]

10. Adachi, Y.; Ishiguro, J.; Kotani, H.; Hisada, T.; Ichikawa, M.; Gondo, N.; Yoshimura, A.; Kondo, N.; Hattori, M.; Sawaki, M.; et al. Comparison of Clinical Outcomes between Luminal Invasive Ductal Carcinoma and Luminal Invasive Lobular Carcinoma. *BMC Cancer* **2016**, *16*, 248. [CrossRef]

11. Du, T.; Zhu, L.; Levine, K.M.; Tasdemir, N.; Lee, A.V.; Vignali, D.A.A.; van Houten, B.; Tseng, G.C.; Oesterreich, S. Invasive Lobular and Ductal Breast Carcinoma Differ in Immune Response, Protein Translation Efficiency and Metabolism. *Sci. Rep.* **2018**, *8*, 7205. [CrossRef]

12. Ortega, M.A.; Fraile-Martínez, O.; Guijarro, L.G.; Casanova, C.; Coca, S.; Álvarez-Mon, M.; Buján, J.; García-Honduvilla, N.; Asúnsolo, Á. Á. The Regulatory Role of Mitochondrial MicroRNAs (MitomiRs) in Breast Cancer: Translational Implications Present and Future. *Cancers* **2020**, *12*, 2443. [CrossRef] [PubMed]

13. Hao, P.; Huang, Y.; Peng, J.; Yu, J.; Guo, X.; Bao, F.; Dian, Z.; An, S.; Xu, T.R. IRS4 Promotes the Progression of Non-Small Cell Lung Cancer and Confers Resistance to EGFR-TKI through the Activation of PI3K/Akt and Ras-MAPK Pathways. *Exp. Cell Res.* **2021**, *403*, 112615. [CrossRef] [PubMed]

14. Ikink, G.J.; Hilkens, J. Insulin Receptor Substrate 4 (IRS4) Is a Constitutive Active Oncogenic Driver Collaborating with HER2 and Causing Therapeutic Resistance. *Mol. Cell. Oncol.* **2017**, *4*, e1279722. [CrossRef] [PubMed]

15. Guijarro, L.G.; Sanmartin-Salinas, P.; Pérez-Cuevas, E.; Toledo-Lobo, M.V.; Monserrat, J.; Zoullas, S.; Sáez, M.A.; Álvarez-Mon, M.A.; Bujan, J.; Noguerales-Fraguas, F.; et al. Possible Role of IRS-4 in the Origin of Multifocal Hepatocellular Carcinoma. *Cancers* **2021**, *13*, 2560. [CrossRef] [PubMed]

16. Ortega, M.A.; Pekarek, L.; Garcia-Montero, C.; Fraile-Martinez, O.; Saez, M.A.; Asúnsolo, A.; Alvarez-Mon, M.A.; Monserrat, J.; Coca, S.; Toledo-Lobo, M.V.; et al. Prognostic Role of IRS-4 in the Survival of Patients with Pancreatic Cancer. *Histol. Histopathol.* **2022**, *9*, 18432. [CrossRef]

17. Jana, D.; Sarkar, D.K.; Ganguly, S.; Saha, S.; Sa, G.; Manna, A.K.; Banerjee, A.; Mandal, S. Role of Cyclooxygenase 2 (COX-2) in Prognosis of Breast Cancer. *Indian J. Surg. Oncol.* **2014**, *5*, 59. [CrossRef]

18. Barnes, D.M.; Gillett, C.E. Cyclin D1 in Breast Cancer. *Breast Cancer Res. Treat.* **1998**, *52*, 1–15. [CrossRef]

19. Robinson, T.J.W.; Liu, J.C.; Vizeacoumar, F.; Sun, T.; Maclean, N.; Egan, S.E.; Schimmer, A.D.; Datti, A.; Zacksenhaus, E. RB1 Status in Triple Negative Breast Cancer Cells Dictates Response to Radiation Treatment and Selective Therapeutic Drugs. *PLoS ONE* **2013**, *8*, e78641. [CrossRef]

20. Lakhani, S.R. *WHO Classification of Breast Tumours: WHO Classification of Tumours*; International Agency for Research on Cancer: Lion, France, 2019; Volume 2.

21. Ortega, M.A.; Fraile-Martínez, O.; Pekarek, L.; Alvarez-Mon, M.A.; Asúnsolo, Á.; Sanchez-Trujillo, L.; Coca, S.; Buján, J.; Álvarez-Mon, M.; García-Honduvilla, N.; et al. Defective expression of the peroxisome regulators PPARα receptors and lysogenesis with increased cellular senescence in the venous wall of chronic venous disorder. *Histol. Histopathol.* **2021**, *36*, 547–558. [CrossRef]

22. Ortega, M.A.; Pekarek, L.; Fraile-Martinez, O.; Garcia-Montero, C.; Saez, M.A.; Asúnsolo, A.; Alvarez-Mon, M.A.; Monserrat, J.; Ruiz-Llorente, L.; García-Honduvilla, N.; et al. Implication of ERBB2 as a Predictive Tool for Survival in Patients with Pancreatic Cancer in Histological Studies. *Curr. Oncol.* **2022**, *29*, 2442–2453. [CrossRef]

23. Ortega, M.A.; Romero, B.; Asúnsolo, Á.; Sainz, F.; Martinez-Vivero, C.; Álvarez-Mon, M.; Buján, J.; García-Honduvilla, N. Behavior of Smooth Muscle Cells under Hypoxic Conditions: Possible Implications on the Varicose Vein Endothelium. *BioMed Res. Int.* **2018**, *2018*, 7156150. [CrossRef] [PubMed]

24. Huang, J.; Chan, P.S.; Lok, V.; Chen, X.; Ding, H.; Jin, Y.; Yuan, J.; Lao, X.Q.; Zheng, Z.J.; Wong, M.C. Global Incidence and Mortality of Breast Cancer: A Trend Analysis. *Aging* **2021**, *13*, 5748. [CrossRef] [PubMed]

25. Bertucci, F.; Orsetti, B.; Nègre, V.; Finetti, P.; Rougé, C.; Ahomadegbe, J.C.; Bibeau, F.; Mathieu, M.C.; Treilleux, I.; Jacquemier, J.; et al. Lobular and Ductal Carcinomas of the Breast Have Distinct Genomic and Expression Profiles. *Oncogene* **2008**, *27*, 5359–5372. [CrossRef] [PubMed]

26. Schnitt, S.J. Will Molecular Classification Replace Traditional Breast Pathology? *Int. J. Surg. Pathol.* **2010**, *18*, 162–166. [CrossRef] [PubMed]

27. Sørlie, T.; Perou, C.M.; Tibshirani, R.; Aas, T.; Geisler, S.; Johnsen, H.; Hastie, T.; Eisen, M.B.; van de Rijn, M.; Jeffrey, S.S.; et al. Gene Expression Patterns of Breast Carcinomas Distinguish Tumor Subclasses with Clinical Implications. *Proc. Natl. Acad. Sci. USA* **2001**, *98*, 10869–10874. [CrossRef] [PubMed]

28. Chumsri, S.; Li, Z.; Shachner, T.; Advani, P.; Sideras, K.; Moreno-Aspitia, A.; Colon-Otero, G.; Knutson, K.L.; Nassar, A.; Perez, E.A.; et al. Outcome and Immune Landscape of HER2-Positive Invasive Lobular Carcinoma in the North Central Cancer Treatment Group (NCCTG) N9831 (Alliance) Trial. *J. Clin. Oncol.* **2021**, *39*, 535. [CrossRef]

29. Ortega, M.A.; Fraile-Martínez, O.; Asúnsolo, Á.; Buján, J.; García-Honduvilla, N.; Coca, S. Signal Transduction Pathways in Breast Cancer: The Important Role of PI3K/Akt/MTOR. *J. Oncol.* **2020**, *2020*, 9258396. [CrossRef]

30. Sanmartín-Salinas, P.; del Val Toledo Lobo, M.; Noguerales-Fraguas, F.; Londoño, M.T.; Jiménez-Ruiz, A.; Guijarro, L.G. Insulin Receptor Substrate-4 Is Overexpressed in Colorectal Cancer and Promotes Retinoblastoma-Cyclin-Dependent Kinase Activation. *J. Gastroenterol.* **2018**, *53*, 932–944. [CrossRef]

31. Qie, S.; Diehl, J.A. Cyclin D1, Cancer Progression, and Opportunities in Cancer Treatment. *J. Mol. Med.* **2016**, *94*, 1313–1326. [CrossRef]

32. Roy, P.G.; Thompson, A.M. Cyclin D1 and Breast Cancer. *Breast* **2006**, *15*, 718–727. [CrossRef]

33. Soslow, R.A.; Carlson, D.L.; Horenstein, M.G.; Osborne, M.P. A Comparison of Cell Cycle Markers in Well-Differentiated Lobular and Ductal Carcinomas. *Breast Cancer Res. Treat.* **2000**, *61*, 161–170. [CrossRef] [PubMed]

34. Pietiläinen, T.; Lipponen, P.; Aaltomaa, S.; Eskelinen, M.; Kosma, V.M.; Syrjänen, K. Expression of Retinoblastoma Gene Protein (Rb) in Breast Cancer as Related to Established Prognostic Factors and Survival. *Eur. J. Cancer* **1995**, *31A*, 329–333. [CrossRef]

35. Ceccarelli, C.; Santini, D.; Chieco, P.; Taffurelli, M.; Gamberini, M.; Pileri, S.A.; Marrano, D. Retinoblastoma (RB1) Gene Product Expression in Breast Carcinoma. Correlation with Ki-67 Growth Fraction and Biopathological Profile. *J. Clin. Pathol.* **1998**, *51*, 818. [CrossRef] [PubMed]

36. Zhang, X.; Pang, X.; Zhang, Z.; Liu, Q.; Zhang, H.; Xiang, Q.; Cui, Y. Co-Expression and Prognosis Analyses of GLUT1-4 and RB1 in Breast Cancer. *BMC Cancer* **2021**, *21*, 1026. [CrossRef]

37. Costa, C.; Soares, R.; Reis-Filho, J.S.; Leitão, D.; Amendoeira, I.; Schmitt, F.C. Cyclo-Oxygenase 2 Expression Is Associated with Angiogenesis and Lymph Node Metastasis in Human Breast Cancer. *J. Clin. Pathol.* **2002**, *55*, 429. [CrossRef]

38. Harris, R.E.; Casto, B.C.; Harris, Z.M. Cyclooxygenase-2 and the Inflammogenesis of Breast Cancer. *World J. Clin. Oncol.* **2014**, *5*, 677. [CrossRef]

39. Hashemi Goradel, N.; Najafi, M.; Salehi, E.; Farhood, B.; Mortezaee, K. Cyclooxygenase-2 in Cancer: A Review. *J. Cell. Physiol.* **2019**, *234*, 5683–5699. [CrossRef]

40. Holmes, M.D.; Chen, W.Y.; Schnitt, S.J.; Collins, L.; Colditz, G.A.; Hankinson, S.E.; Tamimi, R.M. COX-2 Expression Predicts Worse Breast Cancer Prognosis and Does Not Modify the Association with Aspirin. *Breast Cancer Res. Treat.* **2011**, *130*, 657–662. [CrossRef]

41. Singh-Ranger, G.; Salhab, M.; Mokbel, K. The Role of Cyclooxygenase-2 in Breast Cancer: Review. *Breast Cancer Res. Treat.* **2008**, *109*, 189–198. [CrossRef]

42. Hoellen, F.; Kelling, K.; Dittmer, C.; Diedrich, K.; Friedrich, M.; Thill, M. Impact of Cyclooxygenase-2 in Breast Cancer | Anticancer Research. *Anticancer Res.* **2011**, *31*, 4359–4367.

43. Perrone, G.; Zagami, M.; Santini, D.; Vincenzi, B.; Gullotta, G.; Morini, S.; Battista, C.; Guerriero, G.; Altomare, V.; Tonini, G.; et al. COX-2 Expression in Lobular in Situ Neoplasia of the Breast: Correlation with Histopathological Grading System According to the Tavassoli Classification. *Histopathology* **2007**, *51*, 33–39. [CrossRef] [PubMed]