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Article

Inositol (1,4,5)-Trisphosphate Receptors in Invasive Breast Cancer: A New Prognostic Tool?

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Simple Summary: The inositol-trisphosphate receptor (IP₃R) is a key player in physiological and pathological intracellular calcium signaling. The objective of the present study was to assess the putative value of the three IP₃R subtypes as prognostic biomarkers in breast cancer. We found that IP₃R3 is the most strongly expressed subtype in breast cancer tissue. Furthermore, IP₃R3 and IP₃R1 are significantly more expressed in invasive breast cancer tissue than in non-tumor tissue. In contrast to IP₃R1 and IP₃R2, the expression of IP₃R3 was positively correlated with prognostic factors including tumor size, regional node invasion, histologic grade, proliferation index, and hormonal status. By analyzing public databases, we found that the expression of all IP₃R subtypes is significantly correlated with the overall survival and disease-free survival of patients with breast cancer. We conclude that relative to the other two IP₃R subtypes, IP₃R3 expression is upregulated in breast cancer and is correlated with prognostic factors. We strongly believe that our results will open up new perspectives with regard to the link between IP₃R3s and breast cancer aggressiveness.

Abstract: Breast cancer is the leading cause of cancer death among women in worldwide and France. The disease prognosis and treatment differ from one breast cancer subtype to another, and the disease outcome depends on many prognostic factors. Deregulation of ion flux (especially Ca²⁺ flux) is involved in many pathophysiology processes, including carcinogenesis. Inside the cell, the inositol-trisphosphate receptor (IP₃R) is a major player in the regulation of the Ca²⁺ flux from the endoplasmic reticulum to the cytoplasm. The IP₃R3s (and particularly the IP₃R3 subtype) are known to be involved in proliferation, migration, and invasion processes in breast cancer cell lines. The objective of the present study was to evaluate the potential value of IP₃R3s as prognostic biomarkers in breast cancer. We found that expression levels of IP₃R3 and IP₃R1 (but not IP₃R2) were significantly higher in invasive breast cancer of no special type than in non-tumor tissue from the same patient. However, the IP₃R3 subtype was expressed more strongly than the IP₃R1 and IP₃R2 subtypes. Furthermore, the expression of IP₃R3 (but not of IP₃R1 or IP₃R2) was positively correlated with prognostic factors such as tumor size, regional node invasion, histologic grade, proliferation index, and hormone receptor status. In an analysis of public databases, we found that all IP₃R3s types are significantly associated with overall survival and progression-free survival in patients with breast cancer. We conclude that relative to the other two IP₃R subtypes, IP₃R3 expression is upregulated in breast cancer and is correlated with prognostic factors.

Keywords: inositol 1,4,5 trisphosphate; breast cancer; invasive prognostic marker

1. Introduction

In 2020, around 2.2 million new cases of breast cancer (BC) were diagnosed worldwide [1]—making this disease a major public health problem that affects 1 in 9 women at some point in life. Despite recent and constant progress in diagnosis and management, BC is the second deadliest cancer in women. However, the implementation of BC screening programs and the development of systemic treatments have reduced mortality and the incidence of metastatic cancer. Ninety percent of BC deaths are due to metastases [2,3]. Invasive breast carcinoma of no special type (IBC-NST) is the most frequent histologic subtype. Many prognostic criteria are applied when choosing an adjuvant treatment in BC; these criteria are variously clinical (tumor size, axillary lymph node invasion, remote metastases, etc.) and histologic (the Scarff-Bloom-Richardson (SBR) grade, hormone receptor (HmR) status, human epidermal growth factor 2 (HER2) expression, and the Ki67 index) [4–11]. BC is a complex, heterogeneous disease. Perou et al. developed a molecular classification for BC, which included luminal A, luminal B, HER2, and triple-negative (TN) subtypes [12]. The luminal A and B subtypes generally spread more slowly and recur less frequently than the HER2 and TN subtypes [13–15]. Moreover, disease-free survival rates are higher for metastatic luminal BC than for metastatic HER2 or TN BCs [13].

At BC diagnosis, 5% of patients have metastases [16]. Ten to 15% of BC patients will develop remote metastases in the first three years after diagnosis [16,17]. A third of patients without axillary lymph node involvement will still develop metastases [17]. Early-stage (non-metastatic) BC is curable; treatments include local surgery, brachytherapy, and systemic therapies such as chemotherapy and hormone therapy. The choice of treatment for BC is now driven by the tumor's histologic and molecular characteristics. The current policy of care for BC is tending towards less aggressive, more targeted, more personalized treatments [18]. Indeed, the indication for systemic chemotherapy is based on the risk of recurrence risk. This risk is determined by the above-mentioned risk factors and the BC's molecular classification. This therapeutic de-escalation has been made possible by the development of new tools for determining the indication for adjuvant chemotherapy. Genomic tests can now evaluate the 10-year risk of BC recurrence, which in turn can guide the treatment choice [18].

Despite the development of these new tools, some BCs will recur locally or will form metastases. It is therefore essential to find novel treatments and prognostic markers for even more effective patient care. The heterogeneity of BC is now taken into account when developing new therapies and prognostic factors, with a view to making treatment even more personalized.

Calcium ions (Ca^{2+} , from outside the cell or from within the endoplasmic reticulum (ER)) drive the development of metastases. When Ca^{2+} channels are open, the cytosolic Ca^{2+} concentration rises by a factor of 5 to 10 (from 100 nM to 500–1000 nM). Inside the cell, inositol (1,4,5)-trisphosphate (IP_3) generates part of this calcium signaling via the IP_3 receptor (IP_3R), which has three characterized subtypes: $\text{IP}_3\text{R1}$, $\text{IP}_3\text{R2}$, and $\text{IP}_3\text{R3}$. The three subtypes show 60% to 80% homology and are expressed ubiquitously. Interestingly, it was found that each subtype has a specific calcium release signature: strong Ca^{2+} oscillations through $\text{IP}_3\text{R2}$, weaker oscillations through $\text{IP}_3\text{R1}$, and monophasic transients through $\text{IP}_3\text{R3}$ [19].

The (dys)regulation of IP_3Rs expression and activity is involved in many oncogenic processes, including cancer cell growth, migration, proliferation, and survival. $\text{IP}_3\text{R1}$ is involved in apoptosis resistance in prostate cancer cells [20]. $\text{IP}_3\text{R2}$ is overexpressed in acute myeloid leukemia, and the level of expression is significantly correlated with poorer overall survival [21]. $\text{IP}_3\text{R3}$ is involved in glioblastoma cell migration and invasion [22], gastric cancer cell proliferation [23], and many other cancers (pancreatic, colonic, and renal) [24].

With regard to BC more specifically, the IP₃R3 overexpression induced by estradiol promotes MCF-7 BC cell growth in vitro [25]. IP₃R3 also regulates BC cell line proliferation via an interaction with BKCa voltage- and Ca²⁺-dependent K⁺ channels [26]. Moreover, IP₃R3 expression increases the migration capacity of human BC cells by shifting calcium oscillations towards a more sustained signature [27]. IP₃R3 is also able to coordinate the remodeling of the profilin cytoskeleton organization through the ARHGAP18/RhoA/mDia1/FAK pathway [28]. In human BC more specifically, IP₃R1 is not overexpressed. In contrast, Singh et al. found that IP₃R2 and IP₃R3 are more highly expressed in BC tissue than in non-tumor tissue [29]. In this context, we sought to (i) characterize the expression patterns of all IP₃R subtypes within human BC tissue and (ii) evaluate the putative correlation between the IP₃R3 expression level and the BC's proliferative/aggressive profile.

2. Materials and Methods

We conducted a prospective, observational study (named CARCINO study) at Amiens University Medical Center (Amiens, France). The study was approved by the local institutional review board (CPP Nord-Ouest II, Amiens, France; reference: ID-RCB 2015-A00537-42, dated July 2015). We included patients requiring surgery for an invasive BC with a greatest dimension > 15 mm. After resection, the pathologist collected one to three tumor tissue samples (size: 3 to 7 mm) depending on the tumor size. At least one sample per patient included was frozen immediately and stored at −80 °C for Western blot assays, any other samples were then used for immunohistochemistry. Informed consent was obtained from all subjects involved in the study.

2.1. Western Blot

The frozen tissue sample was mechanically dissociated in RIPA buffer (1% Triton 100×, 1% sodium deoxycholate, 150 mM NaCl, 2 mM EDTA, 5 mM PO₄Na₂/K, pH 7.2) supplemented with 0.8% protease inhibitor cocktail (Sigma Aldrich, IL, USA) in a special test tube for dissociation, using the GentleMACSTM system (Miltenyi Biotec, MA, USA) and the "Protein 01_01" protocol. After centrifugation at 15,000× g and 4 °C for 15 min, the protein concentration in the supernatant was assayed using the BCA method (Bio-Rad, CA, USA) according to the manufacturer's instructions. Protein samples were then denatured for 10 min at 95 °C in Laemmli sample buffer. Protein was separated by SDS-PAGE and transferred onto nitrocellulose membranes (Hybond, Wis, GE Healthcare, Chicago, IL, USA). Membranes were blocked in 1% BSA in TBS-T (0.1% Tween 20, 50 mM Tris HCl buffer, 150 mM NaCl, pH 7.5). Next, the membranes were incubated overnight at 4 °C with mouse monoclonal anti-IP₃R1 (1/500, Neuromab, CA, USA), rabbit monoclonal anti-IP₃R2 (1/250, Santa Cruz, CA, USA), mouse monoclonal anti-IP₃R3 (1/500, BD Biosciences, Switzerland), or goat polyclonal anti-actin (1/2500, Santa Cruz, CA, USA) primary antibodies diluted in 1% BSA in PBS-T. Actin primary antibody was used for loading control experiments. Membranes were then incubated for 1 h at RT with respective secondary antibodies (1/2500–1/5000; Santa-Cruz, CA, USA), developed using ECL substrate solution (ECL, RevelBolt Intense, Cell Signaling, Neve Yamin, Israel), exposed with the MF-ChemiBIS (DNR, bio-imaging systems, Neve Yamin, Israel) and analyzed using Quantity One software (Biorad, Hercules, CA, USA).

2.2. Immunohistochemistry

Formalin-fixed, paraffin-embedded sections of BC tissue (thickness: 2 to 3 μm) were deparaffinized in xylene and then rehydrated in ethanol. The endogenous peroxidase activity was blocked before the antigen retrieval. The cell conditioning solution CC1 (BenchMark XT, Ventana, Rotkreuz, Switzerland) was then used for antigen retrieval.

Immunohistochemical staining was carried out on a BenchMark ULTRA system (Ventana, Rotkreuz, Switzerland) using antibodies against the three IP₃Rs (1/50 for IP₃R1 (Neuromab, CA, USA), 1/50 for IP₃R2 (Santa Cruz, CA, USA) and 1/100 for

IP₃R3 (BD Biosciences, Switzerland)). This was followed by avidin–biotin–peroxidase complex treatment. The signals were developed using a chromogenic reaction with 3,3'-diaminobenzidine tetrahydrochloride (iVIEW DAB Detection Kit, Ventana). The tissues were counterstained with hematoxylin. All antibodies were certified for immunohistochemical use. All experiments included a negative control (without the primary antibody).

The results were rated independently by two experienced investigators (PR and AF), using a Leica inverted microscope. The staining intensity score ranged from 0 to 3 (0 = no immunostaining; 1 = weak immunostaining; 2 = moderate immunostaining; 3 = strong immunostaining), and the percentage of stained cells was also recorded. An IP₃R immunohistochemical (IH) expression score was then attributed for each tissue sample by multiplying the intensity score by the percentage of stained cells. The IH expression score therefore ranged from 0 (lowest) to 3 (highest).

We also used immunohistochemistry to assess the molecular subtype. This classification is essentially based on positivity for (and the percentage expression of) HmRs (the ER, in particular), HER2, and Ki67 (Table 1).

Table 1. Breast cancer molecular subtype. ER: estrogen receptor, PR: progesterone receptor.

	ER	PR	HER2	Ki67 Index
Luminal A	+	+	–	Low
Luminal B Her2–	+	+	–	High
Luminal B Her2+ Her2	+	+	+	High
Her2	–	–	+	High
Triple negative	–	–	–	High

2.3. Survival Analysis

Survival analysis was conducted using the SurvExpress online tool (Available in bioinformatica.mty.itesm.mx/SurvExpress). Expression levels of the individual genes (*ITPR1*, *ITPR2*, and *ITPR3*) and the combined signature were analyzed using SurvExpress and the optimized Maximize algorithm, which attributes a minimum *p*-value to a risk group. The hazard ratio (HR) [95% confidence interval (CI)] was also evaluated. Five datasets were used: the “Breast cancer recurrence data, 9 datasets from 7 authors” (1561 patients), “Breast cancer Meta-base: 10 cohorts 22K gene” (1888 patients), “Breast Invasive Carcinoma TCGA” (502 patients), “Miller Bergh Breast GSE3494-GPL96” (236 patients) and “BRCA-TCGA Breast invasive carcinoma—July 2016” (962 patients).

2.4. Statistical Analyses

In a descriptive analysis, normally distributed quantitative variables were quoted as the mean ± standard error of the mean (SEM). Pairs of mean values were compared using a non-parametric Mann–Whitney test. A non-parametric Kruskal–Wallis test was used to compare means of more than two groups. The threshold for statistical significance was set to *p* < 0.05.

3. Results

3.1. Study Population

Between 1 November 2015 and 1 November 2018, 52 patients with IBC-NST treated at Amiens University Medical Center were included in the study. An additional 15 IBC-NST samples from patients at the Jean Godinot Institute cancer center (Reims, France) were included. Non-tumor tissue from the same patient was available for each BC tissue sample. The clinical and histologic characteristics are summarized in Table 2.

Table 2. Characteristics of the cohort of Invasive breast carcinoma of no special type (IBC-NST) samples from the CARCINO study and the Jean Godinot Institute cancer center. Data are quoted as the mean \pm SD or n (%). BMI: body mass index, T: tumor size (T1: \leq 20 mm; T2: 20–50 mm; T3: 50 mm). N: regional lymph nodes (N0: no lymph node invasion, N+: lymph nodes invaded). HmR: hormone receptor. SBR: Scarff-Bloom-Richardson.

		CARCINO IBC-NST Samples (n = 52)	Jean Godinot Institute IBC-NST Samples (n = 15)
		n (%)	n (%)
Age		57 \pm 1.7	65.3 \pm 3
BMI		27.1 \pm 0.75	28.7 \pm 2.4
TNM	T1	16 (30.7)	4 (26.7)
	T2	31 (59.6)	8 (53.3)
	T3	5 (9.7)	3 (20)
	N0	25 (48.1)	8 (53.3)
	N+	27 (51.9)	7 (46.7)
HmR+		43 (82.7)	12 (80)
HER2+++		12 (23.1)	2 (13.3)
Triple-negative		7 (13.5)	2 (13.3)
SBR grade	1	6 (11.5)	2 (13.3)
	2	26 (50)	3 (20)
	3	20 (38.5)	10 (66.7)
Ki67 > 20%		26 (50)	10 (66.7)

3.2. IP₃Rs and Invasive Breast Carcinoma of No Special Type

First, we used Western blotting to evaluate the expression of each IP₃R subtype in IBC-NST samples and non-tumor tissue samples. We found that both IP₃R1 and IP₃R3 expression were significantly higher in BC tissue than in non-tumor tissue (IP₃R1; 1.59 \pm 0.04 ($N = 26$) vs. 1 \pm 0.03 ($N = 12$), respectively; $p = 0.02$; IP₃R3: 3.37 \pm 0.14 ($N = 29$) vs. 1 \pm 0.02 ($N = 12$), respectively; $p < 0.0001$) (Figure 1A). IP₃R3 expression was three times greater in BC tissue than in non-tumor tissue. In contrast, there was no difference in IP₃R2 expression between BC tissue and non-tumor tissue (1.05 \pm 0.06 ($N = 25$) vs. 1 \pm 0.08 ($N = 13$), respectively; $p = 0.8$) (Figure 1A). The same results were obtained when considering the IH expression score. Indeed, the IP₃R1 IH expression score was significantly higher in BC tissue than in non-tumor tissue (0.75 \pm 0.14 ($N = 40$) vs. 0.39 \pm 0.14 ($N = 18$), respectively; $p = 0.05$); the same was true for the IP₃R3 IH expression score (1.48 \pm 0.12 ($N = 41$) vs. 0.22 \pm 0.08 ($N = 17$), respectively; $p < 0.0001$) (Figure 1B). In contrast, there was no difference in the IP₃R2 IH expression score between BC tissue and non-tumor tissue (1.64 \pm 0.11 ($N = 30$) and 1.43 \pm 0.19 ($N = 18$), respectively; $p = 0.3$) (Figure 1B). Taken together, our results showed that IP₃R1 and IP₃R3 are overexpressed in IBC-NST, but not IP₃R2.

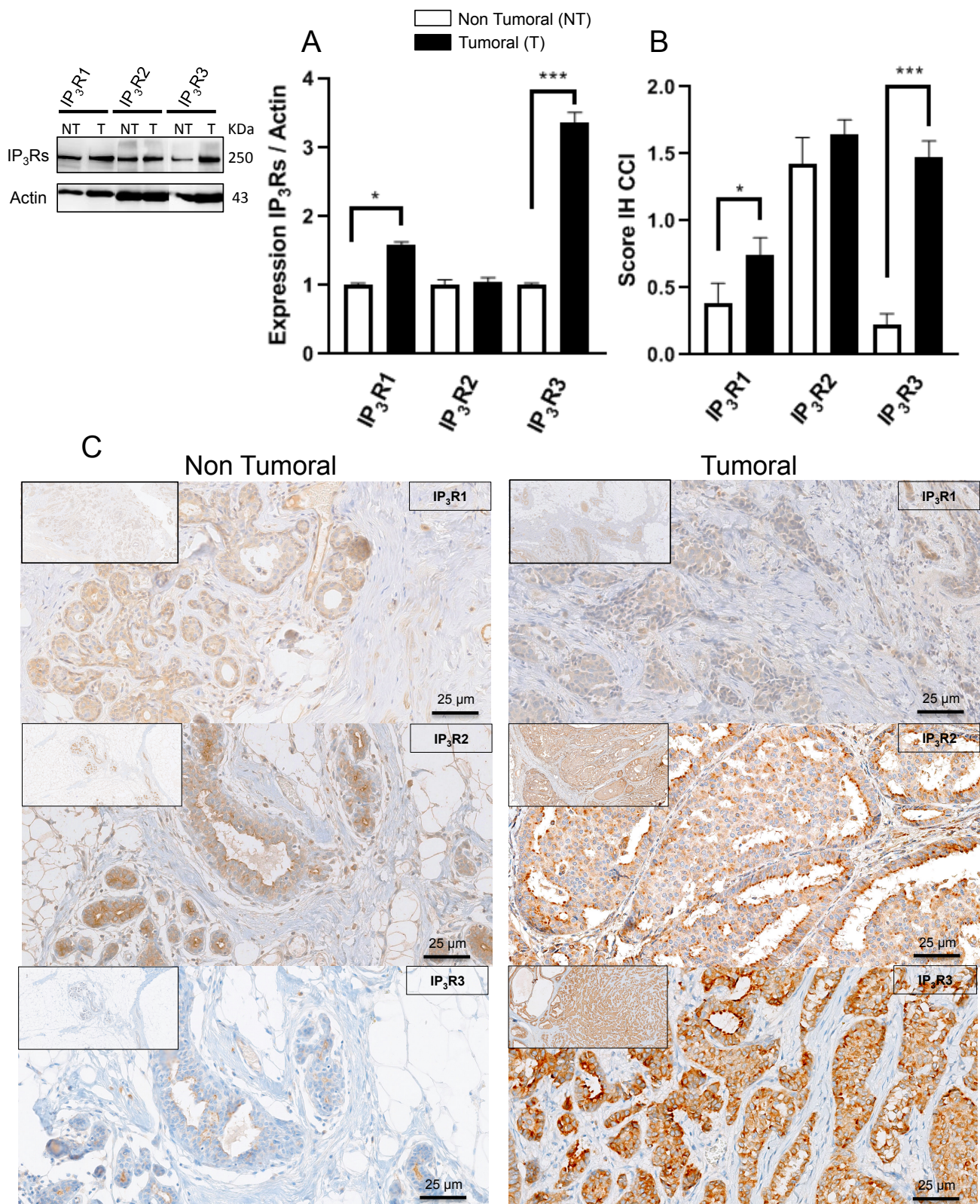


Figure 1. IP₃R expression in BC tissue and non-tumor tissue. The relative expression levels of IP₃R1 and IP₃R3 are significantly higher in BC tissue than in non-tumor tissue; this difference was not observed for IP₃R2 (A). The same results were obtained when considering the IH expression score (B). (A) IP₃R relative expression in IBC-NST, in a Western blot (T: tumor tissue; NT: non-tumor tissue). (B) The IP₃R IH expression score in IBC-NST (T: tumor tissue; NT: non-tumor tissue). (C) A representative IH image (magnification: 200 X; insert: 800 X). * $p < 0.05$; *** $p < 0.001$.

3.3. IP₃R Expression and Predictive Factors

After having found, in Western blot and in IH, that IP₃R1 and IP₃R3 were overexpressed in IBC-NST, we next sought to establish a link between IP₃R expression on the one hand and predictive factors for survival and recurrence in BC patients on the other.

3.4. IP₃R Subtype IH Expression and Tumor Size

Tumor size is a major risk factor for local or remote BC recurrence; the larger the tumor, the greater the risk [30–33]. We compared the IH expression score for the three subtypes, as a function of the tumor size. We also studied the IH expression of IP₃R2, even though its expression levels were similar in the paired tumor and non-tumor samples.

In BCs less than 20 mm in size (T1), there were no statistically significant differences in the IP₃R1, IP₃R2, and IP₃R3 IH expression scores (IH expression score in T1 BCs: 1.08 ± 0.22 ($N = 10$) for IP₃R1; 1.67 ± 0.21 ($N = 9$) for IP₃R2 and 1.36 ± 0.3 ; $p = 0.33$ ($N = 10$) for IP₃R3) (Figure 2A). In T2 BCs (20–50 mm) and T3 BCs (>50 mm), the IP₃R2 and IP₃R3 IH expression scores were significantly higher than the IP₃R1 IH expression score (IH expression score in T2 BCs: 0.73 ± 0.18 ($N = 24$) for IP₃R1 vs. 1.63 ± 0.14 ($N = 19$); $p = 0.0001$ for IP₃R2 and 1.4 ± 0.14 ($N = 25$); $p = 0.004$ for IP₃R3. IH expression score in T3 BCs: 0.18 ± 0.12 ($N = 5$) for IP₃R1 vs. 1.8 ± 0.2 ($N = 3$); $p = 0.04$ for IP₃R2 and 1.92 ± 0.32 ($N = 5$); $p = 0.008$ for IP₃R3) (Figure 2B,C). The IH expression of IP₃R2 was independent of tumor size. Thus, large tumor size was more closely related to IP₃R3 expression than to IP₃R1 expression.

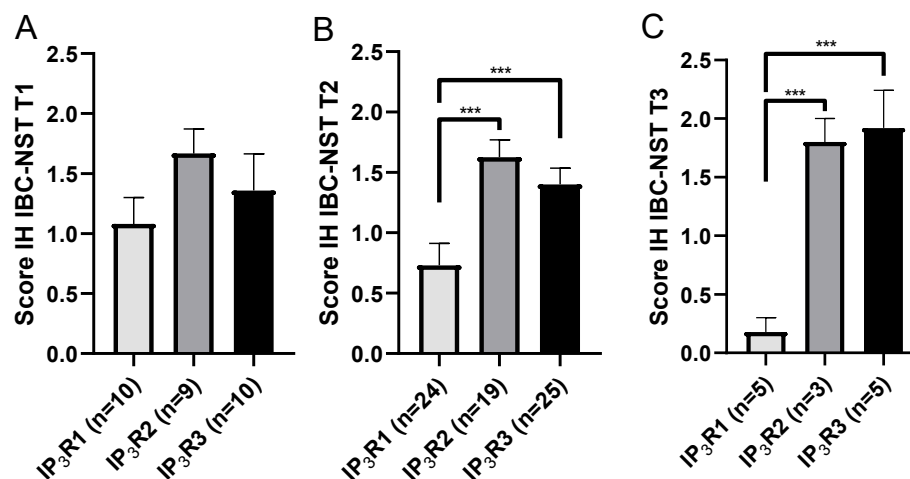


Figure 2. The IP₃R IH expression score, as a function of tumor size (A–C). The IP₃R IH expression scores did not differ significantly in T1 BC (A). The IP₃R2 and IP₃R3 IH expression scores were significantly higher than IP₃R1 score in T2 and T3 BCs (B,C). *** $p < 0.001$.

3.5. IP₃Rs and Lymph Node Involvement

Regional lymph node involvement is a risk factor for local and remote recurrence [30,31]. We compared IBC-NST with (N+) and without (N0) regional lymph node involvement with regard to the IH expression score for each of the three subtypes.

Only the IP₃R3 IH expression score was greater (by a factor of 1.3) in N+ BCs (1.72 ± 0.16 ($N = 20$)) than in N0 BCs (1.3 ± 0.16 ($N = 21$); $p = 0.08$) (Figure 3A). The IP₃R1 and IP₃R2 IH expression scores were similar when comparing N0 and N+ samples. In N0 BCs, the IH expression score was 0.77 ± 0.14 ($N = 20$) for IP₃R1 vs. 1.78 ± 0.17 ($N = 16$; $p < 0.0001$) for IP₃R2, and 1.3 ± 0.16 ($N = 21$; $p = 0.03$) for IP₃R3. In N+ BCs, the IH expression score was 0.73 ± 0.21 ($N = 20$) for IP₃R1, vs. 1.49 ± 0.13 ($N = 14$; $p = 0.001$) for IP₃R2, and 1.72 ± 0.16 ($N = 20$; $p = 0.001$) for IP₃R3. (Figure 3B). Moreover, the IP₃R1 IH expression score was significantly lower than the IP₃R2 and IP₃R3 IH expression scores in both N0 and N+ BCs (Figure 3B). Thus, IP₃R3 appeared to be specifically related to lymph node involvement in IBC-NST.

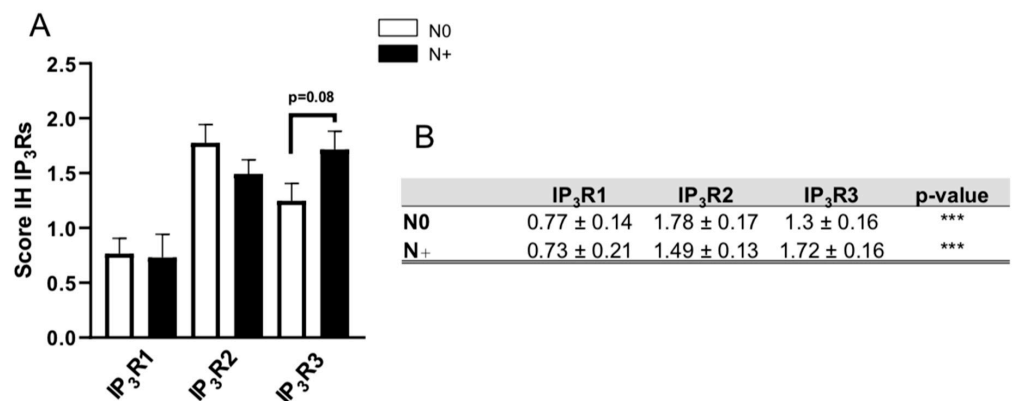


Figure 3. The IP₃R IH expression scores as a function of lymph node status (A,B). *** $p < 0.001$.

3.6. IP₃Rs, Histology Grades, and the Ki67 Proliferation Index

The Ki67 proliferation index and the histologic (SBR) grade are risk factors for BC recurrence: the higher the index or grade, the greater the risk of recurrence [5,30–33]. We therefore compared the SBR histologic grade and Ki67 index with regard to the three subtypes IH expression scores. We found that the IH expression scores for IP3R2 and IP3R3 were significantly higher than that for IP3R1 in grade III SBR samples and samples with a Ki67 index greater than 20% (Figure 4A,B). The IH expression score in grade 3 samples was 0.46 ± 0.12 ($N = 16$) for IP3R1 vs. 1.47 ± 0.26 ($N = 9$; $p = 0.0006$) for IP3R2 and 1.64 ± 0.19 ($N = 17$; $p < 0.0001$) for IP3R3. (B) The IH expression score in samples with a Ki67 index $> 20\%$ was 0.59 ± 0.2 ($N = 20$) for IP3R1 vs. 1.75 ± 0.15 ($N = 13$; $p < 0.0001$) for IP3R2 and 1.59 ± 0.18 ($N = 21$; $p < 0.0001$) for IP3R3 (Figure 4A,B). In both settings, there was no difference between the IP3R2 and IP3R3 IH expression scores. Given that IP3R2 is not overexpressed in BC tissue, IP3R3 expression is thus closely related to BC with a poor prognosis.

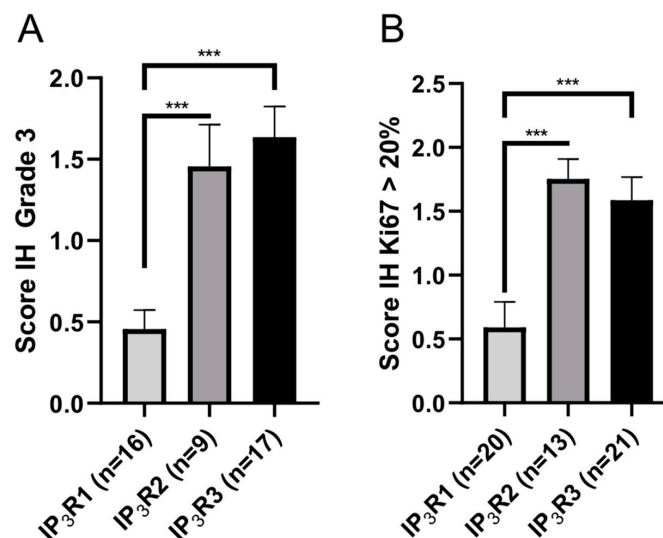


Figure 4. The IP₃R IH expression scores in SBR grade 3 IBC-NST (A) and IBC-NST with a high Ki67 proliferation index (B). *** $p < 0.001$.

3.7. IP₃Rs and the Molecular Classification of BCs

At present, clinicians typically classify carcinomas into five subtypes (luminal A, luminal B HER2⁻, luminal B HER2⁺, HER2, and TN) on the basis of histologic and molecular characteristics, which notably include expression of the estrogen receptor (ER), progesterone receptor (PR), HER2, and Ki67. Tumors that are ER⁺ and/or PR⁺ are referred to as HmR⁺. The prognosis, disease aggressiveness, and risk of remote metastasis and relapse vary from one subtype to another. In fact, the luminal A subtype has the best prognosis [34].

We assessed the three IP₃R IH expression scores in the five BC molecular subtypes. In luminal A BC, there were no significant differences in the IH expression scores for the three IP₃R subtypes (IH expression scores in luminal A BC: 0.97 ± 0.17 ($N = 17$) for IP₃R1, vs. 1.61 ± 0.16 ($N = 14$) for IP₃R2 and: 1.33 ± 0.17 ($N = 17$) for IP₃R3; $p = 0.2$) (Figure 5A). In luminal B and TN samples, the IH expression score was significantly lower for IP₃R1 than for IP₃R2 and IP₃R3 (IH expression scores in luminal B BC: 0.62 ± 0.21 ($N = 14$) for IP₃R1, vs. 1.54 ± 0.18 ($N = 9$; $p = 0.002$) for IP₃R2 and 1.54 ± 0.18 ($N = 15$; $p = 0.0002$) for IP₃R3 and IP₃R1 IH expression scores in TN BC: 0.51 ± 0.36 ($N = 8$) for IP₃R1 vs. 1.97 ± 0.26 ($N = 6$; $p = 0.01$) for IP₃R2 and 1.48 ± 0.32 ($N = 8$; $p = 0.02$) for IP₃R3) (Figure 5B,C). In luminal B HER2- samples, the IH expression score was significantly higher for IP₃R2 than for IP₃R1 (IH expression scores in luminal B HER2-: 0.66 ± 0.42 ($N = 7$) for IP₃R1, vs. 1.67 ± 0.17 ($N = 6$; $p = 0.04$) for IP₃R2 and 1.3 ± 0.31 ($N = 7$) for IP₃R3). In luminal B HER2+ samples, the IH expression score was significantly higher for IP₃R3 than for IP₃R1 (IP₃R1 IH expression scores in luminal B HER2+: 0.56 ± 0.15 ($N = 5$) for IP₃R1, vs. 1.77 ± 0.23 ($N = 6$; $p = 0.004$) for IP₃R3 and 1.38 ± 0.38 ($N = 4$) for IP₃R2) (Figure 5D,E). There were no differences between the IP₃R2 and IP₃R3 IH expression scores in any of the subtypes. This finding suggests that IP₃R3 is associated with a more aggressive disease profile for IBC-NST.

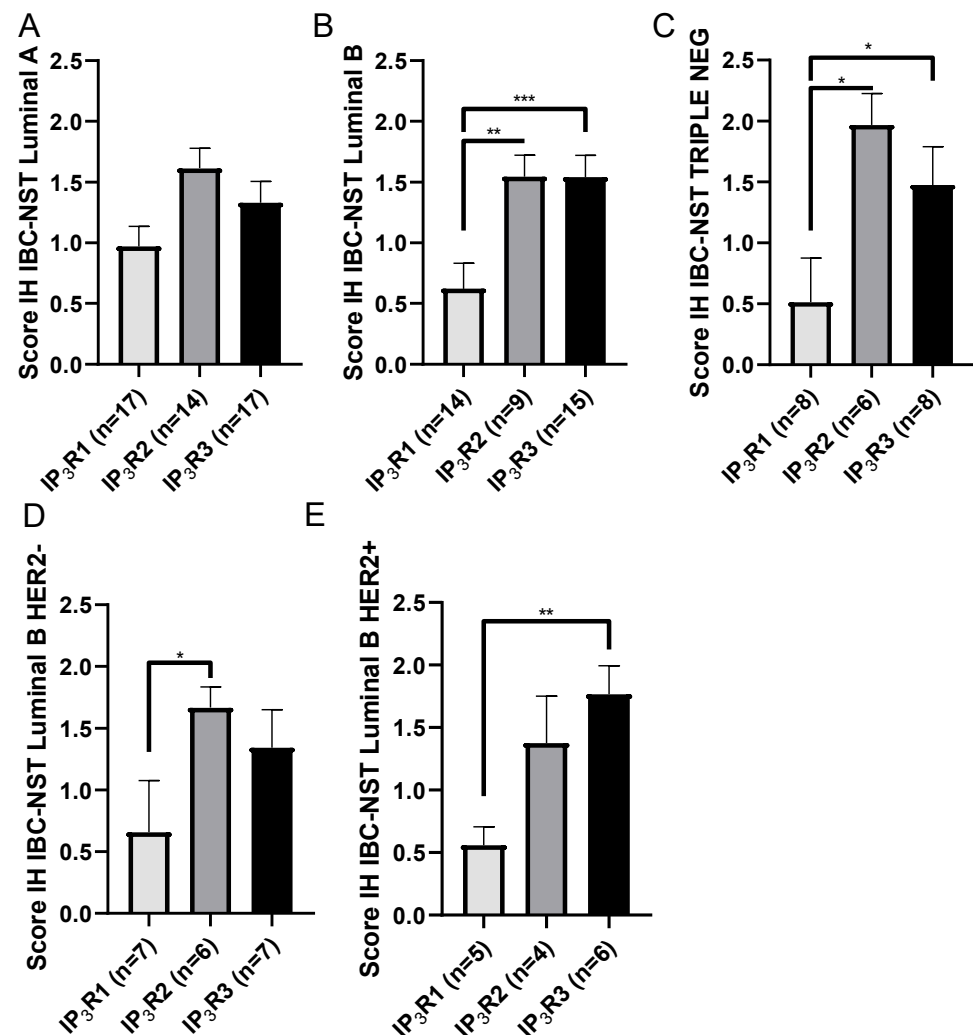


Figure 5. IP₃R expression score as a function of the BC molecular subtype. (A) Luminal A, (B) luminal B, (C) triple negative, (D) luminal B HER2-, and (E) luminal B HER2+ BC. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

3.8. Expression of IP₃Rs in Non-Tumor Tissue

We also compared the expression of IP₃Rs in the non-tumor tissue. Our results, obtained both by IH and Western blot, show that IP₃R2 is strongly expressed in contrast to IP₃R1 and IP₃R3. IH expression score: IP₃R2 = 1.43 ± 0.19 ($N = 18$) vs. 0.39 ± 0.14 ($N = 18$); $p = 0.0001$ for IP₃R1 and 0.22 ± 0.08 ($N = 17$); $p < 0.0001$ for IP₃R3. The IP₃R2 relative expression score is 0.65 ± 0.08 ($N = 13$) vs. 0.24 ± 0.03 ($N = 12$); $p < 0.0001$ for IP₃R1 and 0.25 ± 0.03 ($N = 12$); $p < 0.0001$ for IP₃R3. Indeed, the relative expression and IH expression scores are significantly higher for IP₃R2 than for IP₃R1 or IP₃R3 (Figure 6A,B). Thus, in contrast to IP₃R3, IP₃R2 appears as a highly expressed IP₃R in non-tumoral as in tumoral breast tissue.

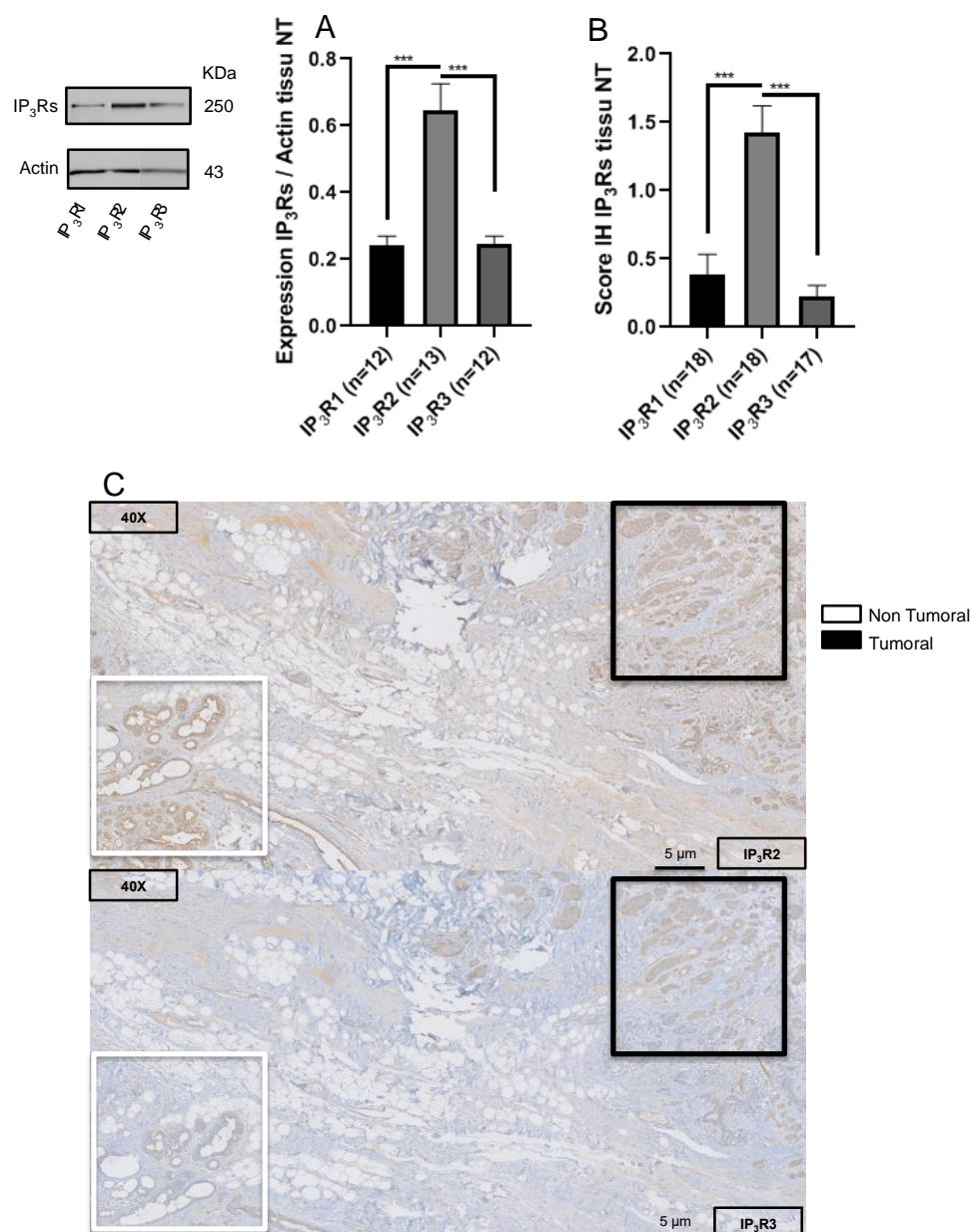


Figure 6. IP₃R expression in non-tumor tissue. (A) Western blot analysis of the relative expression of IP₃R1/2/3. (B) The IH expression score for IP₃R1/2/3. (C) Representative IH images. *** $p < 0.001$.

3.9. IP₃Rs and Patient Survival

We next sought to establish whether or not the expression of the three IP₃R subtypes was correlated with the patients' survival. We compared survival data and the HR for

predefined populations, such as high-risk and low-risk groups for the three receptor subtypes (Figure 7). We used the SurvExpress website’s optimized algorithm to generate risk groups by sorting according to the prognostic index (a higher IP₃R value for a higher risk) and splitting the patients into cohorts where the *p*-value was the lowest [35].

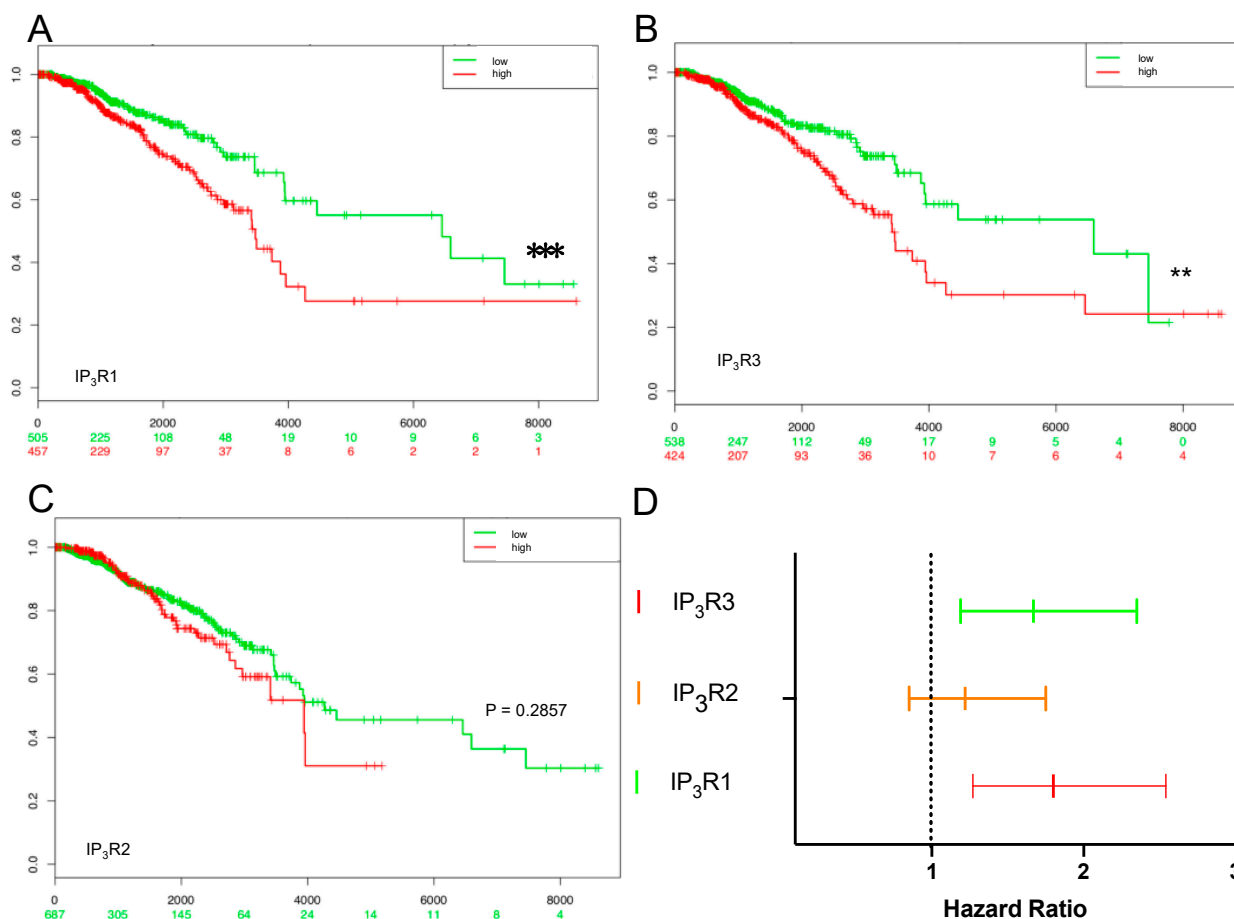


Figure 7. Analysis of the association between IP₃R expression and overall survival, after application of the SurvExpress tool to the “BRCA-TCGA Breast invasive carcinoma—July 2016” database (comprising 962 BC samples). (A,B) IP₃R1 expression and IP₃R3 expression were significantly associated with poor overall survival ($p = 0.0009$ and $p = 0.003$). (C) IP₃R2 expression was not significantly associated with poor overall survival ($p = 0.286$). (D) The HR for each IP₃R appears in an analysis of the “Breast invasive carcinoma—July 2016” database. ** $p < 0.01$; *** $p < 0.001$.

We chose to analyze five databases (listed in the “Survival analysis” section of the Methods) with the largest number of documented patients. In the “BRCA-TCGA Breast invasive carcinoma—July 2016” database, IP₃R1 and IP₃R3 expression were significantly associated with poor overall survival (Figure 7A,B). The results for IP₃R2 were not statistically significant (Figure 7C). IP₃R1 gave a higher HR (1.8; 95%CI [1.27–2.54]; $p = 0.0009$) than IP₃R3 (1.67; 95%CI [1.19–2.35]; $p = 0.003$) or IP₃R2 (1.22; 95%CI [0.85–1.75]; $p = 0.286$) (Figure 7D). IP₃R1 and IP₃R3 expression were significantly associated with poor overall survival in 1358 and 1700 patients, respectively (Table 3), whereas IP₃R2 expression was associated with poor overall survival in only 434 patients (Table 3).

Table 3. Statistically significant correlations between IP₃R subtype expression and survival. IP₃R3 and IP₃R1 expression levels were significantly associated with worse overall survival in three datasets (comprising 1358 patients (A) and 1700 patients (B), respectively), and IP₃R2 was significantly associated with worse overall survival in two datasets (comprising 434 patients) (C). Expression of the three IP₃R subtypes was significantly associated with worse recurrence-free survival in two datasets (comprising 3449 patients) (D). HR: hazard ratio. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$.

A			
Datasets—IP ₃ R3 and Overall Survival	N: Low-Risk Group vs. High-Risk Group	HR [95%CI]	<i>p</i> -Value
Breast—Breast cancer recurrence data, 9 datasets from 7 authors	198; 92 vs. 106	1.74 [1.23–2.46]	**
Breast—Breast Cancer Metabase:10 cohorts 22K genes	198; 86 vs. 112	1.44 [1.02–2.02]	*
Breast—BRCA-TCGA Breast Invasive Carcinoma—July 2016	962; 538 vs. 424	1.67 [1.19–2.35]	**
B			
Datasets—IP ₃ R1 and Overall Survival	N: Low-Risk Group vs. High-Risk Group	HR [95%IC]	<i>p</i> -Value
Breast—Breast Invasive Carcinoma TCGA	502; 376 vs. 126	1.87 [1–3.19]	*
Breast—Miller Bergh Breast GSE3494—GPL96	236; 45 vs. 191	4.64 [1.45–14.87]	**
Breast—BRCA—TCGA Breast invasive carcinoma—July 2016	962; 505 vs. 457	1.8 [1.27–2.54]	***
C			
Datasets—IP ₃ R2 and Overall Survival	N: Low-Risk Group vs. High-Risk Group	HR [95%IC]	<i>p</i> -Value
Breast—Breast Cancer Metabase:10 cohorts 22K genes	198; 167 vs. 31	1.61 [1.03–2.53]	*
Breast—Miller Bergh Breast GSE3494—GPL96	236; 45 vs. 191	4.64 [1.45–14.87]	**
D			
Datasets—Disease-Free Survival	N: Low-Risk Group vs. High-Risk Group	HR [95%IC]	<i>p</i> -Value
IP ₃ R3—Breast cancer recurrence data, 9 datasets from 7 authors	1561; 967 vs. 594	1.28 [1.08–1.51]	**
IP ₃ R3—Breast Cancer Metabase:10 cohorts 22K genes	1888; 1407 vs. 481	1.25 [1.05–1.49]	*
IP ₃ R2—Breast cancer recurrence data, 9 datasets from 7 authors	1561; 1194 vs. 367	1.27 [1.05–1.53]	*
IP ₃ R2—Breast Cancer Metabase:10 cohorts 22K genes	1888; 1614 vs. 274	1.36 [1.11–1.67]	**
IP ₃ R1—Breast cancer recurrence data, 9 datasets from 7 authors	1561; 842 vs. 719	1.43 [1.21–1.69]	***
IP ₃ R1—Breast Cancer Metabase:10 cohorts 22K genes	1888; 1113 vs. 775	1.48 [1.27–1.73]	***

Whenever possible, we evaluated the association between IP₃RS expression and disease-free survival (Table 3); data were available for the “Breast cancer recurrence data, 9 datasets from 7 authors” and “Breast cancer Meta-base: 10 cohorts 22K gene” databases, comprising a total of 3449 samples. IP₃R expression was significantly associated with poor disease-free survival (Table 3).

4. Discussion

Our present results evidenced different IP₃R subtype expression profiles in BC. IP₃R3 was most overexpressed in IBC-NST and was correlated with clinical features such as

tumor size and grade, regional node invasion, proliferation index, and HmR status. IP₃R1 was also overexpressed, albeit to a lesser extent than IP₃R3; however, the expression of IP₃R1 was inversely correlated with tumor size and did not vary as a function of the other prognostic markers. IP₃R2 was more strongly expressed than the other two subtypes in non-tumor tissue without being overexpressed in BC tissue compared to non-tumor tissue. Interestingly, IP₃R2 expression remained high regardless of tumor size, lymph node involvement, histologic grade, tKi67 index, and BC molecular classification. Moreover, we found that high IP₃R expression was significantly associated with worse overall survival among treated patients.

IP₃Rs have a role in the pathophysiology of cancer. In myeloid acute leukemia, the IP₃R2 expression level is correlated with worse overall survival [21]. IP₃R1 is involved in resistance to apoptosis in prostate cancer cells [20] and in the epithelial–mesenchymal transition induced by epidermal growth factor in the MDA-MB-468 human BC cell line [36]. Kang et al. demonstrated that IP₃R3 had prognostic value in glioblastoma in an animal model; the survival rate was higher when IP₃R3 expression was inhibited [22]. IP₃R3 is also involved in the peritoneal dissemination of gastric cancer cells [23]. Moreover, the IP₃R3 expression level is correlated with the aggressiveness of colorectal cancer [37].

More specifically, IP₃R3 is a key factor in many mechanisms of BC oncogenesis. It is involved in BC cell proliferation via estrogen-dependent stimuli [25] and an interaction with the BKCa potassium channel [26]. Moreover, the IP₃R3 expression level influences the migration capacity of human BC cells by changing the calcium signature [27]. IP₃R3 is also able to coordinate the remodeling of the profilin cytoskeleton via the ARHGAP18/RhoA/mDia1/FAK pathway [28]. However, a prognostic role for IP₃R3 (or even a correlation with BC aggressiveness, as observed for glioblastoma or colorectal cancer) has not yet been demonstrated. Studying the tissue expression of the three IP₃R subtypes is thus essential for understanding their mechanism of action. In that context, our results established for the first time a link between the level of expression of IP₃R3 and the aggressiveness of IBC-NST. To the best of our knowledge, our study is the first to have established this link in BC—a link that is already known for cancers such as glioblastoma and gastric cancer. Although the prospective nature of our study consequently limited the number of samples eligible for this work, it enabled us to obtain precise data in terms of overall survival but also in terms of recurrence-free survival at 5 years. These data will be the subject of a future study.

Based on a SurvExpress analysis of five databases, we showed that expression of the three IP₃R subtypes is negatively correlated with overall survival and disease-free survival. In BC, there are many clinical or histologic survival factors. All these criteria should be taken into account when selecting the primary treatment, neo-adjuvant or adjuvant chemotherapy, or a more targeted type of therapy such as hormone therapy. The negative correlation between IP₃R subtype expression on the one hand, and overall survival and disease-free survival on the other, testifies to the receptor's significant involvement in breast carcinogenesis in general and metastatic invasion in particular. It is known that the mortality rate in BC is linked to the occurrence of remote metastases [38,39]. Moreover, our database analyses showed that receptor expression is inversely correlated with overall survival and disease-free survival—suggesting a genuine prognostic role for these receptors.

We found that IP₃R1 and (especially) IP₃R3 are expressed significantly more in BC tissue than in non-tumor tissue (Figure 1). IP₃R3 expression appears to be correlated with all the known prognostic factors for BC. In non-tumor tissue, IP₃R2 is expressed significantly more than IP₃R1 and IP₃R3; however, IP₃R2 expression is also high in all BC subtypes. IP₃R1 is overexpressed in BC but to a lesser extent than IP₃R3. IP₃R1 expression (relative to the two other subtypes) decreases with tumor size but is not correlated with other BC aggressiveness factors (lymph node involvement, histologic grade, and the Ki67 index). IP₃R3 can thus be considered as a marker of aggressiveness in BC as its expression is correlated to the severity of BC.

Our data argue in favor of the involvement of all IP₃R subtypes in breast carcinogenesis processes. Differences in expression between non-tumor tissue and tumor tissue are also observed in colorectal cancer. Indeed, IP₃R3 is not detected in non-tumor tissue (in contrast to the other two subtypes) but is overexpressed in colorectal cancer tissue [37]. The IP₃R subtypes are not expressed in all tissues and are expressed to differing degrees in given cell types. In rat hepatocytes, for example, IP₃R3 is not expressed, IP₃R1 is expressed diffusely in the cytoplasm, and IP₃R2 is concentrated in the pericanalicular region [38]. These subcellular differences in location suggest that the three IP₃R subtypes have distinct functions in the hepatocyte's calcium signaling activity [38]. The differences in expression of the three subtypes between BC tissue and non-tumor tissue suggest that each subtype has a different role in breast carcinogenesis. IP₃R1 and IP₃R3 are involved in the epithelial–mesenchymal transition in BC cells, whereas IP₃R2 is not [36].

Next, we sought to establish a link between IP₃R expression and other predictive factors of survival in BC. There were no significant differences in IP₃R subtype expression in tissue from BCs smaller than 20 mm. However, the IH expression scores were significantly higher for IP₃R2 and IP₃R3 than for IP₃R1 in tumors larger than 20 mm (i.e., T2 and T3). IP₃R3 expression appears to be correlated with tumor size. The IP₃R3 subtype is therefore linked to disease aggressiveness with regard to tumor size—a major factor in the decision to prescribe adjuvant treatment. Based on other prognostic criteria, patients with a small tumor size might not receive chemotherapy. Wallgren et al. showed that tumor size >20 mm was a risk factor for local or remote recurrence [4].

Regional lymph node invasion is a major risk factor for recurrence; the greater the number of invaded axillary lymph nodes, the greater the incidence of local or remote recurrence [4]. IP₃R3 is the most highly involved subtype since it (but not IP₃R1 or IP₃R2) is upregulated in N+ tissue. IP₃R3 expression may therefore be linked to a more aggressive profile with regard to the lymph node invasion criterion.

In SBR grade III IBC-NST, IP₃R1 was expressed significantly less than the other two subtypes. These results suggest that IP₃R3 expression is associated with the histologic prognosis and, again, more aggressive disease in patients with IBC-NST. Likewise, in tissue with a Ki67 proliferation index above 20%, IP₃R1 was expressed significantly less than the other two subtypes. IP₃R3, therefore, appears to be involved in IBC-NST with a high proliferation index and is linked to a more aggressive disease profile. The proliferation index and the histologic grade are predictive of a poor prognosis and are taken into account for certain treatment decisions.

Lastly, IP₃R3 was expressed significantly more than IP₃R1 in BCs overexpressing HmRs and HER2, whereas the expression of IP₃R2 was significantly higher than that of IP₃R1 in TN BC. Moreover, no differences between IP₃R2 expression and IP₃R3 expression were found in these two tumor subtypes. Overexpression of the HER2 receptor and the absence of HmR and HER2 expression are associated with a worse prognosis [10]. Our results therefore suggest that IP₃R3 is associated with more aggressive IBC-NST profiles, as defined by positivity or negativity for HmRs and HER2.

Based on our results, IP₃R3 expression appears to be associated with more aggressive IBC-NST profiles. We established that the level of IP₃R3 expression was significantly higher in BC tissue than in non-tumor tissue. IP₃R2 was not overexpressed in BC tissue but is strongly expressed in all tissues—suggesting that it is essential for the mammary gland's function. Therefore, our findings suggest that (i) IP₃R3 is the subtype most significantly involved in breast carcinogenesis processes, and (ii) IP₃R3 expression is linked to a more aggressive BC profile. Hence, IP₃R3 might be a specific marker of BC aggressiveness. In contrast to the other two subtypes, IP₃R3 is significantly involved in the proliferation and migration of human BC cells [26,27]. Furthermore, IP₃R3 influences the morphology of BC cells. When IP₃R3 expression is low, human BC cells take on a round shape that is much less conducive to migration and invasion [28]. Proliferation, migration, and invasion are three major factors in carcinogenesis. In BC, the prognosis is essentially linked to the presence of regional or remote metastases. When cancer cells lack migratory or invasive abilities,

they are unable to metastasize. IP₃R3 might be involved in cell migration and invasion, with a role in the BC prognosis. IP₃R3 is known to be involved in the carcinogenesis of other cancers. IP₃R3 is thus overexpressed in cholangiocarcinoma, where it is involved in cell migration and proliferation [39]. It also has a major role in invasion processes in glioblastoma [22]. These two cancers have a very poor prognosis, which emphasizes IP₃R3 putative involvement as a prognostic factor in certain cancers.

IP₃Rs (mainly IP₃R3) have a role in apoptosis. In B and T lymphocytes, elevated IP₃R3 expression induces more apoptosis [40]; this is also the case in pancreatic cells [41] and hepatocytes [42]. In some cancers, IP₃R3 becomes anti-apoptotic; this is the case in clear cell kidney cancers, where IP₃R3 has anti-apoptotic activity and IP₃R1 has pro-apoptotic activity [43]. This might also be the case in BC since the inhibition of IP₃R expression in human BC cells induces an increase in apoptosis [29].

The current problem in BC management is the absolute necessity to not “over-treat” patients. The latest results from basic research and clinical studies argue in favor of therapeutic de-escalation and personalized medicine [18]. For example, the advent of genomic testing several years ago has enabled some patients with non-aggressive cancers to avoid chemotherapy. IP₃R3’s involvement in overall survival and disease-free survival in BC remains to be defined. If it is proven that a low IP₃R3 threshold in an invasive breast tumor is correlated with greater survival, this receptor might eventually become a complementary tool in the decision to initiate (or not) adjuvant treatment. IP₃R3 expression could therefore be integrated into genomic-based calculations of the recurrence risk.

In conclusion, our results strongly suggest that IP₃R3 can be considered a prognostic marker in BC. In the longer term, it might be possible to predict tumor aggressiveness and perhaps select an appropriate adjuvant treatment if the primary tumor strongly expresses IP₃R3.

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References

1. World Health Organization. Available online: <https://www.who.int/news-room/fact-sheets/detail/breast-cancer> (accessed on 24 February 2022).
2. Gupta, G.P.; Massague, J. Cancer Metastasis: Building a Framework. *Cell* **2006**, *127*, 679–695. [CrossRef] [PubMed]
3. Bendre, M.; Gaddy, D.; Nicholas, R.W.; Suva, L.J. Breast cancer metastasis to bone: It is not all about PTHrP. *Clin. Orthop. Relat. Res.* **2003**, *415*, S39–S45. [CrossRef] [PubMed]
4. Wallgren, A.; Bonetti, M.; Gelber, R.; Goldhirsch, A.; Castiglione-Gertsch, M.; Holmberg, S.; Lindtner, J.; Thürlimann, B.; Fey, M.; Werner, I.; et al. Risk Factors for Locoregional Recurrence Among Breast Cancer Patients: Results From International Breast Cancer Study Group Trials I Through VII. *J. Clin. Oncol.* **2003**, *21*, 1205–1213. [CrossRef] [PubMed]
5. Kilickap, S.; Kaya, Y.; Yucel, B.; Tuncer, E.; Babacan, N.A.; Elagoz, S. Higher Ki67 expression is associates with unfavorable prognostic factors and shorter survival in breast cancer. *Asian Pac. J. Cancer Prev.* **2014**, *15*, 1381–1385. [CrossRef] [PubMed]

6. Fisher, B.; Redmond, C.; Fisher, E.R.; Caplan, R. Relative worth of estrogen or progesterone receptor and pathologic characteristics of differentiation as indicators of prognosis in node negative breast cancer patients: Findings from National Surgical Adjuvant Breast and Bowel Project Protocol B-06. *J. Clin. Oncol.* **1988**, *6*, 1076–1087. [[CrossRef](#)]
7. Borg, Å.; Tandon, A.K.; Sigurdsson, H.; Clark, G.M.; Fernö, M.; Fuqua, S.A.; Killander, D.; McGuire, W.L. HER-2/neu amplification predicts poor survival in node-positive breast cancer. *Cancer Res.* **1990**, *50*, 4332–4337.
8. Winstanley, J.; Cooke, T.; Murray, G.D.; Platt-Higgins, A.; George, W.D.; Holt, S.; Myskov, M.; Spedding, A.; Barraclough, B.R.; Rudland, P. The long term prognostic significance of c-erbB-2 in primary breast cancer. *Br. J. Cancer* **1991**, *63*, 447–450. [[CrossRef](#)]
9. Paterson, M.C.; Dietrich, K.D.; Danyluk, J.; Paterson, A.H.G.; Lees, A.W.; Jamil, N.; Hanson, J.; Jenkins, H.; Krause, B.E.; McBlain, W.A.; et al. Correlation between c-erbB-2 amplification and risk of recurrent disease in node-negative breast cancer. *Cancer Res.* **1991**, *51*, 556–567.
10. Clark, G.M.; McGuire, W.L. Follow-up study of HER-2/neu amplification in primary breast cancer. *Cancer Res.* **1991**, *51*, 944–948.
11. Ren, Z.; Li, Y.; Hameed, O.; Siegal, G.P.; Wei, S. Prognostic factors in patients with metastatic breast cancer at the time of diagnosis. *Pathol. Res. Pr.* **2014**, *210*, 301–306. [[CrossRef](#)]
12. Perou, C.M.; Sørlie, T.; Eisen, M.B.; Van De Rijn, M.; Jeffrey, S.S.; Rees, C.A.; Pollack, J.R.; Ross, D.T.; Johnsen, H.; Akslen, L.A.; et al. Molecular portraits of human breast tumours. *Nature* **2000**, *406*, 747–752. [[CrossRef](#)] [[PubMed](#)]
13. Kennecke, H.; Yerushalmi, R.; Woods, R.; Cheang, M.C.U.; Voduc, D.; Speers, C.H.; Nielsen, T.O.; Gelmon, K. Metastatic Behavior of Breast Cancer Subtypes. *J. Clin. Oncol.* **2010**, *28*, 3271–3277. [[CrossRef](#)] [[PubMed](#)]
14. Smid, M.; Wang, Y.; Zhang, Y.; Sieuwerts, A.M.; Yu, J.; Klijn, J.G.M.; Foekens, J.A.; Martens, J.W.M. Subtypes of Breast Cancer Show Preferential Site of Relapse. *Cancer Res.* **2008**, *68*, 3108–3114. [[CrossRef](#)]
15. Lowery, A.J.; Kell, M.R.; Glynn, R.W.; Kerin, M.J.; Sweeney, K.J. Locoregional recurrence after breast cancer surgery: A systematic review by receptor phenotype. *Breast Cancer Res. Treat.* **2011**, *133*, 831–841. [[CrossRef](#)]
16. Jemal, A.; Bray, F.; Center, M.M.; Ferlay, J.; Ward, E.; Forman, D. Global cancer statistics. *CA A Cancer J. Clin.* **2011**, *61*, 69–90. [[CrossRef](#)] [[PubMed](#)]
17. Weigelt, B.; Peterse, J.L.; van't Veer, L.J. Breast cancer metastasis: Markers and models. *Nat. Rev. Cancer* **2005**, *5*, 591–602. [[CrossRef](#)] [[PubMed](#)]
18. Foulon, A.; Theret, P.; Rodat-Despoix, L.; Kischel, P. Beyond Chemotherapies: Recent Strategies in Breast Cancer Treatment. *Cancers* **2020**, *12*, 2634. [[CrossRef](#)]
19. Miyakawa, T.; Maeda, A.; Yamazawa, T.; Hirose, K.; Kurosaki, T.; Iino, M. Encoding of Ca²⁺ signals by differential expression of IP3 receptor subtypes. *EMBO J.* **1999**, *18*, 1303–1308. [[CrossRef](#)]
20. Boutin, B.; Tajeddine, N.; Monaco, G.; Molgo, J.; Vertommen, D.; Rider, M.; Parys, J.; Bultynck, G.; Gailly, P. Endoplasmic reticulum Ca²⁺ content decrease by PKA-dependent hyperphosphorylation of type 1 IP3 receptor contributes to prostate cancer cell resistance to androgen deprivation. *Cell Calcium* **2015**, *57*, 312–320. [[CrossRef](#)]
21. Shi, J.-L.; Fu, L.; Wang, W.-D. High expression of Inositol 1,4,5-trisphosphate receptor, type 2 (ITPR2) as a novel biomarker for worse prognosis in cytogenetically normal acute myeloid leukemia. *Oncotarget* **2015**, *6*, 5299–5309. [[CrossRef](#)]
22. Kang, S.S.; Han, K.-S.; Ku, B.M.; Lee, Y.K.; Hong, J.; Shin, H.Y.; Almonte, A.; Woo, D.H.; Brat, D.J.; Hwang, E.M.; et al. Caffeine-Mediated Inhibition of Calcium Release Channel Inositol 1,4,5-Trisphosphate Receptor Subtype 3 Blocks Glioblastoma Invasion and Extends Survival. *Cancer Res.* **2010**, *70*, 1173–1183. [[CrossRef](#)] [[PubMed](#)]
23. Sakakura, C.; Hagiwara, A.; Fukuda, K.; Shimomura, K.; Takagi, T.; Kin, S.; Nakase, Y.; Fujiyama, J.; Mikoshiba, K.; Okazaki, Y.; et al. Possible involvement of inositol 1,4,5-trisphosphate receptor type 3 (IP3R3) in the peritoneal dissemination of gastric cancers. *Anticancer Res.* **2003**, *23*, 3691–3697. [[PubMed](#)]
24. Rosa, N.; Sneyers, F.; Parys, J.B.; Bultynck, G. Type 3 IP3 receptors: The chameleon in cancer. *Int. Rev. Cell Mol. Biol.* **2020**, *351*, 101–148. [[CrossRef](#)] [[PubMed](#)]
25. Szatkowski, C.; Parys, J.B.; Ouadid-Ahidouch, H.; Matifat, F. Inositol 1,4,5-trisphosphate-induced Ca²⁺ signalling is involved in estradiol-induced breast cancer epithelial cell growth. *Mol. Cancer* **2010**, *9*, 156. [[CrossRef](#)] [[PubMed](#)]
26. Mound, A.; Rodat-Despoix, L.; Bougarn, S.; Ouadid-Ahidouch, H.; Matifat, F. Molecular interaction and functional coupling between type 3 inositol 1,4,5-trisphosphate receptor and BKCa channel stimulate breast cancer cell proliferation. *Eur. J. Cancer* **2013**, *49*, 3738–3751. [[CrossRef](#)]
27. Mound, A.; Vautrin-Glabik, A.; Foulon, A.; Botia, B.; Hague, F.; Parys, J.B.; Ouadid-Ahidouch, H.; Rodat-Despoix, L. Downregulation of type 3 inositol (1,4,5)-trisphosphate receptor decreases breast cancer cell migration through an oscillatory Ca²⁺ signal. *Oncotarget* **2017**, *8*, 72324–72341. [[CrossRef](#)]
28. Vautrin-Glabik, A.; Botia, B.; Kischel, P.; Ouadid-Ahidouch, H.; Rodat-Despoix, L. IP3R3 silencing induced actin cytoskeletal reorganization through ARHGAP18/RhoA/mDia1/FAK pathway in breast cancer cell lines. *Biochim. Biophys. Acta Mol. Cell Res.* **2018**, *1865*, 945–958. [[CrossRef](#)]
29. Singh, A.; Sharma, R.K.; Chagtoo, M.; Agarwal, G.; George, N.; Sinha, N.; Godbole, M.M. 1H NMR Metabolomics Reveals Association of High Expression of Inositol 1,4,5 Trisphosphate Receptor and Metabolites in Breast Cancer Patients. *PLoS ONE* **2017**, *12*, e0169330. [[CrossRef](#)]
30. Page, D.L. Prognosis and breast cancer. Recognition of lethal and favorable prognostic types. *Am. J. Surg. Pathol.* **1991**, *15*, 334–349. [[CrossRef](#)]

31. Koscielny, S.; Tubiana, M.; Lê, M.G.; Valleron, A.; Mouriesse, H.; Contesso, G.; Sarrazin, D. Breast cancer: Relationship between the size of the primary tumour and the probability of metastatic dissemination. *Br. J. Cancer* **1984**, *49*, 709–715. [[CrossRef](#)]
32. Carter, C.L.; Allen, C.; Henson, D.E. Relation of tumor size, lymph node status, and survival in 24,740 breast cancer cases. *Cancer* **1989**, *63*, 181–187. [[CrossRef](#)]
33. Elston, C.; Ellis, I. pathological prognostic factors in breast cancer. I. The value of histological grade in breast cancer: Experience from a large study with long-term follow-up. *Histopathology* **1991**, *19*, 403–410. [[CrossRef](#)] [[PubMed](#)]
34. Sørli, T.; Tibshirani, R.; Parker, J.; Hastie, T.; Marron, J.S.; Nobel, A.; Deng, S.; Johnsen, H.; Pesich, R.; Geisler, S.; et al. Repeated observation of breast tumor subtypes in independent gene expression data sets. *Proc. Natl. Acad. Sci. USA* **2003**, *100*, 8418–8423. [[CrossRef](#)]
35. Aguirre-Gamboa, R.; Gomez-Rueda, H.; Martínez-Ledesma, E.; Martínez-Torteya, A.; Chacolla-Huaringa, R.; Rodriguez-Barrientos, A.; Tamez-Pena, J.G.; Treviño, V. SurvExpress: An Online Biomarker Validation Tool and Database for Cancer Gene Expression Data Using Survival Analysis. *PLoS ONE* **2013**, *8*, e74250. [[CrossRef](#)]
36. Davis, F.M.; Parsonage, M.T.; Cabot, P.J.; Parat, M.-O.; Thompson, E.W.; Roberts-Thomson, S.J.; Monteith, G.R. Assessment of gene expression of intracellular calcium channels, pumps and exchangers with epidermal growth factor-induced epithelial-mesenchymal transition in a breast cancer cell line. *Cancer Cell Int.* **2013**, *13*, 76. [[CrossRef](#)]
37. Shibao, K.; Fiedler, M.J.; Nagata, J.; Minagawa, N.; Hirata, K.; Nakayama, Y.; Iwakiri, Y.; Nathanson, M.H.; Yamaguchi, K. The type III inositol 1,4,5-trisphosphate receptor is associated with aggressiveness of colorectal carcinoma. *Cell Calcium* **2010**, *48*, 315–323. [[CrossRef](#)] [[PubMed](#)]
38. Hirata, K.; Dufour, J.-F.; Shibao, K.; Knickelbein, R.; O'Neill, A.F.; Bode, H.-P.; Cassio, D.; St-Pierre, M.V.; LaRusso, N.F.; Leite, M.F.; et al. Regulation of Ca²⁺ signaling in rat bile duct epithelia by inositol 1,4,5-trisphosphate receptor isoforms. *Hepatology* **2002**, *36*, 284–296. [[CrossRef](#)]
39. Ueasilamongkol, P.; Khamphaya, T.; Guerra, M.T.; Rodrigues, M.A.; Gomes, D.; Kong, Y.; Wei, W.; Jain, D.; Trampert, D.C.; Ananthanarayanan, M.; et al. Type 3 Inositol 1,4,5-Trisphosphate Receptor Is Increased and Enhances Malignant Properties in Cholangiocarcinoma. *Hepatology* **2020**, *71*, 583–599. [[CrossRef](#)]
40. Khan, A.A.; Soloski, M.J.; Sharp, A.H.; Schilling, G.; Sabatini, D.M.; Li, S.-H.; Ross, C.A.; Snyder, S.H. Lymphocyte Apoptosis: Mediation by Increased Type 3 Inositol 1,4,5-Trisphosphate Receptor. *Science* **1996**, *273*, 503–507. [[CrossRef](#)]
41. Blondel, O.; Takeda, J.; Janssen, H.; Seino, S.; Bell, G. Sequence and functional characterization of a third inositol trisphosphate receptor subtype, IP3R-3, expressed in pancreatic islets, kidney, gastrointestinal tract, and other tissues. *J. Biol. Chem.* **1993**, *268*, 11356–11363. [[CrossRef](#)]
42. Lail-Trecker, M.R.; Peluso, C.E.; Peluso, J.J. Hepatocyte Growth Factor Disrupts Cell Contact and Stimulates an Increase in Type 3 Inositol Triphosphate Receptor Expression, Intracellular Calcium Levels, and Apoptosis of Rat Ovarian Surface Epithelial Cells. *Endocrine* **2000**, *12*, 303–314. [[CrossRef](#)]
43. Rezuchova, I.; Hudcova, S.; Soltysova, A.; Matuskova, M.; Durinikova, E.; Chovancova, B.; Zuzcak, M.; Cihova, M.; Burikova, M.; Penesova, A.; et al. Type 3 inositol 1,4,5-trisphosphate receptor has antiapoptotic and proliferative role in cancer cells. *Cell Death Dis.* **2019**, *10*, 186. [[CrossRef](#)] [[PubMed](#)]