RESEARCH ARTICLE

Editorial Process: Submission:04/17/2025 Acceptance:11/18/2025 Published:11/24/2025

nNav1.5 mRNA Expression in Triple Negative and Non-Triple Negative Breast Cancer Patients Treated at Hospital Pakar Universiti Sains Malaysia

Ahmad Firdaus Ahmad Sukri[1], Nuramira Syazreen Suhaimi[1], Elis Rosliza Mohd Adzemi[1], Mawaddah Mohd Azlan[1], Maya Mazwin Yahya[2], Wan Faiziah Wan Abdul Rahman[2], Noor Fatmawati Mokhtar[1,2]*

Abstract

Objective: This study aimed to evaluate the expression of *nNav1.5 mRNA* and its association with clinicopathological features in triple-negative breast cancer (TNBC) and non-TNBC patients treated at Hospital Pakar Universiti Sains Malaysia (HPUSM), Kelantan. **Methods:** Tumor tissue samples were collected from TNBC (n = 43) and non-TNBC (n = 19) patients. Real-time PCR was performed to measure *nNav1.5 mRNA* expression levels. Clinicopathological parameters assessed included tumor size, histological grade, cancer stage, hormone receptor status, *HER2* expression, and lymph node involvement. Sanger sequencing was carried out to verify the identity of the amplified *nNav1.5* transcripts. **Results:** *nNav1.5 mRNA* expression was significantly higher in TNBC compared to non-TNBC tumors (p = 0.007). Sanger sequencing confirmed >99% sequence identity with the canonical human *nNav1.5* transcript. Elevated *nNav1.5* expression was significantly associated with TNBC subtype (p = 0.007), advanced tumor stage (p = 0.007), and distant lymph node metastasis (p = 0.002). **Conclusion:** High expression of *nNav1.5 mRNA* in breast tumors is significantly associated with the TNBC subtype and aggressive clinical features.

Keywords: *nNav1.5 mRNA*- TNBC- non-TNBC- patient outcomes- tumour tissue

Asian Pac J Cancer Prev, **26 (11)**, 4061-4067

Introduction

The expression of the neonatal splice variant Nav1.5 (*nNav1.5*) in breast cancer has emerged as a potent marker of aggressiveness and metastasis. Its significance lies in its multifaceted role in driving breast cancer progression, influencing a myriad of cellular and molecular processes as demonstrated in vitro [1–4], and in animal models [5, 6]. Early insights into how *nNav1.5* contributes to breast cancer progression reveal its role in promoting extracellular matrix degradation through increased protease activation [7–9] and facilitating cell death in the surrounding microenvironment by increasing glutamate secretion, akin to neuronal excitotoxicity [10–13].

Elevated expression of *nNav1.5 mRNA* in breast tumor tissues compared to normal, non-cancerous tissues was first reported by Fraser et al. [1]. Subsequent research reinforced its clinical significance, revealing a strong correlation between high *nNav1.5* expression and poor prognostic outcomes such as lymph node metastasis, tumor recurrence, and five-year mortality [1, 14]. Due to its consistent association with aggressive tumor behavior, elevated *nNav1.5* expression has been logically linked to triple-negative breast cancer (TNBC). However, existing studies examining this relationship have been limited to in vitro models, primarily using the MDA-MB-231 TNBC cell line, or in vivo models involving orthotopic implantation of these cells in mice [1, 15]. This underscores a critical gap in the current literature, namely, the lack of clinical data on *nNav1.5* expression in TNBC patient tumor samples.

Understanding tumor biomarker expression across diverse populations is crucial for the development, validation, and successful clinical integration of novel biomarkers. To date, studies on *nNav1.5* expression have been largely confined to specific cohorts, primarily from the United Kingdom [1, 14], limiting the generalizability of findings. Furthermore, there remains a notable gap in the literature regarding the association between *nNav1.5 mRNA* expression and clinicopathological parameters, particularly in distinguishing between breast cancer subtypes such as TNBC and non-triple-negative breast

*[1]Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Health Campus, 16150 Kubang Kerian, Kelantan, Malaysia. [2]Breast Cancer Awareness & Research Unit (BestARi), Hospital Pakar Universiti Sains Malaysia, Universiti Sains Malaysia, Kampus Kesihatan, 16150, Kota Bharu, Kelantan, Malaysia. *For Correspondence: fatmawati@usm.my*

cancer (non-TNBC). Exploring these relationships within a local patient cohort from Hospital Pakar Universiti Sains Malaysia (HPUSM) offers a valuable and timely opportunity to enhance our understanding of *nNav1.5*'s prognostic relevance in a more diverse clinical setting.

Materials and Methods

Sample recruitment, clinicopathological characteristics and study design

This prospective study was approved by the Human Research Ethics Committee of Universiti Sains Malaysia (USM/JEPeM/18120775), which complies with the Declaration of Helsinki [16, 17]. The study subjects were recruited from HPUSM while experimental analyses were carried out at Institute for Research in Molecular Medicine (INFORMM), Universiti Sains Malaysia, Kelantan.

Ten malignant breast cancer patients (TNBC = 5, non-TNBC = 5) and five non-cancerous normal samples were collected from HPUSM after getting informed consent. The patients were recruited based on inclusion criteria, which include: i) female breast cancer patients, ii) histopathologically confirmed breast cancer patients who were negative for estrogen receptor (ER), progesterone receptor (PR) and human epidermal growth factor receptor-2 (*HER2*) (TNBC group), and iii) histopathologically confirmed breast cancer patients who were positive for any combination of the ER, PR and *HER2* (non-TNBC group). Males and those with incomplete or missing clinicopathological data were excluded from this study. The clinical and pathological variables including age at diagnosis, histology type, molecular subtype, staging according to TNM classification, lymph node involvement, menopausal status, presence and location of metastases were retrieved from the patient's medical records.

nNav1.5 mRNA expression analysis

Total RNA was extracted from 100 mg of the breast tissues using Sepasol-RNA I Super G reagent (Nacalai Tesque, Japan). A Nanodrop ND-1000 spectrophotometer (Thermo Fisher Scientific, USA) was used to assess RNA concentrations and quality. 1000 ng of purified RNA was reverse transcribed to cDNA using QuantiNova® Reverse Transcription Kit following the manufacturer's protocol (Qiagen, Germany). The mRNA expression of the target gene, *nNav1.5*, and a housekeeping gene, β-actin, was assessed through real-time PCR. The real-time PCR master-mix reagent used was the SensiFAST™ SYBR® Hi-ROX Mix according to the manufacturers' protocol (Bioline, UK). Accordingly, the master-mix contained 0.8 µl of 0.4 µM forward and reverse primers for the *nNav1.5* gene (forward: 5′-TGATTATCATGGCGTATGTATCAGA-3' and reverse: 5′-TGAGGGCAAAGACGCTGAG-3′) and the housekeeping gene β-actin (forward: 5′-ATTGCCGACAGGATGCAGAAG-3′ and reverse: 5′-TAGAAGCATTTGCGGTGGACG-3′), 10 µl of SensiFAST SYBR Hi-ROX Mix (1X working concentration) and 4.4 µl of RNase-free water. A total of 4.0 µl of 2.5X diluted cDNA template was added to the master mix, making up a total volume of 20 µl. The PCR cycling conditions used include an initial activation step for 5 minutes at 95°C, followed by 40 cycles of denaturation at 95°C for 10 seconds and combined annealing/extension at 60°C for 30 seconds. A pre-set thermal dissociation analysis at 95°C for 30 seconds, 65°C for 30 seconds and 95°C for 30 seconds was included. The thermal dissociation or melting curve analysis was performed with a resolution of 0.5°C and soak time of 5 seconds. The housekeeping gene was used in the same preparation of RNA/cDNA as the target sequence. The use of the housekeeping gene, β-actin as the internal control was required to compensate for any errors in the cDNA conversion step or pipetting errors to ensure more accurate comparative quantification of gene expression. Comparison of gene expression between TNBC, non-TNBC and normal tissues were analyzed using $2^{-\Delta Ct}$ method by using the average Ct values of target gene.

Sanger sequencing

Amplified PCR products undergo the purification process through the Isopropanol PCR Purification method. The method was optimized accordingly to remove residual primers, nucleotides, and enzymes. The quality and quantity confirmation of purified product was identified by subjecting the product to the gel electrophoresis run. Subsequently, the sequence profile of the purified PCR product was amplified through the PCR Sanger Sequencing using the BigDye® Terminator v3.1 Sequencing kit (Applied Biosystems). The reaction of 10µL PCR master mix containing 0.5 µL of BigDye reagent, 1.75 µL of 5× sequencing buffer, 3.3 pmol of sequencing primer (Forward or Reverse), and ~50 ng of purified PCR product. The thermal cycling conditions of 96 °C for 1 minute, followed by 25 cycles of 96 °C for 10 seconds, 50 °C for 5 seconds, and 60 °C for 4 minutes. Afterwards, the sequencing reaction product was clean-up using Ethanol Precipitation method. The final product of Sanger purification was re-suspended in Hi-Di™ Formamide (Applied Biosystems, USA) and denatured at 95 °C for 2 minutes before loading onto an Applied Biosystems 3130xl Genetic Analyzer for capillary electrophoresis. Raw sequence data were analyzed using Sequencing Analysis Software v6.0 (Applied Biosystems, USA) and aligned with reference sequences using BioEdit for base calling and were compared to the reference gene (Homo sapiens partial mRNA for voltage gated sodium channel Nav1.5 (SCN5A gene), D1 neonatal splice variant, cell line MDA-MB-231 (GenBank: AJ310886.1)) using BLAST (NCBI) to confirm specificity and alignment accuracy.

Clinicopathological parameters were analyzed using descriptive statistics [17–19]. Categorical data are presented as counts and corresponding percentages while continuous data are presented as median and ranges. Comparison of categorical data between the two groups of patients (high *nNav1.5* expression versus low *nNav1.5* expression) was performed using the Fisher's exact test or χ^2-test while continuous data were compared between the groups using Mann–Whitney U-test or the Kruskal–Wallis test, in case of more than two groups. Variables with a p-value < 0.05 were considered statistically significant.

Statistical analyses were performed, and graphics were generated using IBM SPSS v.22 and GraphPad Prism v.7 software. All statistical tests were two-sided, and the significance level was set at 0.05.

Results

nNav1.5 mRNA expression in TNBC and non-TNBC

nNav1.5 mRNA were detected in breast cancer tissues, both in TNBC and non-TNBC samples whilst not detected in non-cancerous normal tissue samples. Accordingly, *nNav1.5 mRNA* expression level in TNBC was significantly greater in comparison to non-TNBC ($p = 0.009$, $n = 5$) and non-cancerous normal samples ($p = 0.005$, $n = 5$) (Figure 1). Similarly, the *nNav1.5 mRNA* expression level in non-TNBC samples was significantly higher than in non-cancerous normal samples ($p = 0.005$, $n = 5$) (Figure 1).

DNA sequence identity of the product amplicons

The qPCR amplicons were subjected to Sanger sequencing to determine their sequence identity. All six query sequences (3 TNBC and 3 non-TNBC) had high degrees of similarities (PN1 = 96.8%, PN2 and PN4 = 100%, PT1 = 90.3%, PT2 = 96.8% and PT4 = 100%) (Figure 2 and Table 1). The sequence matches with the reference deposited by Fraser et al. (2005), Homo sapiens partial mRNA for voltage gated sodium channel Nav1.5 (SCN5A gene), D1 neonatal splice variant, cell line MDA-MB-231 (GenBank: AJ310886.1).

Association between nNav1.5 mRNA expression with clinicopathological characteristics

The association between *nNav1.5* expression in tumour tissues with clinicopathologic variables were assessed using the median as the optimal cut-off point [16, 20, 21]. In the group of malignant breast cancer patients, there was a statistically significant association between the level of *nNav1.5 mRNA* expression (high versus low) and clinically detected distant metastases (M0 versus M1), with high *nNav1.5* expression occurring only in M1 cases ($p = 0.002$). Moreover, there was a statistically significant correlation between *nNav1.5* expression and location of metastases, with high *nNav1.5* expression ($p = 0.007$). Similarly, a statistically significant association was observed between the level of *nNav1.5* expression (high versus low) with TNM stage (Stage I/II/III/IV), with high *nNav1.5* expression associated with Stage IV disease ($p = 0.007$) and finally, molecular subtype (Luminal A/

Table 1. Degrees of Similarities against Reference

	Changes in the 31 nucleotides for nNav1.5	Degree of similarities (%)
PN1	10	96.8
PN2	NC	100
PN4	NC	100
PT1	12, 13, 30	90.3
PT2	27	96.8
PT4	NC	100

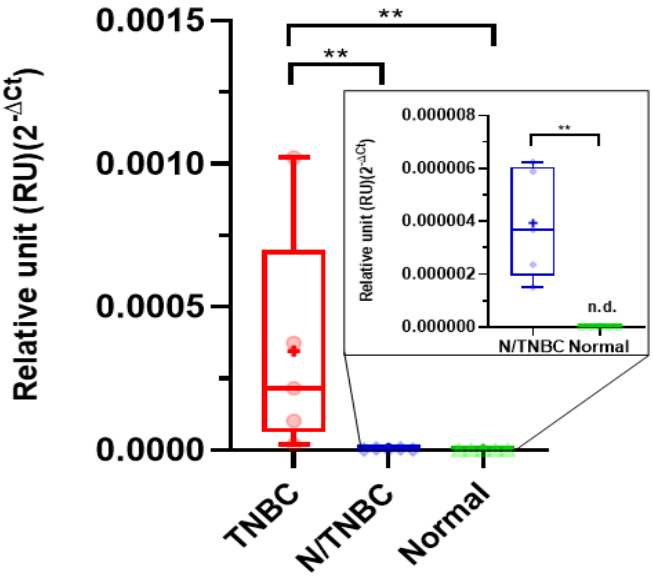

Figure 1. Comparison of the mRNA Expression Level of *nNav1.5* in TNBC, non-TNBC and Normal Tissues Measured by qPCR. $2^{-\Delta Ct}$ was used for the semi-quantitative analysis. The expression of nNav1.5 mRNA in TNBC ($N = 5$) and non-TNBC ($N = 5$) group was significantly higher compared to the normal group ($N = 5$) ($p < 0.05$). The expression of nNav1.5 mRNA in TNBC group was also significantly higher than in the non-TNBC group ($p < 0.05$). The top and bottom of the box represent the third quartile (Q3) and first quartile (Q1), respectively. The box covers the interquartile interval, where 50% of the data is found. The vertical line that splits the box into two is the median of the RUs. The mean relative unit (RU) is indicated by a cross (+) on the box plot. The two lines outside the box, called the whiskers, go from each quartile to the maximum or minimum RU. The shapes (●, ♦, ▲) represent the RU for each individual sample. Data were collected from $n = 3$ independent experiments, presented as mean ± standard error of the mean (SEM) and were compared between groups using the Mann Whitney U-test. (**) indicates significance level at $p < 0.05$.

Luminal B/*HER2*-enriched/TNBC), with high *nNav1.5 mRNA* expression was associated with TNBC subtype (p = 0.007) (Table 2).

Discussion

The foundation for elevated expression of neonatal Nav1.5 (*nNav1.5*) mRNA in breast cancer has been primarily characterized using two widely studied

Table 2. Association between *nNav1.5* Expression (High vs Low) and Clinicopathological Characteristics in Malignant Breast Cancer Patients (n = 10).

Patient characteristics	All patients (N = 10)		
	nNav1.5 high expression N (%)	*nNav1.5* low expression N (%)	p-value
Total	5 (50.0)	5 (50.0)	
Histology			
Invasive ductal carcinoma/no special type	5 (100.0)	5 (100.0)	-[b]
Invasive lobular carcinoma	-[a]	-[a]	
Metaplastic carcinoma (Squamous cell type)	-[a]	-[a]	
Histological grade			
Grade I (Well differentiated)	-[a]	1 (20.0)	0.656
Grade II (Moderately differentiated)	1 (20.0)	1 (20.0)	
Grade III (poorly differentiated)	4 (80.0)	3 (60.0)	
Tumour size			
T1 (0-2 cm)	1 (20.0)	-[a]	0.446
T2 (2-5 cm)	2 (40.0)	4 (80.0)	
T3 (>5 cm)	1 (20.0)	-[a]	
T4 (attached to chest wall)	1 (20.0)	1 (20.0)	
Lymph node metastases (N)			
N0 (0)	1 (20.0)	1 (20.0)	0.261
N1 (<3)	-[a]	2 (40.0)	
N2 (4-9)	2 (40.0)	2 (40.0)	
N3 (>10)	2 (40.0)	-[a]	
Lymphovascular invasion			
Yes	4 (80.0)	3 (60.0)	0.49
No	1 (20.0)	2 (40.0)	
Distant metastases			
M0 (No)	-[a]	5 (100.0)	0.002[c]
M1 (Yes)	5 (100.0)	-[a]	
Location of metastases			
Visceral (soft organ)	4 (80.0)	-[a]	0.007[c]
Non-visceral	1 (20.0)	-[a]	
None	-[a]	5 (100.0)	
TNM Stage			
Stage I	-[a]	-[a]	
Stage II	-[a]	2 (40.0)	0.007[c]
Stage III	-[a]	3 (60.0)	
Stage IV	5 (100.0)	-[a]	
Molecular subtype			
Luminal A (ER+/ PR+/ HER2-)	-[a]	4 (80.0)	0.007[c]
Luminal B (ER+/ PR-/ HER2-)	-[a]	1 (20.0)	
HER2-enriched (ER-/PR-/HER2+)	-[a]	-[a]	
TNBC	5 (100.0)	-[a]	

N, number; TNBC, Triple negative breast cancer; [a]No cases were recorded; [b]No statistics are computed as the independent variable is a constant; [c]Significant when p-value is less than 0.05.

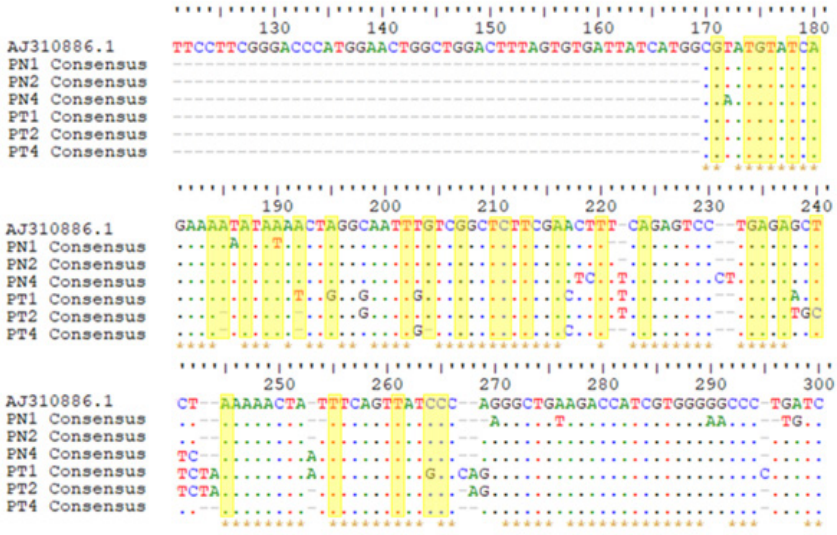

Figure 2. Dot Conservation Plot for All Samples (PN1, PN2, PN4, PT1, PT2, PT4) using the Homo sapiens partial mRNA for voltage gated sodium channel Nav1.5 (SCN5A gene), D1 neonatal splice variant, cell line MDA-MB-231 (AJ310886.1) as the reference sequence. The stars (*) indicate the residues that are 100% matched among all sequences. The nucleotides highlighted in yellow indicate the 31-nucleotide difference between adult and neonatal Nav1.5. PN- non-TNBC samples; PT - TNBC samples; NC – no change.

human breast cancer cell lines: the highly aggressive, triple-negative breast cancer (TNBC) MDA-MB-231 and the less aggressive, hormone receptor–positive MCF-7 [1, 4, 22–24]. Notably, *nNav1.5 mRNA* expression is over 1000-fold higher in MDA-MB-231 cells, which lack estrogen receptor (ER), progesterone receptor (PR), and human epidermal growth factor receptor 2 (*HER2*), compared to MCF-7 cells, which express all three receptors [1]. In contrast, *nNav1.5* expression is undetectable in non-cancerous mammary epithelial cells such as MCF-10A [25], supporting its tumor-specific expression. More recently, significant upregulation of *nNav1.5 mRNA* has also been reported in the murine 4T1 mammary carcinoma cell line, another model associated with TNBC [15].

The earliest report of *nNav1.5 mRNA* expression in breast tumor biopsies was by Fraser et al. [1], employing real-time PCR followed by DNA sequencing. The findings revealed that 10 out of 11 (91%) tumor samples expressed *nNav1.5* . Subsequent studies have further evaluated *nNav1.5 mRNA* expression in breast cancer tissues [22, 25], reinforcing its role in tumor aggressiveness and metastatic potential. However, to date, no study has explicitly investigated the association between *nNav1.5* expression and the triple-negative breast cancer (TNBC) subtype, underscoring a critical gap in the current literature.

This study compared *nNav1.5 mRNA* expression levels between TNBC and non-TNBC patient samples. While both subtypes exhibited detectable expression, TNBC samples showed a markedly higher expression, approximately 137.5-fold compared to non-TNBC samples. In contrast, *nNav1.5 mRNA* was undetectable in non-cancerous breast tissue, supporting its cancer-specific expression pattern. These findings are consistent with previous in vitro studies; however, this is the first clinical study to establish a significant association between elevated *nNav1.5 mRNA* expression in the TNBC subtype of patient-derived tumor tissues.

DNA sequencing confirmed that the amplified *nNav1.5* products from both TNBC and non-TNBC tumor tissues closely aligned with the sequence reported by Fraser et al. [1] for the Homo sapiens partial mRNA of the voltage-gated sodium channel Nav1.5 (SCN5A gene), D1 neonatal splice variant, originally derived from the MDA-MB-231 breast cancer cell line (GenBank: AJ310886.1). Sequence alignment within the 31-nucleotide region specific to *nNav1.5* demonstrated a high degree of conservation, ranging from 97–100% in TNBC samples and 90–100% in non-TNBC samples. These results confirm the presence of *nNav1.5* in clinical breast cancer tissues and support its relevance across molecular subtypes. However, the observed sequence variations between TNBC and non-TNBC samples warrant further investigation to determine their biological significance. Such differences may reflect underlying subtype-specific molecular heterogeneity and could potentially influence the functional behavior of the channel in the tumor microenvironment.

Fraser et al. [1] were not only the first to employ DNA sequencing to confirm the identity of *nNav1.5 mRNA* in human breast tumor biopsies, but importantly, the first to demonstrate the clinical relevance of *nNav1.5* expression. Accordingly, increased *nNav1.5 mRNA* expression was shown to strongly associated with lymph node metastasis, disease recurrence, and mortality within five years. Building on this foundational work, the present study extends the molecular profiling of *nNav1.5* in a distinct patient cohort and across breast cancer molecular subtypes. Notably, multivariate analysis of clinicopathological parameters revealed a significant association between elevated *nNav1.5 mRNA* expression and the triple-negative breast cancer (TNBC) subtype (p

= 0.007). In addition, high *nNav1.5* expression correlated with distant metastasis (p = 0.002) and advanced tumor stage IV (p = 0.007). These findings are in line with those reported by Fraser et al. (2005), further supporting the role of *nNav1.5 mRNA* as a potential molecular marker of aggressive breast cancer phenotypes.

nNav1.5 in breast cancer is typically expressed alongside an adult isoform, Nav1.5, although, *nNav1.5* is the predominant form expressed [1]. In vitro studies have shown that both isoforms contribute similarly to tumor progression by promoting cellular invasiveness and metastatic potential. Due to the availability of commercial antibodies targeting the adult Nav1.5 protein, most studies examining clinical relevance have focused on Nav1.5 protein expression. A recent study by Leslie et al. [26] reported that Nav1.5 protein expression in breast cancer was significantly associated with adverse clinicopathological features, including larger tumor size, lymph node metastasis, distant metastasis, and higher tumor stage . Moreover, Nav1.5 expression demonstrated a negative correlation with estrogen receptor (ER) and progesterone receptor (PR) status, and a positive correlation with human epidermal growth factor receptor 2 (*HER2*) expression. Nevertheless, the study did not find a direct association between Nav1.5 protein expression and the triple-negative breast cancer (TNBC) subtype. In contrast, the lack of antibodies specific to *nNav1.5* has hindered the clinical translation and broader investigation of this variant.

The primary limitation of this study is the relatively small sample size, which may limit the generalizability of the findings. Nonetheless, the results provide a valuable foundation for future investigations, and larger-scale studies are warranted to validate and expand upon these preliminary observations.

In conclusion, the present study reveals that *nNav1.5 mRNA* expression was exclusively detected in cancerous breast tissues, with no presence in normal, non-cancerous tissues. High *nNav1.5 mRNA* expression was significantly associated with TNBC subtype, distant metastasis and Stage IV disease. These findings suggest the potential value of *nNav1.5* as a prognostic marker and therapeutic target in breast cancer, underscoring its association with aggressive tumor phenotypes and unfavorable clinical outcomes.

Author Contribution Statement

AFAS & NNS – writing the initial draft of the paper, performed the experiments, derived the models, and analyzed the data; ERMA – assisting in sample collection and record acquisition; MMA – assisting in real-time PCR and DNA sequencing experiments; MMY – surgeon assisting in sample collection and record acquisition; WFWAR – pathologist assisting in sample preparation and record acquisition; NFM – conceived the project and funding, overseeing and guiding the research project, revising the manuscript for intellectual content and accuracy..

Acknowledgements

The authors extend their sincere gratitude to all researchers who contributed to this study. Special thanks to Noor Fatmawati Mokhtar for conceiving the project, securing funding, providing overall supervision, and critically revising the manuscript for intellectual content and accuracy. We acknowledge Ahmad Firdaus Ahmad Sukri and Nuramira Syazreen Suhaimi for drafting the initial manuscript, performing the experiments, developing the models, and conducting data analysis. Elis Rosliza Mohd Adzemi is gratefully acknowledged for her support in sample collection and data acquisition. We also thank Mawaddah Mohd Azlan for her assistance in the real-time PCR and DNA sequencing experiments. Appreciation is due to Maya Mazwin Yahya, the attending surgeon, and Wan Faiziah Wan Abdul Rahman, the pathologist, for their contributions to clinical sample collection, preparation, and record acquisition. We also thank the members of INFORMM laboratories and the BestARi staff at Hospital Pakar Universiti Sains Malaysia (HPUSM) for their technical support and valuable discussions throughout the project. This research was supported by the Fundamental Research Grant Scheme (FRGS) from the Ministry of Education Malaysia (FRGS/1/2022/SKK10/USM/02/25) and by the Higher Institution Centre of Excellence (HICoE) Grant (Grant No: 311/CIPPM/4401005). We are also grateful to the Human Research Ethics Committee of Universiti Sains Malaysia for providing ethical approval and oversight (USM/JEPeM/18120775). The authors declare no conflicts of interest.

Funding

This work was supported by Fundamental Research Grant (FRGS) under the Ministry of Education, Malaysia (FRGS/1/2022/SKK10/USM/02/25) and the Malaysian Ministry of Education through the Higher Institution Centre of Excellence (HICoE) Grant No: 311/CIPPM/4401005.

Conflict of Interest

The authors declare that there is no conflict of interest

References

1. Fraser SP, Diss JK, Chioni AM, Mycielska ME, Pan H, Yamaci RF, et al. Voltage-gated sodium channel expression and potentiation of human breast cancer metastasis. Clin Cancer Res. 2005;11(15):5381-9. https://doi.org/10.1158/1078-0432.Ccr-05-0327.
2. Gillet L, Roger S, Bougnoux P, Le Guennec JY, Besson P. Beneficial effects of omega-3 long-chain fatty acids in breast cancer and cardiovascular diseases: Voltage-gated sodium channels as a common feature? Biochimie. 2011;93(1):4-6. https://doi.org/10.1016/j.biochi.2010.02.005.
3. Isbilen B, Fraser SP, Djamgoz MB. Docosahexaenoic acid (omega-3) blocks voltage-gated sodium channel activity and migration of mda-mb-231 human breast cancer cells. Int J Biochem Cell Biol. 2006;38(12):2173-82. https://doi.org/10.1016/j.biocel.2006.06.014.
4. Brackenbury WJ, Chioni AM, Diss JK, Djamgoz MB. The neonatal splice variant of nav1.5 potentiates in vitro invasive

behaviour of mda-mb-231 human breast cancer cells. Breast Cancer Res Treat. 2007;101(2):149-60. https://doi.org/10.1007/s10549-006-9281-1.
5. Nelson M, Yang M, Dowle AA, Thomas JR, Brackenbury WJ. The sodium channel-blocking antiepileptic drug phenytoin inhibits breast tumour growth and metastasis. Mol Cancer. 2015;14(1):13. https://doi.org/10.1186/s12943-014-0277-x.
6. Nelson M, Yang M, Millican-Slater R, Brackenbury WJ. Nav1.5 regulates breast tumor growth and metastatic dissemination in vivo. Oncotarget. 2015;6(32):32914-29. https://doi.org/10.18632/oncotarget.5441.
7. Besson P, Driffort V, Bon É, Gradek F, Chevalier S, Roger S. How do voltage-gated sodium channels enhance migration and invasiveness in cancer cells? Biochim Biophys Acta. 2015;1848(10 Pt B):2493-501. https://doi.org/10.1016/j.bbamem.2015.04.013.
8. Brisson L, Driffort V, Benoist L, Poet M, Counillon L, Antelmi E, et al. Nav1.5 na^+ channels allosterically regulate the nhe-1 exchanger and promote the activity of breast cancer cell invadopodia. J Cell Sci. 2013;126(Pt 21):4835-42. https://doi.org/10.1242/jcs.123901.
9. Eddy RJ, Weidmann MD, Sharma VP, Condeelis JS. Tumor cell invadopodia: Invasive protrusions that orchestrate metastasis. Trends Cell Biol. 2017;27(8):595-607. https://doi.org/10.1016/j.tcb.2017.03.003.
10. Takano T, Lin JH, Arcuino G, Gao Q, Yang J, Nedergaard M. Glutamate release promotes growth of malignant gliomas. Nat Med. 2001;7(9):1010-5. https://doi.org/10.1038/nm0901-1010.
11. de Groot J, Sontheimer H. Glutamate and the biology of gliomas. Glia. 2011;59(8):1181-9. https://doi.org/10.1002/glia.21113.
12. Willard SS, Koochekpour S. Glutamate signaling in benign and malignant disorders: Current status, future perspectives, and therapeutic implications. Int J Biol Sci. 2013;9(7):728-42. https://doi.org/10.7150/ijbs.6475.
13. Azahar, II, Sharudin NA, Noor Din AHM, Che Has AT, Mohd Nafi SN, Jaafar H, et al. Nnav1.5 expression is associated with glutamate level in breast cancer cells. Biol Res. 2022;55(1):18. https://doi.org/10.1186/s40659-022-00387-1.
14. Yang M, Kozminski DJ, Wold LA, Modak R, Calhoun JD, Isom LL, et al. Therapeutic potential for phenytoin: Targeting na(v)1.5 sodium channels to reduce migration and invasion in metastatic breast cancer. Breast Cancer Res Treat. 2012;134(2):603-15. https://doi.org/10.1007/s10549-012-2102-9.
15. Sharudin NA, Murtadha Noor Din AH, Azahar, II, Mohd Azlan M, Yaacob NS, Sarmiento ME, et al. Invasion and metastasis suppression by anti-neonatal nav1.5 antibodies in breast cancer. Asian Pac J Cancer Prev. 2022;23(9):2953-64. https://doi.org/10.31557/apjcp.2022.23.9.2953.
16. Koutras AK, Kalogeras KT, Dimopoulos MA, Wirtz RM, Dafni U, Briasoulis E, et al. Evaluation of the prognostic and predictive value of her family mrna expression in high-risk early breast cancer: A hellenic cooperative oncology group (hecog) study. Br J Cancer. 2008;99(11):1775-85. https://doi.org/10.1038/sj.bjc.6604769.
17. Peng J, Ou Q, Wu X, Zhang R, Zhao Q, Jiang W, et al. Expression of voltage-gated sodium channel nav1.5 in non-metastatic colon cancer and its associations with estrogen receptor (er)-β expression and clinical outcomes. Chin J Cancer. 2017;36(1):89. https://doi.org/10.1186/s40880-017-0253-0.
18. Gonçalves H, Jr., Guerra MR, Duarte Cintra JR, Fayer VA, Brum IV, Bustamante Teixeira MT. Survival study of triple-negative and non-triple-negative breast cancer in a brazilian cohort. Clin Med Insights Oncol. 2018;12:1179554918790563. https://doi.org/10.1177/1179554918790563.
19. Hsu JY, Chang CJ, Cheng JS. Survival, treatment regimens and medical costs of women newly diagnosed with metastatic triple-negative breast cancer. Sci Rep. 2022;12(1):729. https://doi.org/10.1038/s41598-021-04316-2.
20. Li H, Long J, Xie F, Kang K, Shi Y, Xu W, et al. Transcriptomic analysis and identification of prognostic biomarkers in cholangiocarcinoma. Oncol Rep. 2019;42(5):1833-42. https://doi.org/10.3892/or.2019.7318.
21. Rapti V, Moirogiorgou E, Koliou GA, Papadopoulou K, Binas I, Pentheroudakis G, et al. Mrna expression of specific her ligands and their association with clinical outcome in patients with metastatic breast cancer treated with trastuzumab. Oncol Lett. 2022;23(1):23. https://doi.org/10.3892/ol.2021.13141.
22. Yamaci RF, Fraser SP, Battaloglu E, Kaya H, Erguler K, Foster CS, et al. Neonatal nav1.5 protein expression in normal adult human tissues and breast cancer. Pathol Res Pract. 2017;213(8):900-7. https://doi.org/10.1016/j.prp.2017.06.003.
23. Kamarulzaman NS, Dewadas HD, Leow CY, Yaacob NS, Mokhtar NF. The role of rest and hdac2 in epigenetic dysregulation of nav1.5 and nnav1.5 expression in breast cancer. Cancer Cell Int. 2017;17:74. https://doi.org/10.1186/s12935-017-0442-6.
24. Erdoğan M, Ozpolat B. Targeting of nav1.5 channel in metastatic breast cancer models in vitro and in vivo mice as a novel therapy. Eur J Cancer. 2017;72:S43. https://doi.org/10.1016/S0959-8049(17)30221-6.
25. Chioni AM, Fraser SP, Pani F, Foran P, Wilkin GP, Diss JK, et al. A novel polyclonal antibody specific for the na(v)1.5 voltage-gated na(+) channel 'neonatal' splice form. J Neurosci Methods. 2005;147(2):88-98. https://doi.org/10.1016/j.jneumeth.2005.03.010.
26. Leslie TK, Tripp A, James AD, Fraser SP, Nelson M, Sajjaboontawee N, et al. A novel na(v)1.5-dependent feedback mechanism driving glycolytic acidification in breast cancer metastasis. Oncogene. 2024;43(34):2578-94. https://doi.org/10.1038/s41388-024-03098-x.