UNDERSTANDING THE ROLE OF nNav1.5 IN BREAST CANCER VIA siRNA APPROACH

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by

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DECLARATION

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(SHAREEZMA BINTI KAMARUL ZAMAN)

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LIST OF SYMBOLS AND ABBREVIATIONS

%	Percentage
°	Degree
°C	Degree celsius
µl	Microlitre
µM	Micrometre
g	Gram
mL	Millilitre
mm	Millimetre
ng/µL	Microgram per milliliter
α	Alpha
β	Beta
Δ	Delta
BRCA	Breast cancer gene
BSC	Biosafety cabinet
cDNA	Complementary DNA
CK5/6	Cytokeratin 5/6
CO_2	Carbon dioxide
DCIS	Ductal carcinoma in situ
DI: S3	Domain I segment 3 region
DMEM	Dulbecco's Minimum Essential Medium
DMSO	Dimethyl Sulfoxide
DNA	Deoxyribonucleic acid
ER	Oestrogen receptors
FBS	Foetal bovine serum

HER1/2	Human epidermal growth factor receptors 1/2
Mol	Motility index
mRNA	Messenger ribonucleic acid
n.s	Not significant
nNav1.5	Neonatal Nav1.5
PBS	Phosphate buffered saline
PCR	Polymerase Chain Reaction
PR	Progesterone receptors
RISC	RNA-Induced Silencing Complex
RNA	Ribonucleic Acid
RNAi	RNA interference
rpm	Revolutions per minute
RT-qPCR	Real-time Polymerase Chain Reaction
SD	Standard deviation
siRNA	Small interfering RNA
TGFβ1	Transforming Growth Factor Beta 1
TNBC	Triple-negative breast cancer
TTX	Tetrodoxin
VGSC	Voltage gated sodium channels
WHO	World Health Organization

MENYIASAT MOTILITI SEL DAN EKSPRESI mRNA nNav1.5

MENGGUNAKAN siRNA

ABSTRAK

Neonatal Nav1.5 (nNav1.5) memainkan peranan penting dalam perkembangan dan penyebaran kanser payudara. Kajian ini menekankan potensinya sebagai penanda biomolekul dan sasaran rawatan. Kanser payudara, yang merupakan salah satu kanser paling lazim di seluruh dunia, dicirikan oleh pertumbuhan sel yang tidak terkawal. Terapi yang disasarkan sangat diperlukan kerana metastasis bertanggungjawab terhadap kira-kira 90% kematian berkaitan kanser, walaupun terdapat kemajuan dalam pengesanan awal dan rawatan. Kajian ini meneroka mekanisme molekul terhadap peranan nNav1.5 dalam kebolehan invasif kanser payudara. Dalam sel MDA-MB-231, perencatan nNav1.5 menggunakan siRNA digunakan untuk menilai kesannya terhadap ekspresi mRNA nNav1.5 dan tingkah laku metastasis, motiliti sel. Hasil kajian terdahulu menunjukkan bahawa nNav1.5 diekspresikan secara berlebihan dalam sel kanser payudara metastatik berbanding dengan sel bukan metastatik, yang dikaitkan dengan peningkatan kebolehan invasif dan migrasi. Di dalam kajian ini potensi terapeutik penargetan nNav1.5 disokong apabila motiliti sel kanser menurun oleh perencatan nNav1.5 melalui siRNA. Kajian ini juga menyokong potensi ekspresi molekul nNav1.5 sebagai penanda prognostik berguna bagi subjenis kanser payudara yang agresif, kanser payu dara 'triple-negative'. Kajian ini menyediakan asas kukuh untuk penyelidikan masa depan dalam mengintegrasikan pendekatan yang disasarkan kepada nNav1.5 ke dalam rangka kerja perubatan presisi, dan ia menyumbang kepada pemahaman biologi metastasis. Penemuan ini berpotensi untuk meningkatkan kadar kelangsungan hidup pesakit dan penjagaan kanser payudara dengan menangani masalah kritikal metastasis.

INVESTIGATING CELL MOTILITY AND mRNA EXPRESSION OF nNav1.5 BY siRNA

ABSTRACT

Neonatal Nav1.5 (nNav1.5) plays a crucial role in the development and spread of breast cancer. This work highlights its potential as a biomarker and treatment target. The hallmarks of breast cancer, a cancer that is common around the world, are unchecked cell growth and metastasis. Targeted therapies are needed since metastasis is responsible for around 90% of cancer-related fatalities, despite the advancements in early identification and therapy. The study explored the molecular mechanisms underlying nNav1.5's role in the invasive property of breast cancer. In MDA-MB-231 cells, siRNA-mediated knockdown was used to assess its effect of nNav1.5 mRNA expression and metastatic behaviour, cell motility. Previous research results showed that nNav1.5 is substantially overexpressed in metastatic breast cancer cells relative to non-metastatic cells, which is associated with increased invasive and migratory capabilities. In this study, the potential role of nNav1.5 in the development of metastases was further supported by the significant decrease in cancer cell motility which siRNA-mediated silencing of nNav1.5. This study also supports nNav1.5 expression as a useful prognostic indicator for aggressive breast cancer subtypes, the triple-negative breast cancer. A solid basis for future research aiming at incorporating nNav1.5-targeted tactics into precision medicine frameworks is established by this work, which also contributes to our understanding of metastatic biology. These results could greatly improve patient survival and breast cancer care by tackling the crucial problem of metastasis.

CHAPTER 1: INTRODUCTION

1.1 Background of study

Cancer is characterised by unchecked cell growth and metastasis, or the spread of cancer to other parts of the body (National Cancer Institute 2021). The ability of cancer cells to proliferate in the absence of a growth signal is one of the many ways that they differ from healthy cells. They can divide even in the absence of external growth cues and are essentially "self-sufficient" in terms of growth (Gentry & Jackson, 2013). Anywhere in the body, cancerous cells can develop into tissue lumps that have the potential to develop into tumours. These tumours can be classified as either benign or malignant. Malignant tumours, are created when cancer cells invade neighbouring tissues, spreading to other parts of the body (Cooper, 2025). Because of its invasive nature, which disrupts the function of essential organs, cancer becomes even more dangerous.

1.1.1 Breast Cancer

Breast cancer is typified by aberrant cells that proliferate and spread uncontrollably at the breast and probably the surrounding tissues (World, 2024). Histologically, it is categorised based on whether the cells are of invaded cancers from other location, and whether the cancer started in the ducts or lobules (Makki, 2015). Inflammatory and pregnancy-related breast tumours are the most dangerous of these, and at diagnosis they frequently exhibit evidence of widespread invasion or metastasis (Lefrère et al., 2021). Additionally, breast cancer can be characterised according to molecular subtypes, each with unique characteristics based on the expression of oestrogen receptors (ER), progesterone receptors (PR), cytokeratin 5/6 (CK5/6), and human epidermal growth factor receptors 1 and 2 (HER1 and HER2) (Rivenbark et al., 2013).

Advances in gene expression profiling further led to the five intrinsic molecular subtypes: claudin-low, HER2-enriched, basal-like (triple-negative breast cancer (TNBC)), Luminal A (expressing the estrogen receptor), and Luminal B (expressing the estrogen receptor) (Lefrère et al., 2021).

1.1.2 Aetiology factor of breast cancer

There is no single cause of breast cancer, but the most common causes of breast cancer, is genetics, family history, aging, poor lifestyle (exposure to cancer related agents) (Djamgoz et al., 2019). Germ-line mutations in high-penetrance genes, including *BRCA1*, *BRCA2*, and others, are genetically responsible for 5–10% of all breast cancers (Gao et al. 2021). Breast cancer is also influenced by hormonal factors, including late menopause and early menstruation (Anderson et al., 2017). Women who are nulliparous or who become pregnant later in life are also at a higher risk of developing breast cancer. The aetiology of breast cancer is also influenced by diet and alcohol, with alcohol use linked to DNA damage and the development of breast cancer (Agostinetto et al., 2021). Diets high in fat contain a lot of cholesterol, raises the risk of breast cancer. A daily intake of 35–45g of dietary fibre can provide protection against the resorption of oestrogen (Gaskins et al., 2009).

1.1.3 Metastasis as a Hallmark of Cancer

It is reported that about one-third of women who are diagnosed with no disease go on to develop metastases (Djamgoz et al., 2019). Ninety percent of cancer-related deaths are caused by metastasis, a hallmark of the disease (Dillekås et al., 2019). In achieving metastasis, cancer cells split off, migrate through tissue, invade, enter the bloodstream or lymphatic system, re-attach, spread out, and develop into secondary tumours (Martin et al., 2017). The interplay and orchestration of oncogenes, adhesion molecules, cell cycle markers, and steroid hormones etc. are among the genes associated with metastasis (Djamgoz et al., 2019). Furthermore, due to cancer genetically unstable, they able to spreads and adjusting to the microenvironments of distant tissues (Lambert et al., 2017). Because of their heterogeneity, metastases can appear ten years or more after diagnosis, making it difficult to assess risk factors and find a cure (Feng et al., 2018). Indirect indicators of metastatic progression are frequently used in clinical management; however, new functional biomarkers are required to precisely forecast metastatic potential.

1.1.4 Voltage Gated Sodium Channels (VGSCs) and cascade

It has been discovered that voltage-gated sodium channels (VGSCs) play a role in a number of the metastatic cascade's regulating elements (Angus & Ruben, 2019). The VGSC was expressed by cancers of the breast, colon, lung, prostate, cervix, ovary, lymphomas, and melanomas. Although VGSC are known to generate and propagate action potentials in excitable cells like neurones and muscle fibre, their role in cancer is still unknown. This VGSC is conserved and highly upregulated in a wide variety of cancer

types, suggesting that it gives cancer cells some advantages or survivability (Argus & Ruben, 2019).

The neonatal splice form of the Nav1.5 protein, which belongs to the VGSCα family, is highly expressed in breast cancer. The pathobiology of breast cancer and the expression of functional nNav1.5 are consistent with an early event in the acquisition of metastatic potential. The finding of this Nav1.5 predominately express in the neonatal form in the breast cancer suggest it may be useful for biomarker for the cancer progression such as the prognostic marker and also can be the therapeutic target to reduce the metastasis (Nelson et al, 2015; Yamachi et al, 2017).

1.2 Problem statement

Breast cancer is known as one of the most prevalent and hazardous types of cancer globally. In terms of incidence, it refers to the number of new cases of breast cancer that occur in a specific population during a defined period. Breast cancer ranks among the top three most common cancers in terms of incidence, reflecting its high frequency of new cases diagnosed in the year 2022 (GLOBOCAN, 2022). Mortality, on the other hand, is the total number of deaths from breast cancer during a given time frame. Globally, 6.8% number of cancer mortality meanwhile, 11.1% cases in year 2022 for both sexes and all ages. It indicates the fatality rate of the disease and is often used to assess the impact of breast cancer on overall survival rates and public health. The necessity for ongoing research into the underlying processes and potential treatments of breast cancer is underscored by its high incidence. Breast cancer is still a significant cause of death despite improvements in early detection and treatment, primarily because of its propensity to spread. The metastasis process, in which cancer cells migrate from the initial tumour site

to other parts of the body, is responsible for about 90% of cancer-related deaths, making treatment considerably more complex (Chaffer & Weinberg, 2021).

Emerging research has gradually emphasized the voltage-gated sodium channel nNav1.5 as a dangerous factor in the metastatic potential of breast cancer (Brackenbury et al., 2022). *In vitro*, nNav1.5 plays a crucial role in enhancing cancer cell migration and invasion, while *in vivo*, it aids in the movement of cancer cells to distant organs by promoting metastasis (Brackenbury et al., 2022). Studies have indicated that nNav1.5 overexpression in breast cancer is linked to poorer patient outcomes and heightened metastatic activity, marking it as a critical factor in the aggressiveness of metastatic breast cancer (Nelson et al., 2022). Therefore, nNav1.5 could be a novel biomarker for detecting and predicting breast cancer metastasis. This research seeks to deepen our understanding of how nNav1.5 drives metastatic processes and explore its potential as a target for diagnostic and therapeutic strategies by studying its molecular mechanisms. These findings could lead to developing targeted therapies to inhibit cancer spread and improve prognostic tools, ultimately benefiting patients. Moreover, our work aims to provide valuable insights into cancer metastasis biology while contributing to the creation of effective treatments for metastatic breast cancer.

1.3 Rationale of study

The rationale behind this study stems from the urgent need to improve the clinical management of breast cancer, particularly in addressing metastasis, which is responsible for most breast cancer-related deaths (Chaffer & Weinberg, 2021). By focusing on the role of nNav1.5, a voltage-gated sodium channel that has been strongly associated with metastatic behaviour, this study aims to evaluate whether targeting nNav1.5 can slow

down the progression of cancer cells from the primary tumour in the breast to distant organs (Brackenbury et al., 2022). This research could provide valuable evidence supporting the role of nNav1.5 as a biomarker for metastatic breast cancer if this research is accomplished. This study highlights the findings related to nNav1.5, contributing to increased cell metastasis and intrusion. This is known as the critical process in cancer metastasis. By modulating nNav1.5, the ability to slow down and can also inhibit the metastasis in the cell. This could lead to its validation as a critical biomarker for early detection of metastatic progression. In turn, this would add to the growing body of evidence positioning nNav1.5 as a diagnostic marker and a therapeutic target for breast cancer treatment (Nelson et al., 2022). The clinical treatment of breast cancer could be significantly enhanced by this study's confirmation of nNav1.5's involvement in metastasis. Creating therapeutic interventions that target nNav1.5 may lead to new treatment options, improve patient outcomes, and lessen the spread of metastatic disease.

1.4 Objective

1.4.1 General Objective

To conduct cell culture and siRNA nNav1.5 treatment on MDA-MB-231 cells.

1.4.2 Specific Objective

i. To assess the effect of siRNA-nNav1.5 on nNav1.5 mRNA expression level.

ii. To measure the effect of siRNA-nNav1.5 on the motility of MDA-MB-231 cells.

1.5 Hypothesis

1.5.1 Null Hypothesis (H0)

i. There is no effect of siRNA nNav1.5 treatment on the viability or characteristics of the MDA-MB-231 cell culture.

ii. There is no reduction in the nNav1.5 mRNA expression level in MDA-MB-231 cells after siRNA-nNav1.5 treatment.

iii. There is no effect of siRNA-nNav1.5 treatment on the motility of MDA-MB-231 cells.

1.5.2 Alternative Hypothesis (HA)

i. There is an effect of siRNA nNav1.5 treatment on the viability or characteristics of the MDA-MB-231 cell culture.

ii. There is a reduction in the nNav1.5 mRNA expression level in MDA-MB-231 cells after siRNA-nNav1.5 treatment.

iii. There is an effect of siRNA-nNav1.5 treatment on the motility of dtfiMDA-MB-231 cells.