DOI: https://doi.org/10.53350/pjmhs2115123764

ORIGINAL ARTICLE

The Expression of Sodium Channel Alpha Subunit (Nav1.7) Receptors in Intracranial Meningioma and Its Comparison to Urothelial, Prostate and Ovarian Cancers

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ABSTRACT

Nav1.7/SCN9A is a voltage gated sodium ion channel (VGSC), expressed by nociceptive neurons. Its role in pain mechanism was well- established but its involvement in the carcinogenic pathways is still under investigation. We aimed to explore the expression of Nav1.7/ SCN9A receptors in intracranial meningiomas and compare it with urothelial, prostate, and ovarian cancers.

Methods: Ten paraffin embedded tissue samples of brain meningioma and 5 cases each of bladder, prostate and ovarian carcinomas were utilized. Immunohistochemistry (IHC) was performed using anti-SCN9A antibody. Cases were scored based on the intensity of expression and number of positive tumour cells. A score of (+3) indicated highest intensity, (+2) as moderate and (+1) as weak intensity. Similarly, 50-100% expression in cells was labelled as (+3), moderate, 30-50% as (+2) and 10-20% as weak or (+1) expression, and (0) as negative.

Results: Nine cases of meningioma were in grade I and single case was grade III. The nine grade I meningioma were negative for SCN9/Nav1.7 expression while the single grade III case was positive. Tumour cells in urothelial, prostate, and ovarian carcinomas were all strongly positive for SCN9/Nav1.7, having intensity expression as (+3).

Conclusions: This study suggests an emerging role of Nav1.7/SCN9A receptor expression in urothelial, prostate, and ovarian cancers as well as grade III meningiomas compared to grade I meningioma. This clarifies that Nav1.7/SCN9A has a possible role in carcinogenesis of most body tumours.

Key Words: Nav1.7, sodium channels, Ion channels, Channelopathy, meningioma, carcinoma

INTRODUCTION

Voltage-gated sodium channels (VGSCs) have been associated to disorders in which neurons get excited e.g. epilepsy and chronic pain. The role of these channels in physiological and pathological pathways related to autism, migraine, multiple sclerosis, cancers and immunological disorders has also been known (1). VGSCs increase the action potentials in neurons, monocytes, glial cells and other excitable cells (2).

Family of VGSCs comprise of nine principal alpha subunits (VGSCα) and auxiliary 4ß subunits (VGSC-ß). Proteins encoded by VGSC genes are named Nav1.1 though Nav 1.9. These genes are referred to as SCN1A through SCN11A. SCN6/7A gene is part of Nax subfamily and its function is unknown. VGSC genes are usually have single copy and termed as singletons and have small protein interaction networks. It needs to be further investigated (3). Sodium channels are integral membrane protein that form ion channels, conducting sodium ions. They are classified according to the trigger that opens these channels for such ions i.e., voltage dependent or Nav and are ligand gated channels (3).

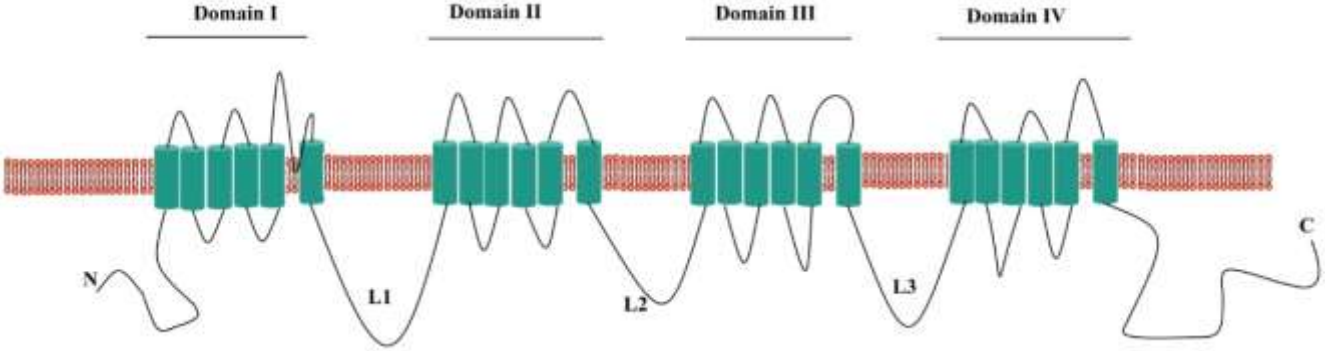

Figure 1: Schematic diagram of Nav1.7 alpha subunit structure showing the four domains, I-IV and 6 transmembrane segments of each domain

VGSCs regulate the conduction of sodium ions across the cell membrane and generate action potential. After activation, VGSC will open, conduct sodium ion and cause depolarization, which will further activate the surrounding potassium gated channels responsible for repolarization. Hence, VGSCs are very essential for the transmission of nerve impulses (4,8,5). VGSC mediate inward sodium current during action potential in the excitable cells (3). Three Na channels (1.7, 1.8 and 1.9) are preferentially expressed in peripheral neurons. Nav1.7 is required for synaptic signaling and the release of substance P or Glutamate from nociceptive neurons (**Figure 2**)(6). In this study, we have focused on Nav1.7, to explore its immunolocalization and expression in urothelial, prostate, and ovarian carcinomas and compared it with the intracranial meningiomas. We are comparing the brain and non-brain tumors and at the same time, we have studied the differences of expression of Nav1.7 among these carcinomas.

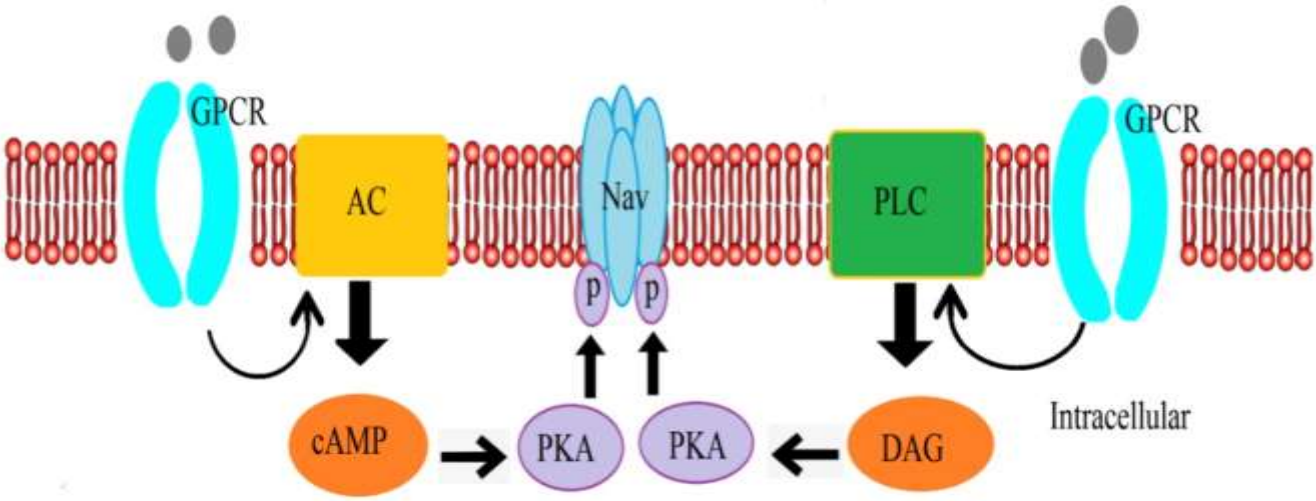

Figure 2: Cartoon diagram of signaling pathways regulating the Nav ion channels. G-Protein coupled receptor (GPCR) is activated upon binding of ligands, consequently stimulating the adenylyl cyclase (AC) and Phospholipase C (PLC) producing cyclic adenosine monophosphate C (PKC). PKA and PKC modulates the Nav channel to perform its function, derived from Wang et al., [6]

MATERIAL AND METHODS

Tissue Collection: The study was performed after getting the approval from ERB of the Hospital. Complete history was taken from all the patients after taking written informed consent. Samples of meningioma were collected from the subjects of both sexes having age from 35 y to 81 y. Being meeting the diagnostic criteria, totally there were 10 cases of meningioma (grade I cases 9 & grade III cases 1). The grading and staging were not deliberated in other cases of cancers. Tissues of brain were taken as a control for Nav1.7 immunohistochemistry (Table 2).

Immunohistochemical (IHC) staining protocol: Size of 3 µm to 4µm thick tissue pieces of the brain tissue, bladder, intracranial meningioma, ovary prostate were attached on APES (3-aminopropyltriethoxysilane) coated slides, rehydrated by solutions of graded ethanol and deparaffinized in xylene. When non-specific reactions were blocked by 10% normal serum of rabbit, the fragments were gestated by primary Anti-Nav1.7 antibody (Rabbit polyclonal, 1:100, ab65167, Abcam) for one h. Manually staining was done. Then pieces were incubated by secondary antibody for thirty minutes & stained by DAB & straddling with rising medium. Slides were assessed via two histopathologists & the average score was measured.

Evaluation of Immunohistochemical staining: The examination of slides was done under light microscopy, product of the immunohistochemical reaction was identified inside the cytoplasm. The intensity of cytoplasmic staining was counted semi-quantitatively, as previously discussed method(8), as 0% to 10% = negative, 10% to 30% = 1+ (weak staining), 30% to 60% = 2+ (moderate staining), 60% to 100% = 3+ (strong staining).

Statistical Analysis: Using SPSS 25 data was analyzed. Quantitative parameters were showed by frequencies and quantitative parameters in mean $\pm$ SD. Fishers exact and was chi-square test was smeared to analyze the relationship between stain intensity and grade of cancer.

RESULTS

Ten paraffin embedded tissue samples of diagnosed meningioma cases and 5 cases each of bladder, prostate and ovarian carcinoma were immunohistochemically analyzed. The mean age of the patients with meningioma was 47±14 years (age range 35-81 years), prostate patients was 55±15 years, ovarian carcinoma was 50.3±3 years and urothelial carcinoma was 61.0 $\pm$ 3.0 years respectively. There were 6 females and 4 males in meningioma cases (Table 1). Nine cases were grade I and single case was grade III for meningioma (Table 2). Brain tissue was used as a normal control and all the cases of normal brain tissue were strongly positive (+3) (Figure 3A-C). Nav1.7 expression appeared to be cytoplasmic in neuronal cells in the brain tissue (Table 2, Figure3A-C).

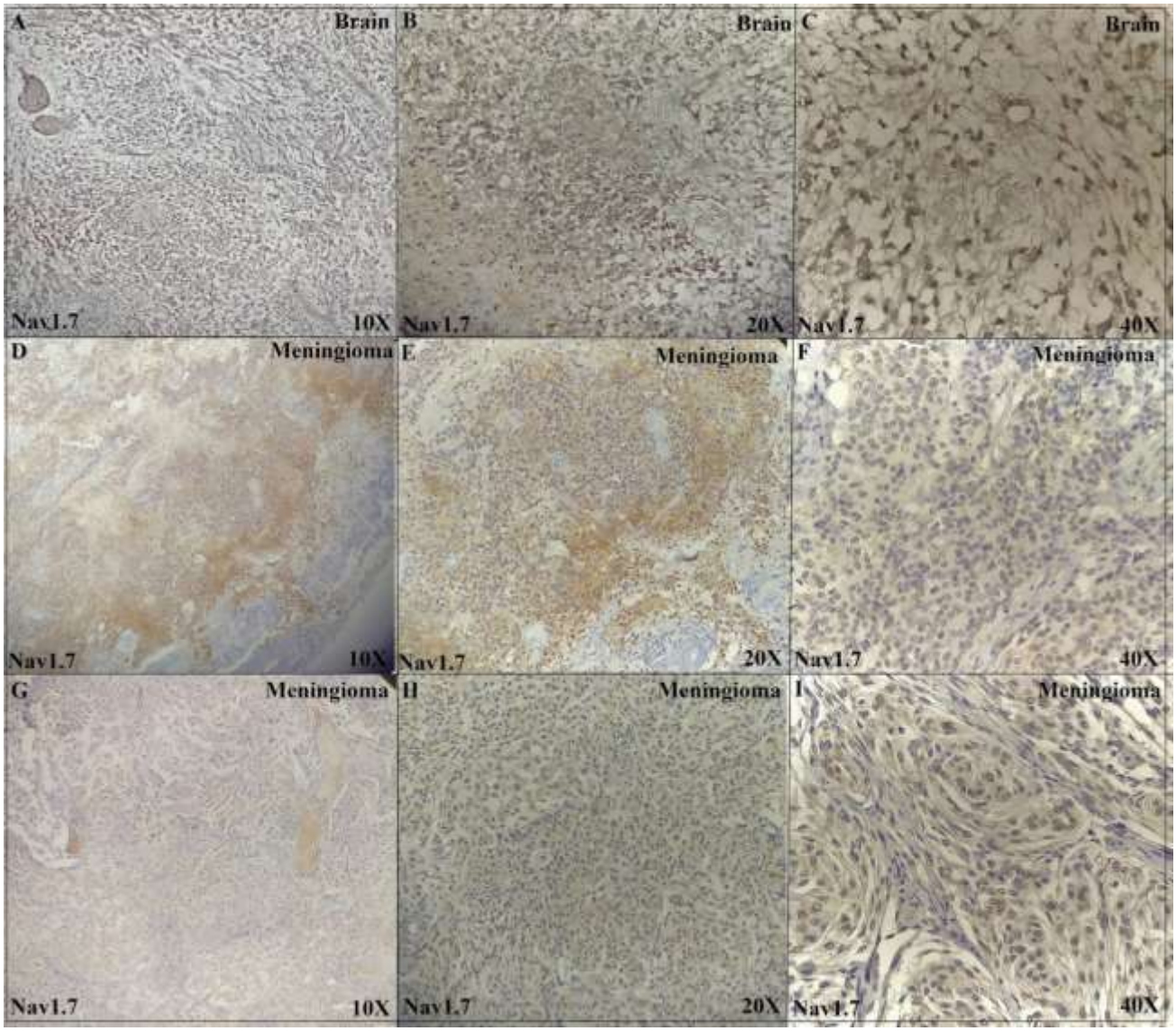

Figure 3: Imunohistochemistry for Nav1.7 A-C) Photomicrograph of brain tissue showing immunohistochemical expression of Nav1.7/ SCN9A at 10X, 20X and 40X (Intensity and expression +3 score), Note cytoplasmic expression of Nav1.7, D-F) Meningioma Grade III at 10X 20X, 40X were also positive, +1 intensity and +1 expression, also note the extracellular expression of Nav1.7, G-I) Meningioma Grade I at 10X, 20X, 40X, showing negative intensity and expression

Table 1: Biographical data. Mean age and Gender in all cancers

	Cancer Type				
	Overall (n=40)	Bladder(n=10)	Ovary(n=10)	Prostate(n=10)	Meningioma(n=10)
Age (Y)	53.25 ± 11.5	61.0 ± 3.0	50.3 ± 3.0	55 ± 15.0	47.0 ± 14.0
Male	19(47.5%)	5(50.0%)	0(0.0%)	10(100.0%)	4(40.0%)
Female	21(52..5%)	5(50.0%)	10(100.0%)	0(0.0%)	6(60.0%)

Table 2: The association between stain Intensity/Expression and tumour type or grading.

Tumor Type		Nav1.7 Intensity/Expression			Total	Pattern of Staining
		Weak Intensity	Moderate Intensity	Highest Intensity		
Bladder		0	0	10	10	Cytoplasmic
Ovary		0	0	10	10	Cytoplasmic
Prostate		0	0	10	10	Cytoplasmic
Meningioma	G1	0	0	0	9	Extracellular
	G3	0	1	0	1	
Brain control		0	0	10	10	Cytoplasmic

The immunohistochemical analysis revealed negative expression of Nav1.7/SCN9A in all the grade I meningiomas (Figure 3 D-I) while it was strongly positive (+3) in all the non-brain cancers (Table 2, Figure 4 A-I). The single case of grade III meningioma was also positive for Nav1.7/SCN9A (Figure 4 D-F). Immunolocalization of Nav1.7 among non-brain tumors appeared to by cytoplasmic in all the cases of bladder, prostate and ovary (Table 2, Figure 4A-I). These findings are very interesting because this is the first time such a comparison among brain and non-brain tumors has been conducted and has discussed the immunolocalization, intensity and expression. This comparison clarifies that Nav1.7/SCN9A receptor expression is associated with high grade tumours as well as cancers. A significant association has also been observed between grade of tumor and Nav1.7 expression and intensity among the non-brain tumors.

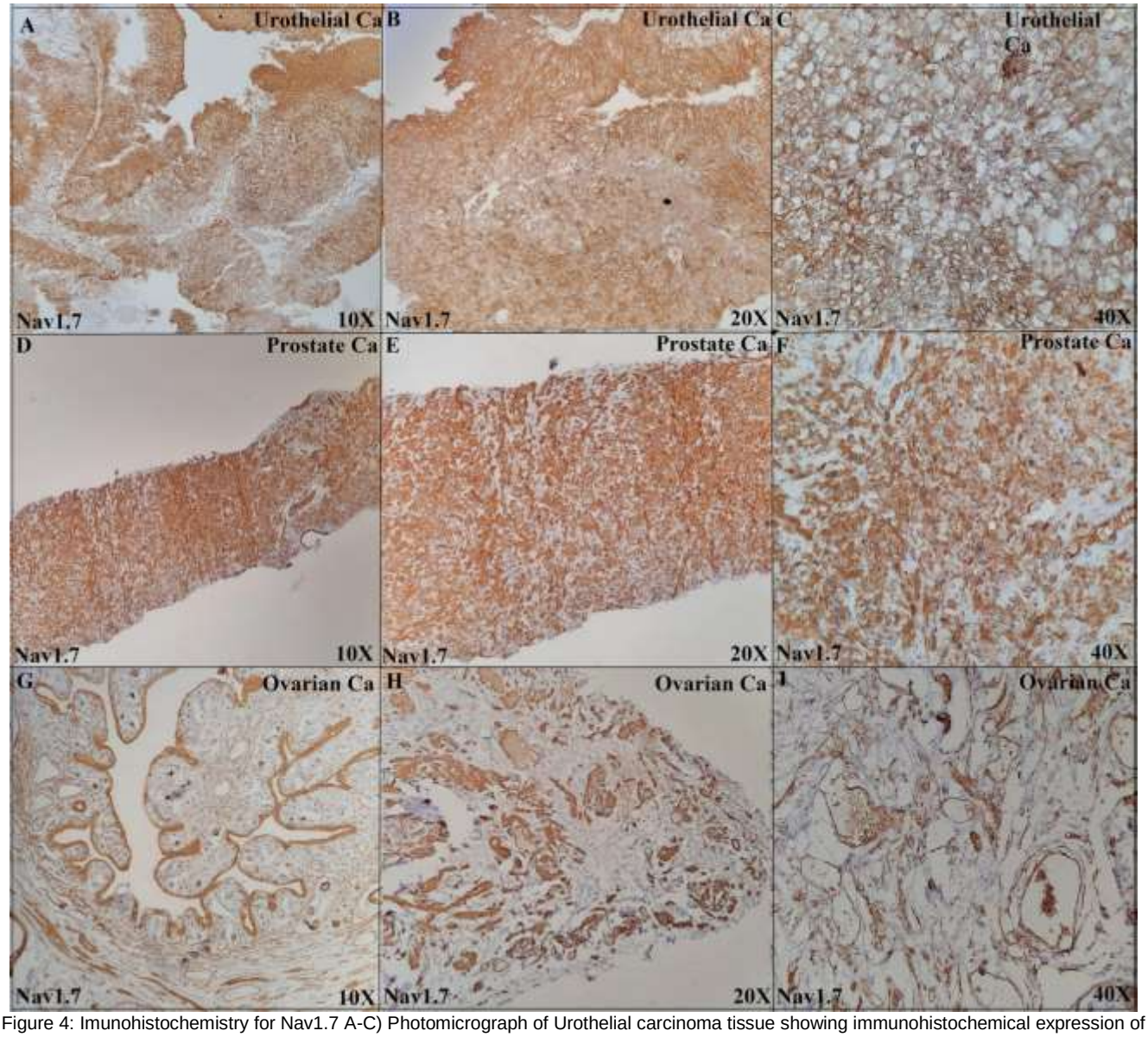

Figure 4: Imunohistochemistry for Nav1.7 A-C) Photomicrograph of Urothelial carcinoma tissue showing immunohistochemical expression of Nav1.7/ SCN9A at 10X, 20X and 40X (Intensity and expression +3 score), Note cytoplasmic expression of Nav1.7, D-F) Prostate Carcinoma-IHC at 10X 20X, 40X showing strong positive, +3 intensity and +3 expression, also note the cytoplasmic expression of Nav1.7, G-I) Ovarian Carcinoma-IHC at 10X, 20X, 40X, showing strong expression +3 and intensity +3

DISCUSSION

Cancer is a multifactorial disease, the etiological factors include a combination of epidemiological factors, genetics, and epigenetics. Epigenetics plays its role from initiation and continues till progression of carcinogenesis (9). Hence, it may be possible that VGSCs may have differential expression in different cancers and different tissues due to epigenetics involvements which must be elucidated. Ion

channels are being discovered in cancer cell lines and in-vivo models but little is known related to the expression, role, mechanisms, and behavior of these channels in different cancers (10). VGSCs are functionally expressed in epithelial cancers, breast, colon, lung, skin and prostate etc in-vivo and they promote the disease progression and metastasis (11). Different sodium channel alpha subunits are expressed differently in different diseases and in different cancers. Nav1.5 is expressed in breast (12) and colon carcinomas (13). Nav1.7 was expressed in breast (12), prostate (11) and lung cancers (14). These were in-vitro studies.

VGSCs may contribute to many of the cell behaviors which are required for metastasis, such as migration (15),proliferation (5), invasion (16), grouping of cells in the form of a colony (17), cell-cell adhesion (18), vascularization (19), nitric oxide synthesis (20) etc. The underlying mechanisms for these cell behaviors related to VGSCs is not understood. VGSCs are regulated by hormones and growth factors e.g. in breast and prostate cancer, estrogen and androgen respectively, regulate the disease progression (10). Immune cells also contribute to these regulation mechanisms in the pathway of cancer (10). Known facts regarding the role and mechanisms of VGSCs in cancer are less clear and it's an ongoing area of research. A relatively recent review published in 2019 stated, 'invadopodia', which is the leading edge of metastatic cancer cells, as a possible role in cancer.

VGSCs are responsible for membrane depolarization and initiating the action potential across the membrane and may activate the VGSC. These mechanisms have functional implications for invadopodia and a possible mechanism between VGSC expression and cancer invasiveness (5). VGSCα subunits are mainly involved in the potentiation and progression of cancer and the use of its antagonist may block the function and expression of these channels, thereby, reducing the metastatic and other cancerous activities of cell (10). Hormone based therapies along with the VGSC blockers in combination may be an effective treatment regimen for the treatment of such cancers (10).

Nav1.7 has been found to be associated with endometrial carcinoma and suggested as a prognostic marker in a study published in 2019 (21). In a study on rat prostate cancer cell lines Mat-Lylu, suggested a role of Nav1.7 dependent regulation of Rho-GTPase activity in cell migration and invasion during carcinogenesis (22). Nav1.7 was studied in gastric cancer tissue and gastric cancer cell lines (BGC-823 and MKN-28 cells) and Real time PCR analysis revealed an upregulated expression of Nav1.7 in cancerous tissue and cell lines as compared to the non-malignant tissues. Immunohistochemical evaluation also showed an elevated Nav1.7 expression in these tissues and associated with cancer progression and poor prognosis (23). In another study, nav1.7 expression was evaluated in normal, hyperplasia and prostatic cancer cell lines. PCR analysis showed an upregulated expression of Nav1.7 in prostate cell lines (24). Therefore, Nav1.7 is a potential prognostic marker and/or therapeutic target for GC.VGSC expression and intensity may be used as a successful marker to grade tumor stage and its metastatic potential as reported in a study conducted on rat model of prostate cancer (25). When there are patients with same grade of tumor, with varying intensity and expression of VGSC, the cases with more expression and intensity of VGSCs may have more chances of recurrence, poor prognosis and metastasis as reported in a breast cancer study with Nav1.5 expression (26). It is also worth-mentioning that VGSCs are although abundantly expressed in neurons but are also expressed in later stages of cancers, the reason is unclear. Hence, VGSCs may be a potential drug target against the cancerous cell behavior of cells in order to treat this deadly disease and improve the prognosis in such patients (27).

Our results show, an elevated expression of Nav1.7 in urothelial, prostate and ovarian carcinoma, and grade III meningioma while decreased expression in grade I benign meningioma. Hence, we may speculate that Nav1.7, is expressed in cancers and high-grade tumours while it is not expressed in benign tumours. However, the expression of Nav1.7 in normal brain tissue explains that these receptors are present in normal tissues. To our knowledge, this is the first study highlights the immunolocalization, expression and the significance of VGNC in different tumours including brain meningioma. Further studies are required elucidate the underlying mechanisms of VGNC in tumours.

CONCLUSIONS

Increased expression of sodium channel alpha subunit 9, Nav1.7 may be involved in the progression of carcinoma of bladder, ovary, and prostate. It may also be involved in grade III meningioma where its decreased expression may be responsible for disease progression. The underlying pathways, mechanisms and genetics must be further investigated in future studies. It may serve as an important prognostic biomarker in tumour progression.

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