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Article

Whole Exome Sequencing of Meningioma in Sudanese Patients

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Abstract: Introduction Meningioma is the second most common primary intracranial tumor of the central nervous system. in Sudan, meningioma is the most common primary brain tumor, among Sudanese patients**,** Surgical total excision of meningioma offers a better survival, however, Chemotherapy has largely been unsuccessful for meningioma treatment, and therefore, refractory and recurrence meningioma treated with palliative surgery and radiotherapy. Monosomy of chromosome 22 is the utmost common genetic alteration among meningioma and many genes were investigated and described in the development of this tumor of which , *NF2 hSNF5/INI1*gene, *P53.* In addition to this, Molecular signal pathways in meningioma, and that contribute to the survival, proliferation, self-renewal, and differentiation properties of normal stem cells which are abnormally activated and nominated as cancerous stem cell (CSCs)**. Objectives** 1/ To identify the possible pathway of candidate genes in both setting and whole genes hypothesis free approach 2/ To assess the presence of mutations in candidate genes among histological subtypes of meningioma in Sudan**. Material and methods** This is a prospective cross-sectional study done at the National Center of Neurological Sciences (NCNS) Khartoum, from 2018 to 2021. The study included all cerebral tumors that were radio- logically diagnosed preoperatively as meningioma. Ethical approval was taken from Institute of Endemic disease, Khartoum University, and written consent was obtained Three DNA extracts were randomly selected from 20 meningioma tissue samples and processed for WES. All genes harboring exonic and splice site variations stratified into SNVs and INDELs sets were prepared. The 1000G online database was used to explore novel mutations per 3 samples. SIFT and PolyPhen-2 programs were used to predict possible impact of amino acids substitution. To address meticulous genes interaction and particular centrality, all shared genes within the class INDEL and SNVs were used as input on Network Analysis to predict protein- protein interaction and functional gene ontology. Finally, shared mutations within genes of centrality were detected via Sanger sequencing and mutations were correlated between histological sub-types and recurrenceThree non meningioma brain tumor used as control. **Results** WES analysis revealed high number for mutational signature within 2 classes (INDEL and SNVs) per 3 samples, the findings of 1000 genome online database showed number of novel mutations; 5618 in fibrous , 6032 in meningothelial while in recurrent fibrous sample 5930. Network analysis of shared genes per each class (INDEL, SNVs), showed *UBC* is the most protein of remarkable degree centrality over thousand of genes. Whereas, all shared genes per 3 samples within the 2 classes (INDEL and SNVs) revealed *Akt1* with the highest degree of centrality and betweenness. ***Akt1*** rs17846829was detected in 20% of the samples, while *CD44* stemness gene revealed mutation A>G (rs9666607) in all meningioma samples (100%), this mutation was associated with 60% of the recurrence. **Conclusions** Nevertheless, the present study, highlight how such complex molecular processes might contribute to tumorogenesis, in attempt to shed light over thousands of genes and multifaceted network analysis rather than a solitary gene which is challenging question.

Keywords: WES; *AKT1*; *CD44;* meningioma; candidate genes**;** NCNS

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Introduction

Meningioma is the second most common primary intracranial tumor of the central nervous system, comprising nearly 30% of all primary brain tumors. [1,2,] in Sudan, meningioma is the most common primary brain tumor, among Sudanese patients.[3]Surgical total excision of meningioma offers a better survival and clinical outcome to patients; however, up to 18% of benign meningioma, 40% of atypical meningioma, and 80% of malignant meningioma recur within 5 years despite of complete excision. [4] Chemotherapy has largely been unsuccessful for meningioma treatment, and therefore, refractory and recurrence meningioma treated with palliative surgery and radiotherapy.[5,6]In this context need for definite therapeutic approaches based on effective molecular targets in order to improve outcome and or long-term control of meningioma is urgent. However; still, in-adequate of clinical predictive power remains one of the most critical impediments in the development of novel study models. [7]

Genetic of Meningioma:

Monosomy of chromosome 22 is the utmost common genetic alteration among meningioma and was one of thefirst cytogenetic alterations described in solid tumors. Mutations within the *NF2* gene were detected in up to 60% of sporadic meningioma and are typically associated with loss of heterozygosity(LOH) 22q.[8]

The majority*NF2*mutations have a shortening effect, which indicate inactivation of the *NF2*gene product Merlin(schwannomin), this gene is functionally important inmeningioma pathogenesis, absent or reduced immune- reactivity in some studiesforMerlin was found in the bulk of meningioma,[9] and was strongly associated with loss of heterozygosis 22q.Although the suppressive activity of this gene is poorly understood, the signaling cascade disruption is considered in tumor formation. One study showed that, molecular genetics and protein of *NF2* was differ among fibroblastic, transitional and meningiothelial meningioma which are the common benignvariant, merlin reduction was observed in the first two variant but rarely in the last one, this observation may suggests the genetic origin of this tumor is independent of *NF2* gene alteration. Similarly study done in Sudan at the National center for neurological sciences (unpublished data), in meningioma among Sudanese patients, revealed mutations in *NF2* gene is not involved in meningioma progression.

In addition to this, some candidate tumor suppressor genes on 22q were screened for mutations in meningioma, one of these was *hSNF5/INI1*gene, was frequently mutated in atypical teratoid/rhabdoidtumors. [10], *LARGE* gene which maps on (22q12.3) and denotes another candidate gene.[11]

Nevertheless, the well-known tumor suppressor gene, *P53*, which is located on chromosome 17 short arm, is one of most frequent mutated gene in human cancer and brain astrocytoma, however, mutation in this gene are rarely seen in meningioma.[12] Several large studies reported some mutations, among which study done in Sudan by Gassoum et al. [13]

Molecular Signaling Pathways

Molecular signal pathways in meningioma, are many and among which is mitogenic signals transduction, approximately all growth factor receptors and kinases are recognized to be involved in tumor growth and being contributing factors in meningioma cell lines, of which epidermal growth factor receptor (*EGFR*), platelet-derived growth factor receptor β (*PDGFRβ*), vascular endothelial growth factor receptor (*VEGFR*) and insulin-like growth factor receptor (*IGFR*).[14]

Moreover, there are many signaling pathways that contribute to the survival, proliferation, self-renewal, and differentiation properties of normal stem cells which are abnormally activated and nominated as cancerous stem cell (CSCs) and thus causing tumorigenesis. In this context, various endogenous or exogenous genes and microRNAs regulate these complex pathways. Eventually, these signaling pathways can induce downstream gene expression, such as cytokines, growth factors, apoptosis genes, anti - apoptotic genes, proliferation genes, and metastasis genes in CSCs. The

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importance of these signaling pathways regulates and linked networks of signaling mediators to regulate cancerous stem cell growth.

These signaling pathways include, Wnts pathway which include large protein ligands, plays an important role in the dedifferentiation of CSCs, metastasis and regulating CSC apoptosis,[15]It affects miscellaneous processes, such as the generation of cell polarity, and cells fate, Aberrant Wnt signaling is found in many aggressive cancers,[16] beside this, activation of Wnt induce the transformation of dormant CSCs into active CSCs which eventually enhancing cell cycle progression through β-catenin.[17]In addition, long noncoding RNAs and microRNAs also promote self-renewal of CSCs through Wnt signaling pathway, this occurred in many cancers such as gastric. [18]

Objectives

1/ To identify the possible pathway of candidate genes in both setting and whole genes hypothesis free approach

2/ To assess the presence of mutations in candidate genes among histological subtypes of meningioma in Sudan

Material and Methods

This is a prospective cross-sectional study done at the National Center of Neurological Sciences (NCNS) Khartoum, from 2018 to 2021. The study included all cerebral tumors that were radiologically diagnosed preoperatively as meningioma. Ethical approval was taken from Institute of Endemic disease, Khartoum University, and written consent was obtained from each patient.

The tumor specimens were obtained immediately from the operating room, before surgery was completed, and sent to the research laboratory in the center within 2-5 minutes. At arrival to the laboratory, small portion from 20 tissue samples were processed for DNA extraction, three of which were selected for whole exome analysis and each was processed further for detection of gene of centrality by using PCR and thus sequencing analysis. Three non meningioma brain tumor were used as control

Whole Exome Sequencing (WES)

Sample Preparation

After evaluating the detailed histopathology of our tumor specimens, 3 samples were selected for DNA extraction for whole exome analysis and each was proceeded further for detection genes of centrality.DNA quality and quantity were assessed using Nano-Drop ND-1000 (Nano-Drop Products, Wilmington, DE, USA) spectrophotometer at wavelength spectrum of 220-750nm and standard 1% agarose gel electrophoresis. Each sample was labeled with a unique accession number, and DNA was sent to Beijing Genomics Institute (BGI) for whole exome sequencing.

DNA Extraction Method

DNA was extracted from tissues obtained from meningioma tumors using Guanidine chloride method. (According to the National Center of Neurological sciences standard protocol)

Exploratory Analysis

Genes set were prepared by filtering for all genes harboring exonic and splice site variations stratified into SNVs and INDELs set genes for each sample. In addition to that, we used 1000G online database to explore novel mutations. And also we explored the excess variant of allele frequency per 3 samples.

To predict possible impact of an amino acid substitution on the structure and function of a human protein, SIFT and PolyPhen-2 programs were used.

In addition to this, shared genes filtering for all genes which stratified into two classes (SNVs and INDELs) per 3 sample, were constructed by utilizing R software (version 4.1.0, 2021-05-18)

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Network Analysis Using Network Analyst 2021

In this study, Network analyst 2021 was used to put up individual functional interaction network of genes per each sample, and for gene set which harboring damaging effect. Although there was number of mutated genes, they were not all included in network analysis, we managed shared gene analysis in both class (INDEL and SNVs) per 3 sample, when needed , high and moderate, low and modifier impact were defined in SNVs. On topography of network analysis, genes of centrality can be conveyed.

To address meticulous genes interaction, and whether these interactions with particular centrality, all shared genes within the class INDEL and SNVs were used as input on Network Analyst (GenMania, Reactome and STRING) to predict protein- protein interaction and functional gene onto

Pathway and GO Enrichment Analysis

Based on enrichment analysis online tools (Network Analyst 2021), which was used to examine pathways enrichment for sets of genes in various data base, and application to forecast diseases associated.

Additionally, shared mutations within genes of centrality were detected using polymerase chain reaction by means of specific forward and reverse primers.

The primers were designed using Primer 3 online program. The table below is containing the list of primers used in this study.

AKT1	F	CCCCTCAGATGATCTCTCCA
	R	TGAAGAATTTGGAGGGAAGG
CD44	F	AGGAACAGTGGTTTGGCAAC
	R	AACTGGCTTGTATCCATTCCT

Results

In this study, we present the result of genetic whole exome sequence analysis of 3 samples from our patients. DNA was labeled as: sample 1B denote fibrous subtype, sample 2B indicates meningiothelial subtype while 4A is fibrous recurrence.

Result of Exploratory Analysis

There was high number for mutational signature within 2 classes is shown in Tables 3 and 4.

Moreover, when we utilized 1000 genome online database to categorize novel mutations, our results showed that, number of mutations in fibrous were 5618, in meningiothelialwere 6032 while in fibrous recurrence were 5930. The result of excess transition C>T, in fibrous recurrencewas 1063, whereas in fibrouswas 1049 and meningiothelialwas 1127, the low allele frequency was T>A, in fibrous 189, meningiothelial 204and fibrous recurrence 213 (Table 5).

Table 3. number of single nucleotide variations and insertion deletion per 3 samples in each class.

Class	Sample	All	Novel	Miss	Intronic	3UTR	5UTR	Splicing
SNVs	1B	116490	930	11323	74276	3481	2125	2560
	2B	119465	6032	11512	75940	3734	379	2386
	4A	118987	5930	10719	77996	3689	369	2157
INDEL				Frs				
	1B	15103	4884	662	11638	554	282	660
	2B	15579	5007	94	3743	173	76	278
	4A	17114	5745	627	13269	660	336	677

1B is fibrous type, 2B is meningiothelial and 4A is fibrous recurrence, Miss=missense, Frs=frame-shift.

Table 4. number of single nucleotide variations and insertion deletion with high and moderate impact per 3 samples.

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Sample	Total high impact SNVs/INDEL	Total moderate impact SNVs/INDEL	P value
1B	17	253	<0.001
2B	17	267	<0.001
4A	11	242	<0.001

1B is fibrous type, 2B is meningiothelial and 4A is fibrous recurrence.

As shown in Table 6, the total number of missense variants with high and moderate impact in class (SNVs) was 253 in fibrous meningioma. SIFT deleterious effect was detected in 54 mutations, among which 16 variants showed damaging effect by PolyPhen-2, as the same time, meningiothelial subtype which included 267 missense variants, by SIFT 63 were deleterious, among which, 18 variants were damaging. SIFT mutations in fibrous recurrence revealed that, 48 mutations were deleterious, among which 30 were damaging by PolyPhen-2.

Table 6. number of single nucleotide variations and the prediction effect of SIFT and PolyPhen-2 per 3 samples.

Sample	SIFT/Total deleterious effect	PolyPhen-2/total D	PolyPhen-2/total P/D	PolyPhen-2/total/B
1B/253SNVs	54	16	10	21
2B/267SNVs	63	18	18	20
4A/242SNVs	48	30	16	25

1B is fibrous type, 2B is meningiothelial and 4A is fibrous recurrence, D=damaging, P/D=probably damaging, B=benign. SIFT score <0.05=deleterious, PolyPhen p=>0. 5 (probably damaging/ damaging).

Network analysis results of class insertion/ deletion (INDEL), the most fabulous features is UBC protein of remarkable degree centrality over thousand of genes, the centrality was considered using degree of centrality and betweenness. Furthermore, other genes of centrality AKt1 and *CD44* were detected.

When we used all shared genes in class SNVs, the network analysis, revealed that, UBC showed the highest degree of centrality, this is with other central hub genes of stemness and non stemenesswhich included*STAT3*, *CD44*, *GLI1*, *KDR*, *KITTP53*, *Akt1* and*MAPK1*,these genes interacting with nearly 2000 of proteins, direct interaction of gene is shown in

Despite cancer is multiple complex processes, we aimed to verify number of mutations that might be related to cancer. In this context we used all shared gene in 2 classes (INDEL and SNVs), and based on Network Analyst, we demonstrated that, 172 number of proteins were interacting, AKt1 protein showed the highest degree centrality and betweenness (degree=52, betweenness=1657.89) flowed by *EGFR*, *PIK3CA*, *UBC*, *SOS1* and varying number of stemness proteins among which were *STAT3*, *MAPK*, *CD44*, *NOTCH* and *SMO* (Figure 8a).Additionally, to take in hand whether these interaction were true particularly of centrality among huge partner, we utilized random selection of genes set, striking finding *Akt1* and *UBC* showed the highest centrality and betweeness (degree= 52, betweenness=1657.89) and (degree=42 betweenness=1317.9) respectively, along with other stemness genes (Figure 8b).

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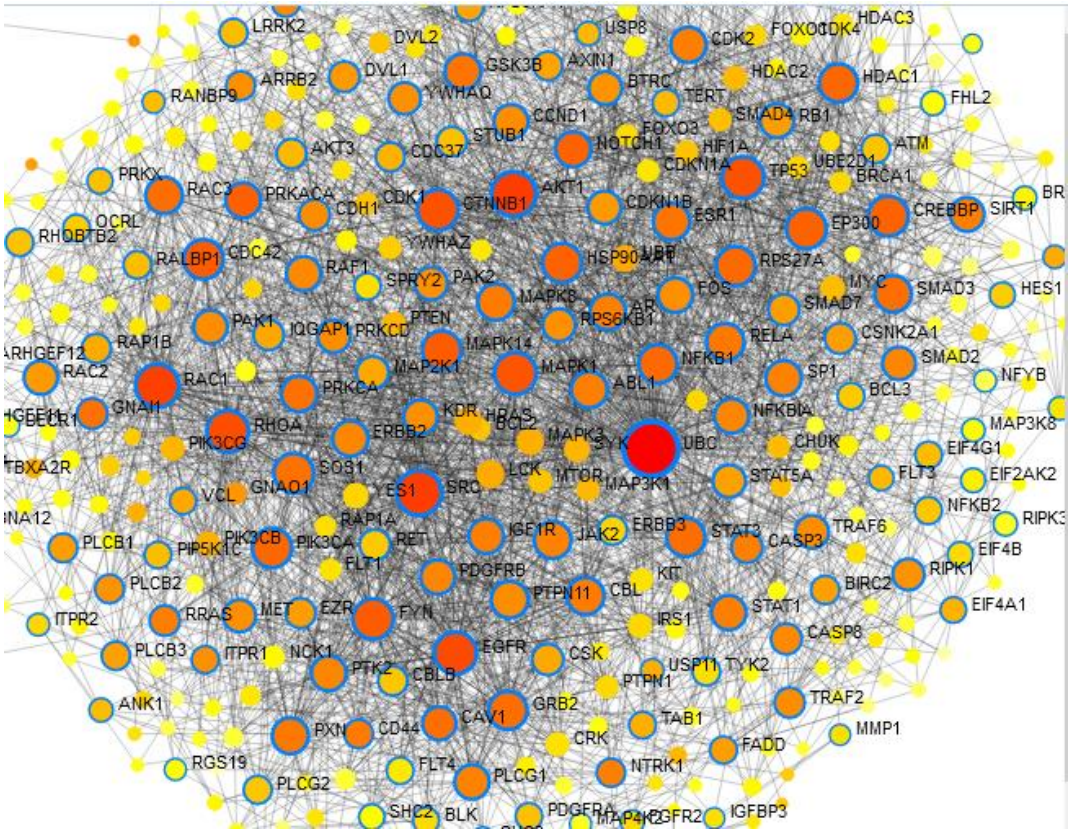

Figure 8a. This figure shows *UBC*, *Akt1* and *CD44* network interaction together with other hub genes per 3 samples, input included all shared genes harboring effect on both classes (SNVs and INDEL), *UBC* and *Akt1* are red in color while *CD44* is brown and highlighted with blue circle color, the interacted proteins are present in yellow color.

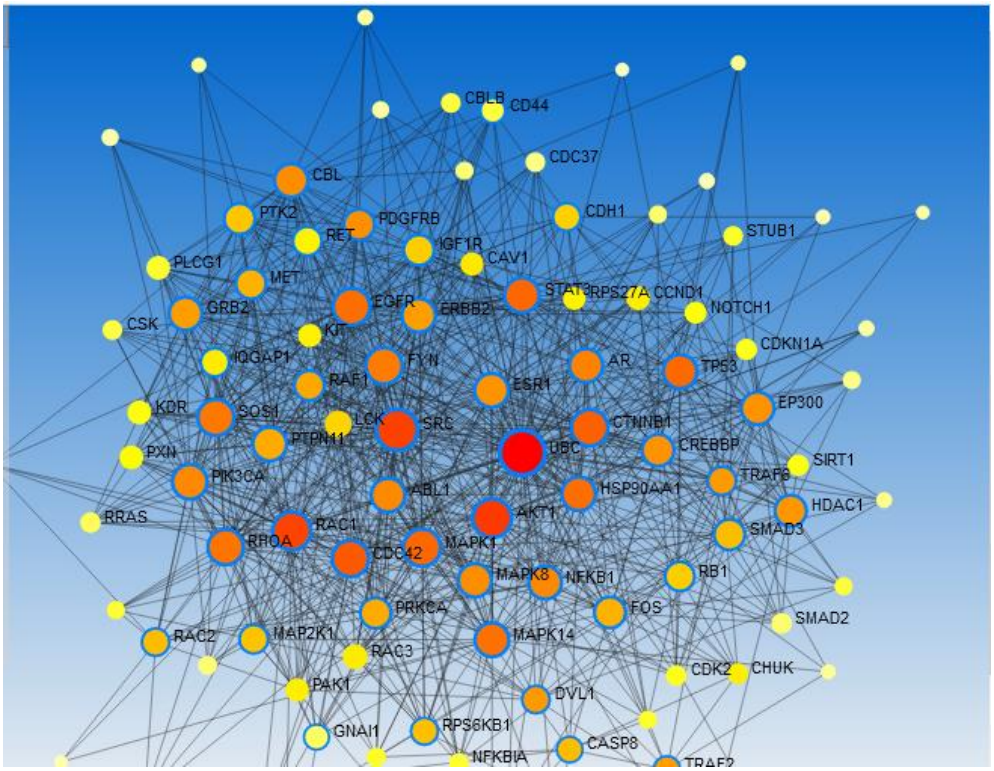

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Figure 8b. random selection shows *UBC*, *Akt1* and *CD44* network interaction together with other hub genes per 3 samples, input included random selection of shared genes harboring effect on both classes (SNVs and INDEL), *UBC* and *Akt1* are red in color while *CD44* is yellow and highlighted with blue circle color.

To figure out, physiological functions, as in put, all shared genes within 2 classes were used, our results by using signaling network, *Akt1* showed high degree centrality and connected to PI3K pathway and yelled its proteins family (Figure 9). Nevertheless, when we utilized gene regularity network (GRN) Aug 2020, to predict miRNA, our findings for instance, revealed number of stemness proteins and non stemness interact with each other(Figure 10).

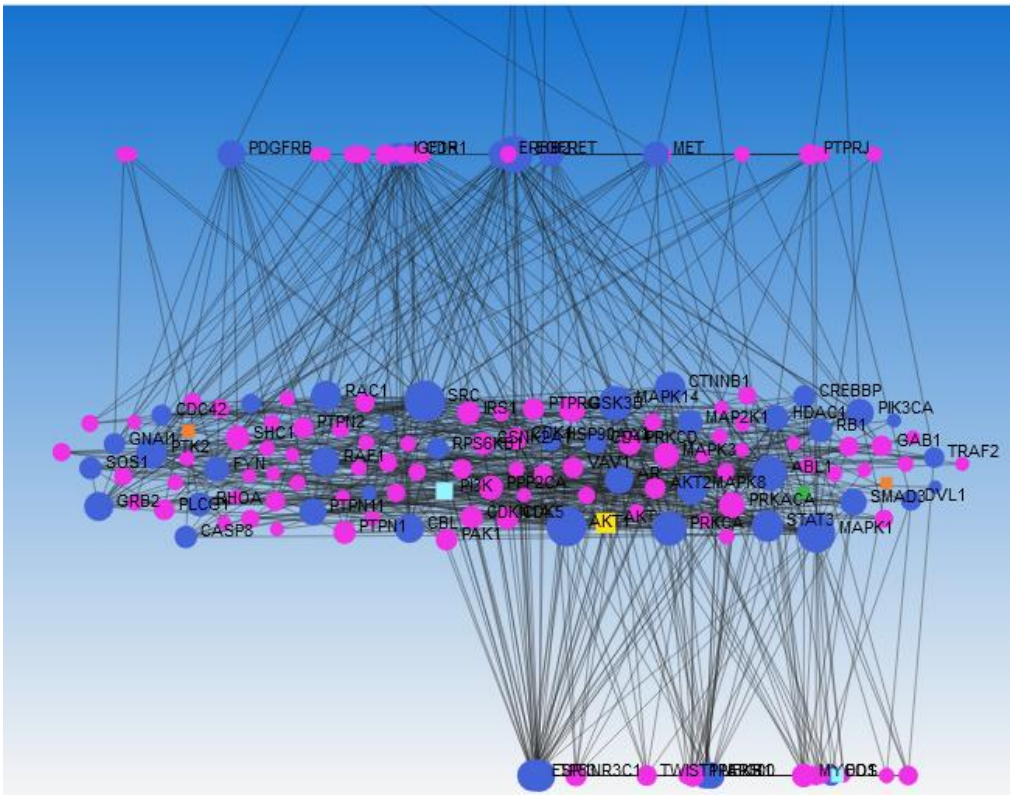

Figure 9. signaling pathway shows physiological function of shared genes per 2 classes. *Akt1* protein and protein family shows high degree centrality.Violet: protein, blue: chemical, green: complex, yellow: protein family, dark brown: small molecule, brown: undefined.

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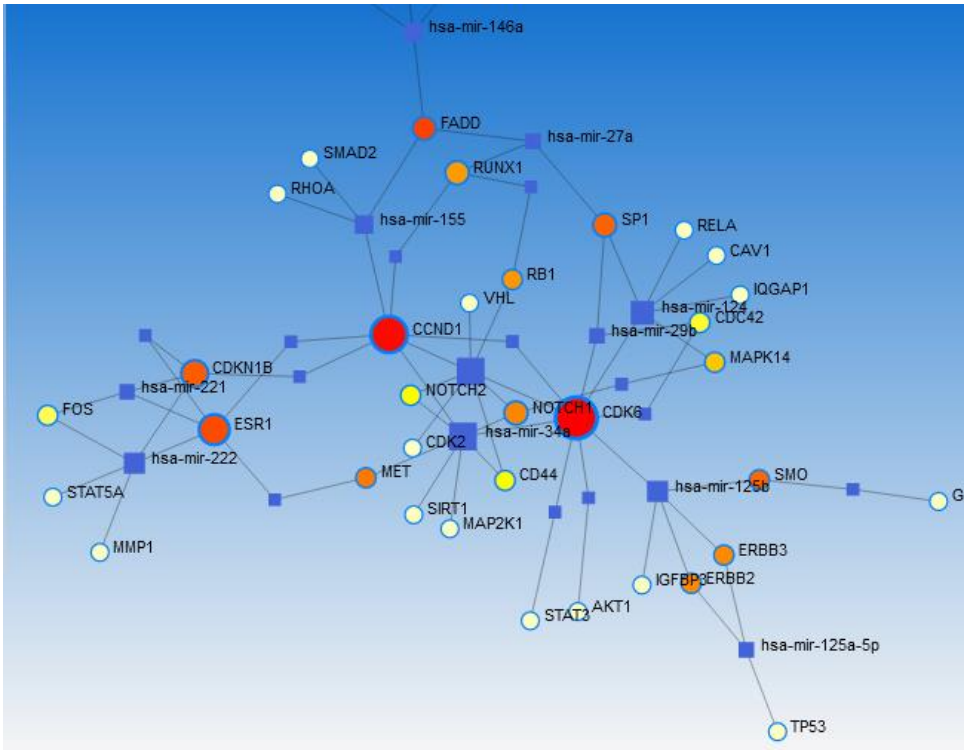

Figure 10. Gene regulatory network (GRN) showed Gene-miRNA-interactions. MicroRNA (miRNA)–gene interactions are well-recognized as involved in the progression of almost all cancer types. Red= nodes, purple=miRna.

Our result after genes application provided significant pathways related to cancer (Table7). Striking finding, enrichment analysis of gene/disease association revealed that, most of shared genes per 3 samples in 2 classes are related to cancers development, among which is meningioma brain tumor, white test was used to define p value (Table 8).

Table 7. Enriched pathways and p value in mutated shared genes per 3 meningioma samples.

Pathway	P value
Pathways in cancer	1.61E-70
Proteoglycans in cancer	2.83E-40
ErbB signaling pathway	2.14E-28
Focal adhesion	6.22E-28
MAPK signaling pathway	2.03E-27
Prostate cancer	1.71E-26
Pancreatic cancer	0.02E-25
Breast cancer	1.21E-25
Colorectal cancer	2.4E-25
Glioma	4.72E-20
Wnt signaling pathway	8.79E-18
MicroRNAs in cancer	2.03E-17
PI3K-Akt signaling pathway	9.42E-17
Signaling pathways regulating pluripotency of stem cells	5.32E-11
mTOR signaling pathway	9.57E-08
Hedgehog signaling pathway	4.05E-07
Phosphatidylinositol signaling system	5.85E-06

P value is written using White test.

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Table 8. Disease in shared genes in 2 classes per 3 samples Enrichr.

Disease Name	P value
Malignant neoplasm of thyroid	2.060e-20
Ovarian Carcinoma	2.770e-26
Transitional Meningioma	0.00001274
Rhabdomyosarcoma	1.382e-18
Neuroectodermal Tumors	9.353e-8
GlioblastomaMultiforme	2.024e-22
Endometrial Neoplasms	1.741e-15
Sphenoid Wing Meningioma	0.00001588
Clear Cell Meningioma	0.00001588
Squamous cell carcinoma of the head and neck	1.458e-22
Carcinoma of bladder	2.235e-23
Malignant Peripheral Nerve Sheath Tumor	4.794e-14

P value is written using White test.

PCR and Sanger Sequencing Results of Genes of Centrality (CD44 and Akt1)

In the present study, 20 meningioma samples (11 fibrous, 4 meningiothelial, 2 atypical and 3 clear cell meningioma) were amplified for *Akt1* and *CD44* genes, 40 PCR product (20 *Akt1* and 20 *CD44*) were analyzed by standard sequencing, the findings of *Akt1* gene showed that, the rs17846829 was detected in 20% of the samples (2 fibrous and 2 atypical meningioma), this mutation (rs17846829) was found in sample 1B (fibrous meningioma) only among our WES data.In addition to that, deletion A at position (chr14:105235886_105235886delT) in *Akt1* was detected in 85% of our samples (8 fibrous, 4 meningiothelial, 3 atypical and 2 clear cell meningioma), moreover rs58565216 was seen in 63% of the fibrous meningioma.

The findings of *CD44* gene revealed that, A>G (rs9666607) was detected in all our meningioma samples (100%), interestingly, this mutation A>G (rs9666607) was sharedper 3 samples ofour WES data, frame shift mutation (D419Tfs*61) was detected in 45% of the samples (6 fibrous, 2 clear cell and 1 meningiothelial meningioma) (Table 9 and Figures 11, 12, 13a, 13b, 14, 15a, 15b).

Table 9. | histopathology, WHO grades, recurrence and mutations of *Akt1* and *CD44* genes in our meningioma samples.

NO	Histopathology variant	WHO Grades	Recurrence	Mutation *Akt1*	Mutation *CD44*
1	Meningiothelial	1	NO	Del A	A>G (rs9666607)
2	Clear cell	11	NO		A>G (rs9666607) D419Tfs*61 InsC
3	Meningiothelial	1	NO	Del A	A>G (rs9666607)
4	Meningiothelial	1	NO	Del A/ InsC	A>G (rs9666607)
5	Fibrous	1	NO	G>A (rs17846829)	A>G (rs9666607) D419Tfs*61
6	Clear cell	11	Yes	Del A	A>G (rs9666607)
7	Fibrous	1	Yes	Del A G>A(rs17846829)	A>G (rs9666607) D419Tfs*61 InsC
8	Fibrous	1	Yes	Del A	A>G (rs9666607) D419Tfs*61
9	Fibrous	1	Yes	Del A	A>G (rs9666607)
10	Atypical	11	Yes	Del A	A>G (rs9666607)

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				G>A (rs17846829)	
11	Fibrous	1	Yes	Del A InsC	A>G (rs9666607) InsC
12	Fibrous	1	NO		A>G (rs9666607) D419Tfs*61
13	Fibrous	1	Yes	Del A	A>G (rs9666607)
14	Fibrous	1	Yes	Del A	A>G (rs9666607) D419Tfs*61
15	Fibrous	1	NO	Del A	A>G (rs9666607) InsC
16	Fibrous	1	NO		A>G (rs9666607) D419Tfs*61
17	Clear cell	11	Yes	Del A	A>G (rs9666607) D419Tfs*61
18	Fibrous	1	Yes	Del A	A>G (rs9666607)
19	Atypical	11	Yes	Del A G>A(rs17846829)	A>G (rs9666607)
20	Meningiothelial	1	Yes	Del A	A>G (rs9666607) D419Tfs*61

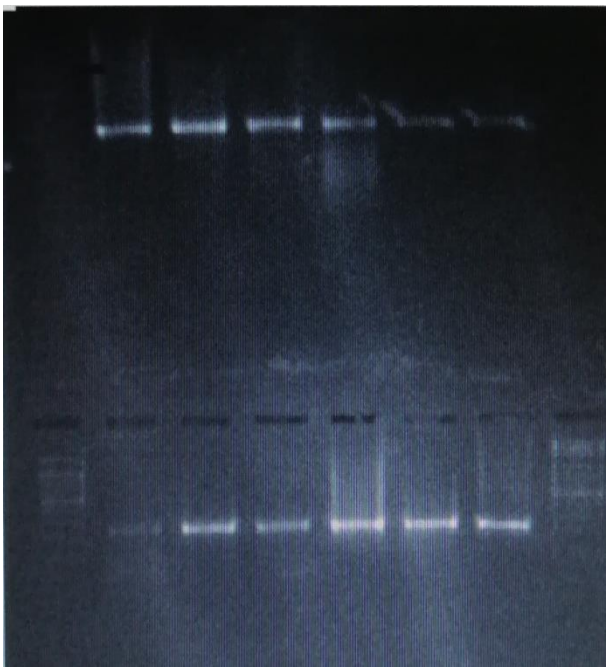

Figure 11. Gel electrophoresis of *Akt1*gene, lanes 1-6 shows band size 232 bp.

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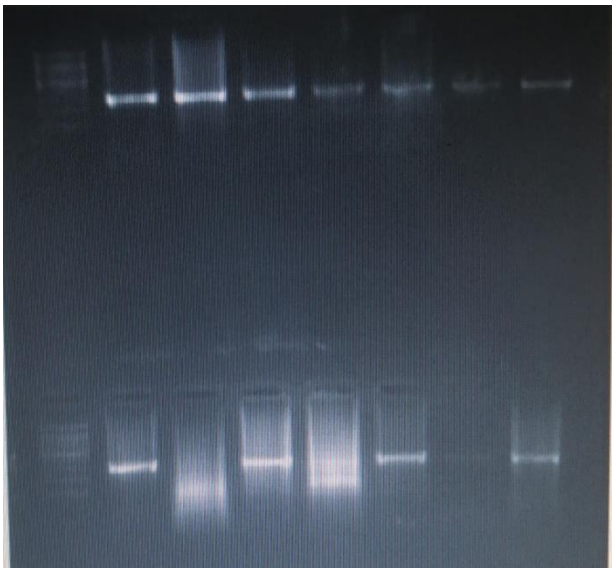

Figure 12. Gel electrophoresis of *CD44* gene, lanes 1-7 shows band size 286 bp.

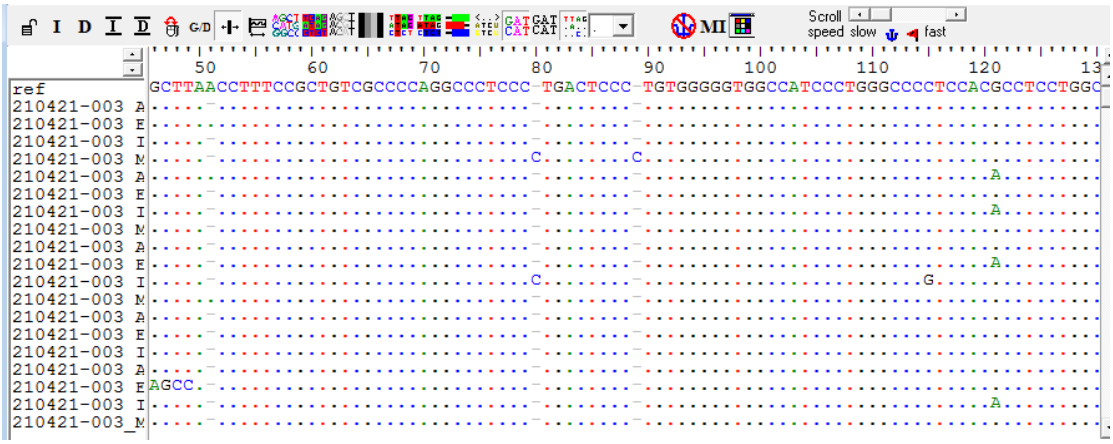

Figure 13a. alignment of *Akt1*gene sequencing shows, deletion A at position (chr14:105235886_105235886delT) and G>A (rs17846829).

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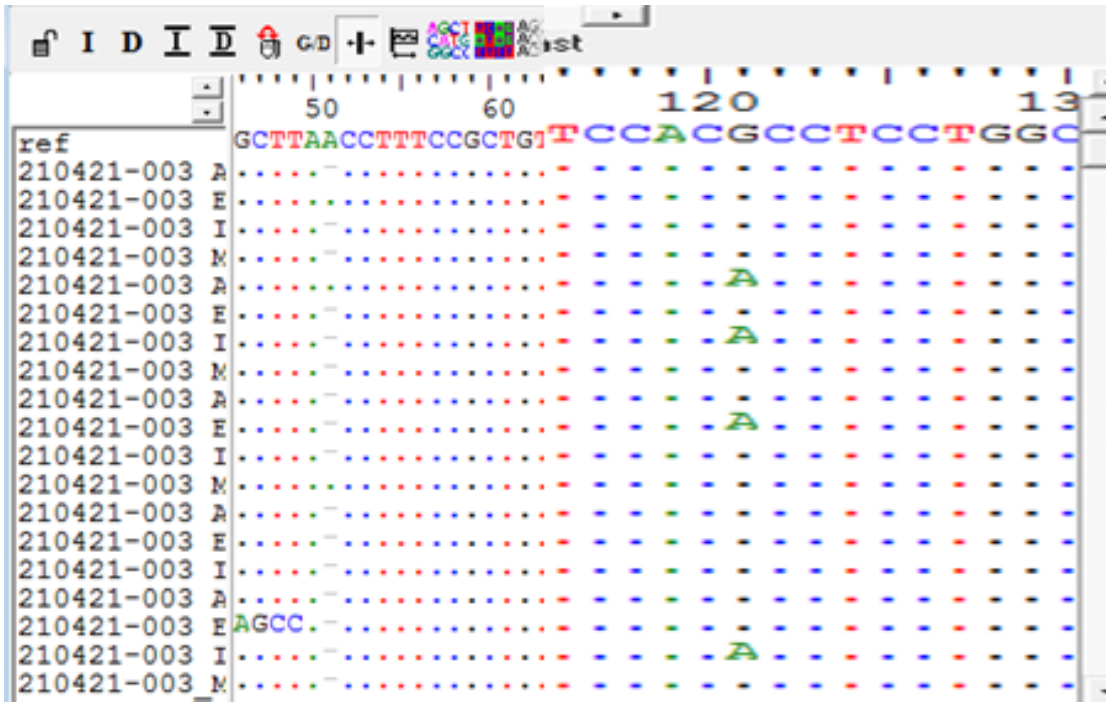

Figure 13b. magnified alignment of *Akt1*gene sequencing shows, deletion A at position (chr14:105235886_105235886delT) and G>A (rs17846829).

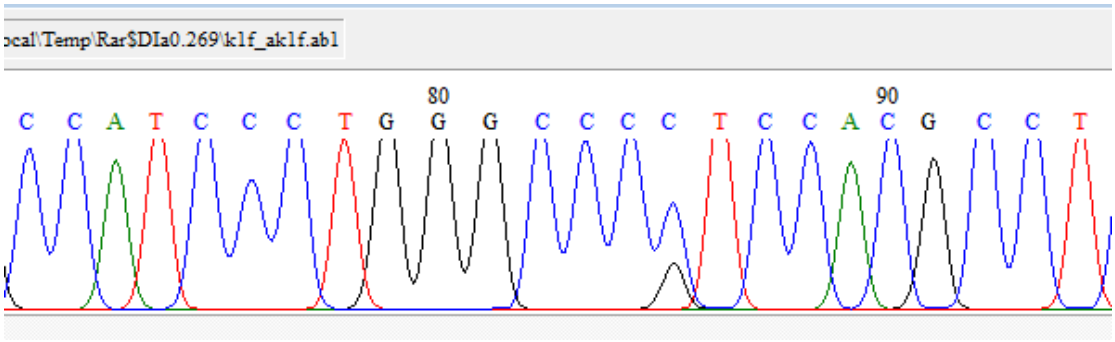

Figure 14. Chromogram of *Akt1*gene sequencing shows, polymorphism C>G (rs58565216).

Discussion:

Meningioma is the most common brain tumor in Sudan and mostly reported in female, [3] similarly, in this current study, there was clear cut towards female predominance. Most of these tumors are slowly growing and associated with symptoms of gradual increase in intracranial pressure, in this context, headache was the main cardinal feature among our patients. Understanding of the normal development of the nervous system has noticeably improved in recent years, it has a complex differentiation hierarchy ranging from a neural stem cell that can give rise to all of the major lineages in the brain parenchyma to lineage committed progenitors that have a more restricted differentiation potential to terminally differentiated cells. Stem cell concepts can influence the understanding of brain cancer. The cancer stem cell hypothesis proposes that established tumors consist of a cellular hierarchy with a subpopulation of tumor cells able to maintain and propagate the tumor. In this current study, thousands of mutations were predicted by whole exome sequencing analysis, of these high C>T of transversions mutations followed by G>A were observed per fibrous, meningiothelial and fibrous recurrence, our results goes with international results, that different cancer type have different mutational signature similar to gastric cancer in which the prevalence of C>T and C>A transversions were high.[19]What is more, among our samples, numerous mutations

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that harboring deleterious poly phen 2 effects were found, which highlight the genetic complexity on this type of brain tumor. Are these thousands mutation are being claimed with development of tumorigenesis process on this type of brain tumor, a question is not merely answered. Despite the similarity, further investigations are considered necessary in our study to investigate prospective functions.

In recent years, the availability of large-scale network and genomic data sets allow studying the association between the position of proteins within molecular networks and their patterns of molecular evolution,[20] the consequence of these studies have shown the individual genes are affected by the position that their encoded products occupy in molecular networks. Genes acting at the center of protein–protein interaction networks and metabolic networks evolve under higher levels of purifying selection than those acting at fundamentals of a network periphery, and those genes are so named genes/ proteins of Centrality. Hence Protein interactions play important roles in vital biological processes such as cell cycle control, metabolic and signaling pathways and disease pathways. These interactions can be represented as complex networks, where the nodes are the proteins and the edges represent the interactions between the pairs of proteins they connect.[21]

With regard to the potential of our datasets to guide precision analysis, genes/ protein of centrality are *Akt1*, *UBC* and numerous stemness genes like *CD44*, which emphasize the significance of network.The significance of *Akt1* gene encodes one of the three members of the human *Akt* serine-threonine protein kinase family which are often referred to as protein kinase B alpha, beta, and gamma. These proteins are phosphorylated by phosphoinositide 3-kinase (PI3K), AKT/PI3K forms a key in component of many signaling pathways that involve the binding of membrane-bound ligands resembling receptor tyrosine kinases, G-protein coupled receptors, and integrin-linked kinase. Therefore, these *Akt* proteins regulate wide variety of cellular functions of which cell proliferation, survival, metabolism, and angiogenesis together in normal and malignant cells, this is along with, *Akt* proteins are recruited to the cell membrane by phosphatidylinositol 3, 4, 5-trisphosphate (PIP3, 4, 5).Mutations in this gene are associated with multiple types of cancer like breast, colorectal, and ovarian cancers.[22]

UBC genewhich is located on 12q24.3, consisting of 2 exons,the promoter of it contains heat shock element which renders and mediates *UBC* stimulation upon stress. It also plays key role in maintaining cellular ubiquitin level under stress condition.[23]

Ubiquitin (*Ub*), was first identified by Gideon Goldstein et al. in 1975 and further confirmed over the next several decades in the human genome. It is highly conserved regulatory protein containing 76 amino acids, can be covalently tagged to target proteins via cascade of enzymatic reactions and regulates numerous physiological and pathological functions.[24,25]

Moreover, this gene is associated with various signaling pathways, of which important nutrient and environmental stimulus. The amino acid plays critical role in the mechanistic target of rapamycin complex 1 (mTORC1) signaling pathway. [26,27] In cancerous stem cell, it regulates and maintains stemness of genes expression likewise *Oct4*, *Sox2* and *Nanog*.[28]It also plays fundamental role in CSC characteristics, such as self-renewal, maintenance, differentiation and tumor-genesis.[29]

Notably, this gene is up regulated in several cancer, meanwhile the role of *UBC* in meningioma should be addressed, in this current study, *UBC* per 2 classes is mutated and represented gene of centrality within each class individually, however, it is not the case, when all shared being the case.

Tumor is the disease which is characterized by multiple phenotypes of which is abnormal cell growth and miscellaneous biological progression. The interaction of biological entities of genes, proteins and metabolites is of great interest in cancer development, as shown in our results, signaling pathway that yelled protein of *Akt* family. Recently, our results on using next generation sequencing, identified multiple genetic signaling pathways of which is Pathways in cancer, Proteoglycans in cancer and PI3K-Akt signaling pathway.

It is known that signaling pathways control cell cycle, however, the mechanism and interactions among these pathways vary between individual tumor and tumor types. For instance, our results among meningioma histological subtypes, taking into account pathways, more than 10 pathways are significantly co expressed and have been compared in a number of pathway data base. Interestingly,

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these pathways are of considerable similarity between our 3 meningioma sample, and this might denote genetic resemblance, since most of input genes did not show variation. Furthermore, our exome analysis highlight number of pathways that related to cancer development; of these, proteoglycan pathway which provided connection between cell membrane and surrounding extra cellular membrane, thus playing vital role in regulating cell cancer, adhesion and migration. Another detected pathway in our sample was miRNA signaling pathway, miRNAs are family of small non coding RNAs which regulate wide range of biological processes that encoding carcinogenesis, and thus have been known dysregulated in cancer. Generously, abnormal expression of miRNA in tumor, it believed that this tumor dysregulation might affect several cancers hallmarks on other words, initiation, and progression. Besides, functionally these miRNAs incorporate into cell cycle proliferation pathway, and the dysregulated miRNAs are accountable for evading growth suppresser and hence sustaining proliferating signaling pathway in cancer.

Nevertheless, *Akt* pathway is intracellular pathway promoted metabolism, proliferation, cell survival growth and angiogenesis in response to extracellular signals. Yet, this pathwayregulates different cell functions and play conservative role through mTORcomplex.

Remarkably, stemness is regarded as the key factor of carcinogenesis and resistance to chemotherapy. [30]Although the cancer stem cells were yet to be isolated from meningioma, cancer stem cell and embryonic stem cell markers have been widely identified from meningioma and strongly associated withpatients' outcomes. [31]

The majority of exome analysis focused on analyzing cancer etiology depending on the mutations, however with no or little look over nature of these genes, few of these studies questioned stem cell genes.

Outstandingly, from our results, stem cell genes were mutated equally per our 3 meningioma samples which might suggest that, cancerous stem cell were probably drive the force of meningioma development, and taken together the distinct histological variant of meningioma (fibrous, meningiothelial and fibrous recurrence) which are the most common variant, suggesting the biological behavior other than recurrence. In absolute numbers of meningioma, most recurrence meningiomaare corresponded to histological benign variant (WHO grade1), however, their potential aggressive behavior still need to be evaluated.

Various cancers are associated with mutations on *Akt1* gene, and some mutations have been associated with the meningioma recurrence. Interestingly, in this current study, *Akt1* has shown number of mutations among 20 meningioma variants. While *Akt1* plays role in modulating synaptic plasticity and signal transduction pathways,[32] deficiency in *Akt1* may lead to abnormal prefrontal cortical structure and deficits in cognitive functions and risk for schizophrenia.[33]This might be claimed to behavioral changes among our patients with this kind of brain tumor.

Overall, our finding implicated rs17846829 which was seen in fibrous and atypical meningioma variant and rs58565216 that was seen within the fibrous variant might explain cognitive decline in meningioma patients. A recent robust study using array comparative genomic hybridization (aCGH), exposed that, identification of *SMO a*nd *Akt1* in meningioma increased the possibility of targeting therapy among meningioma patients.[34]Another German a study, concluded that, mutation of *Akt1* in skull base meningioma designate shorter time of recurrence and activate mTOR and ERK1/2 signaling and targeting mTOR pathway might be of benefit to patients with recurrent skull base meningioma.[35] Furthermore, case study from Switzerland exhibited presence of activated mutations of *Akt1in* sub-population of meningioma by using sequencing, and culturing of meningioma discovered sensitivity to AZD5363, which is a selective inhibitor to kinase activity. Thus clinical therapy of AZD5363 was given to the patients for tumor control. [36] Our study promotes the view, that mutations in *Akt1* denote recurrence and might be future challenges in clinical therapy; however, big data might be needed to consolidate our findings. Moreover, *CD44* A>G (rs9666607 mutation was detected in all our tumor samples, this mutation is located within stretch of residues annotated in UniProt as a distinct region, and the differences in amino acid properties can disturb this region and eventually disturb its function. Furthermore, this mutation is located in a region with known splice variants, and is described K > R (in dbSNP: rs9666607). Despite the mutant residue

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among the observed residue types at this position in other homologous sequences, and sometimes suggests that, this variant was not damaging for the protein's structure and function, however, this mutation was already established as having harmful effect.[37]In one study which analyzed SNPs within stemness-related genes for association with overall survival in the prostate, lung, colorectal and ovarian cancer screening and thus identified SNPs of *CD44* (rs9666607)were evaluated for association with gene expression.[38]In light of the above this rs9666607 in *CD44*among our meningioma samples was detected in *CD44* involves with *Akt1* gene in a proteoglycan pathway in cancer.[39]Nevertheless, *CD44* frame shift mutation (D419Tfs*61) in our samples, which is located within stretch of residues annotated in UniProt as special region consequently differences in amino acid properties can disturb this region and disturb its function, and as well this mutation showed difference in charge between the wild-type and mutant amino acid. Additionally, the charge of the wild-type residue was lost, this be able to cause loss of interactions with other molecules or residues.[40]

Conclusion

Nevertheless, the present study, highlight how such complex molecular processes might contribute to tumorogenesis, in attempt to shed light over thousands of genes and multifaceted network analysis rather than a solitary gene which is challenging question.

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