

Original Investigation

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Comparison of Clinical and Molecular Wnt and SHH Subgroups in Medulloblastoma Tumor Cases

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ABSTRACT

AIM: Medulloblastoma is the most widely seen malign brain tumor in childhood. In addition to surgery, treatment for this tumor involves radiotherapy and chemotherapy. However, there is no specific chemotherapy for medulloblastomas. Recent studies have showed that medulloblastoma cases have varying properties due to different subgroups in the molecular level. These subgroups are divided into the Wnt, SHH, group 3, and group 4 and are associated with different prognosis for different patients. Generally, these group distinctions based on the biological subtypes of medulloblastomas can be made clinically. It is thought that clinically determined subgroups can be important for molecular identification and support, to differentiate patient groups, and to determine the treatment protocols from this respect. The rates of subgroups of medulloblastomas in the gene expression level of the Turkish population are unknown. In this study, we aimed to determine the Wnt and SHH subtypes at the molecular level and to compare them clinically by examining the changes in CTNNB1, AXIN, PTCH1, SMO, SUFU, and GL11 mRNA expression in the medulloblastoma of a Turkish population determined according to patient selection criteria. In this context, the clinical distinction between Wnt and SHH groups are realized by considering the age, gender, survival time, location of the lesion, and radiological features of the patients.

MATERIAL and METHODS: Molecular separation was performed by RT-PCR analysis of CTNNB1, AXIN, PTCH1, SMO, SUFU, and GLI1 mRNA expression changes.

RESULTS: About 17.8% and 22.2% of the cases were included in the Wnt and the SHH group, respectively. When comparing group differences based on clinical and molecular data, 72.7% and 66.6% of matches were observed in the Wnt and the SHH group, respectively.

CONCLUSION: It has been revealed that molecular analysis and grouping of patients with medulloblastoma can provide support for clinically determined subgroups.

KEYWORDS: Medulloblastoma, Wnt, SHH, Molecular diagnosis

ABBREVIATIONS: Wnt: Wingless, SHH: Sonic hedgehog, CSF: Cerebrospinal fluid, WHO: World Health Organization, cDNA: Complementary DNA, PCR: Polymerase chain reaction, RT-PCR: Real time polymerase chain reaction, CTNNB1: Catenin Beta 1, AXIN: Axis inhibitor protein, PTCH1: Protein patched homolog 1, SMO: Smoothened, Frizzled Class Receptor, SUFU: Suppressor of fused, GLI 1: Glioma associated oncogene homolog 1, MYC: MYC proto-oncogene, MYCN: N-myc proto-oncogene protein, DDX3X: DEAD-Box Helicase 3 X-Linked, TP53: Tumor protein 53, APC: Adenomatous polyposis Coli

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INTRODUCTION

edulloblastoma is a highly aggressive brain tumor and is the most common brain tumor in infants and children (6). The current treatment method consists of surgical resection, craniospinal irradiation, and chemotherapy (6). However, this treatment protocol is not specific to medulloblastoma patients and is limited to standard drugs used in other pediatric glial tumors. According to some rare cases, most medulloblastoma cases occur sporadically despite being associated with hereditary diseases such as Li-Fraumeni, Turcot or Gorlin syndrome (8). Genomic and clinical studies conducted by many independent research groups show medulloblastomas vary, and these tumors are divided into several different subgroups at the molecular level namely, Wingless (Wnt), Sonic Heggehog (SHH), group 3, and group 4. Each group has different characteristics regarding survival, age demographics, and genetic changes (10,12). It is not known whether the mechanisms that affect the formation of Wnt and SHH medulloblastomas are related to the clinical features and whether the targeted treatmentcan be performed.

Wnt group medulloblastoma are the rarest subgroup and accounts for 10% of all medulloblastoma. The Wnt signaling pathway plays an important role in embryonic development, as it controls cell proliferation and cell migration (14). In the developing brain, the Wnt path has broad regulatory effects on neuronal maturation and synapse formation (31). The Wnt signal pathway is activated by the binding of Wnt ligands to the receptors. APC and AXIN, which prevent CTNNB1 accumulation in the nucleus, are important negative regulators involved in this pathway (4). Wnt medulloblastoma tumors are determined by examining CTNNB1, DDX3X, SMARCA4, TP53 gene mutations, or CTNNB1, APC, and AXIN mRNA expression levels. The key protein that determines the activity of this signal pathway is the Beta-catenin, which is encoded by the CTNNB1 gene (16). Elevated levels of this protein increases cell proliferation, leading to tumor formation (9,17).

SHH group medulloblastoma constitute approximately 30% of all medulloblastoma cases, and the five-year survival rate is determined as 60%–80% (10). During early cerebellar development, Purkinje cells secrete SHH glycoproteins and stimulate proliferation and migration of granule cells (31). Overactivation of the SHH pathway leads to increased expression of the GLI transcription factor that initiates uncontrolled proliferation of granule cells and subsequent tumor formation (15). The most common genetic changes in this group are mutations in *PTCH1*, *SMO*, and *SUFU* tumor suppressor genes. The inactivation of TP53 as well as the simultaneous amplification of MYCN and GLI has been associated with an increased risk for SHH group medulloblastoma. There are also different molecular changes and risk factors specific to different age groups in the SHH subgroup (17).

Since medulloblastoma tumors do not have the same characteristic features, tumor classification should be made at the molecular level, and treatment protocols should be shaped according to these subgroups. We predict that the main reason why Turkish patients with medulloblastoma respond poorly to treatment is that medulloblastoma treatment is performed with standard protocols based on a single tumor type and the absence of a specific chemotherapy for these tumors. For this reason, treatments based on new molecular studies should be crafted and reach a clinically significant stage. The present study aimed to determine and compare the clinical and molecular distinctions of patients with Wnt and SHH medulloblastoma obtained from a single center of the Turkish population and reveal the relationship of molecularly-determined Wnt and SHH patient groups with prognosis. It is thought that this study will contribute to the separation of medulloblastoma tumors into subgroups as well as the creation of new studies aimed at shaping the treatment protocols toward this direction.

MATERIAL and METHODS

Patients

This retrospective study included 45 patients who were treated in Uludag University Hospital, diagnosed with primary medulloblastoma between 2005 and 2016. Tumor tissue samples were obtained by surgical resection before treatment (radiation and chemotherapy). Surgical procedures were performed by neurosurgeons with the use of a microscope (Zeiss OPMI Pentero Carl Zeiss Inc., Oberkochen, Germany). The resection material of these 45 patients was paraffinized in the Department of Pathology and classified according to the WHO criteria. For the control group, 5 brain tissues removed to reach tissue during epilepsy operations and evaluated as normal by the Department of Pathology were used. Patients who underwent stereotactic biopsy only, and whose pathology preparations were not suitable for epigenetic study, had familial cases, and had concurrent malignancies were excluded from the study. All materials to be studied were approved by the Uludag University Ethics Committee (Decision number 2020-12/16).

Clinical Subgrouping of Medulloblastoma Patient

The clinical progress of the patients was investigated retrospectively. Patients' age, gender, survival times, presence of spinal metastasis, need for CSF diversion, Ki-67 index in histopathological examination, location of the lesion in radiological examinations, vascularity, cystic formation, edema, necrosis, and contrast enhancement intensity were evaluated to predict the genetic subgroups of the cases.

Patients whose tumor site extends from the midline to the basal cisternae, foramen luschkaya, and/or cerebellopontine angle, often in the age range of 10–12 years, with good prognosis, and without CSF diversion were included in the Wnt subgroup. Patients with a tumor located in the cerebellar cortex, with a bimodal age distribution (<3, >15 years), good prognosis in infants but moderate prognosis in older children, and those who may need a shunt were included in the SHH subgroup.

RNA Extraction

Paraffin-embedded tumor and normal tissue blocks were determined by expert pathologists. First, the removal of paraffin was performed using Xylene and 95% alcohol. Total RNA was

isolated using the RNeasy FFPE Mini Kit (Qiagen, Germany) according to the manufacturer's protocol. The concentration and quantity of each RNA sample obtained was determined using the Beckman Coulter DU-640 spectrophotometer device. A260/A280 absorbance ratio of ideal purity quality RNA is expected to be from 1.9 to 2.1.

Reverse Transcription Quantitative PCR (RT-qPCR) Analysis

cDNA synthesis was performed from a total of 100 ng RNA using Transcriptor First Strand cDNA Synthesis Kit (New England Biolabs, MA, US). The PCR conditions for cDNA synthesis were as follows: 25°C for 10 min, 37°C for 2 h, and 85°C for 5 min. Then, expression levels of mRNAs were determined using primers specific to CTNNB1, AXIN, PTCH1, SMO, GLI, and SUFU mRNAs, while using the primers specific to GAPDH genes as controls. RT-qPCR analysis was performed with ABI StepOnePlus[™] Real Time PCR System (Applied Biosystems).

Statistics Analysis

Expression profiles of the genes evaluated in the present study were performed in the web-based RT2 PCR Array Data Analysis (https://dataanalysis2.qiagen.com/) program. After determining the expression profile of each gene in all patients, graphs were drawn in Graphpad version 8 (Graphpad Software, California, USA). The effects of the groups on life expectancy were analyzed using the Kaplan–Meier test using SPSS 25.0 (IBM, New York, USA) program. Data less than p < 0.05 from the analysis result were considered significant.

RESULTS

Patient Characteristics

In our study, 45 patients diagnosed with medulloblastoma were analyzed. Twenty-six (57.7%) of the patients were male, while 19 (42.2%) were female. The age of the patients in the study ranged from 4 months to 17 years (median 8.68 years). Nineteen of the tumors (42.2%) were stage IV. was located in the ventricle, 11 (24.4%) were located in the midline to the basal cisternae, foramen luschkaya, and/or cerebellopontine angle, and 15 (33.3%) were located in the cerebellar cortex. Postoperative MRI examinations revealed gross total resection in all patients. Spinal metastasis was detected in 12 (26.6%) patients, while CSF diversion was required in 23 (51.1%) patients.

It was found that 28 (62.2%) patients died during their followup, and 17 (37.7%) patients were still alive. Average survival was calculated as 29.6 months in patients who died. It was observed that the survival times of 17 patients who were still living on June 2020, were at least 41 months and at most 180 months (average 101 months). The mean survival was found to be 29.6 months (1.5–105 \pm 27.7) in the patients who died. The average Ki-67 index was calculated as 453.8/1000. While a value above the average Ki-67 value was found in 25 (55.6%) patients, the Ki-67 value was below the average in 20 (44.6%) patients (Table I).

Clinical Subgrouping of Medulloblastoma Patients

The clinical progress of the patients was examined in detail. Patients' age, gender, survival times, survival more or less than their average survival time (1,5), presence or absence of spinal metastasis (26), need for CSF diversion (27), and Ki-67 in histopathological examination were all analyzed. The genetic subgroups of the patients were predicted based on the evaluation of clinicopathological data, such as lower or higher index values (29). location of the lesion in radiological examinations, vascularity, cystic formation, presence of edema, necrosis, and contrast enhancement density (21). Patients whose tumor site extends from the midline to the basal cisternae, foramen luschkaya, and/or cerebellopontine angle, often between the ages of 10-12, who have a good prognosis, and who do not need CSF diversion were included in the Wnt subgroup. Patients with tumors located in the cerebellar cortex, bimodal age distribution (<3, >15 years), good prognosis in infants but moderate prognosis in older children, and who may need a shunt were included in the SHH subgroup. Vermian midline and IV. Infants and young pediatric patients with spinal metastases, those often with a poor prognosis, or with a tumor located in the ventricle, rich in vascularity, as well as spinal metastases in the group 3 subgroup. A vermian midline tumor with a rare contrast enhancing tumor. Male patients with moderate and poor prognosis, mostly around the age of 10, were separated from group 3 and included in the group 4 subgroup.

Of the 11 patients included in the Wnt subgroup, 7 were female and 4 were male (Female/Male ratio = 1.7/1). The patients ranged from 2.5 years to 15.5 years, and the average age was calculated as 8.95 years. Only 1 of the patients died. When the survival times of 10 living patients calculated based on June 2020 were included, the average survival was calculated as 9.36 years.

Of the 15 patients included in the SHH subgroup, 6 were female and 9 were male (Female/Male ratio = 2/3). The patients were between 1.5 and 17 years old, and the mean age was calculated as 10.8 years. Twelve of the patients died, and the average survival was calculated as 4.16 years when the survival times calculated based on June 2020 of the 3 patients were included.

Molecular Subgrouping of Medulloblastoma Patients

The grouping of medulloblastoma patients into Wnt and SHH molecular subgroups was made by evaluating the expression profiles of specific genes in these pathways. Expression levels of AXIN and CTNNB1 genes associated with Wnt were examined in molecularly-determined subgroups of medulloblastoma patients. When tumor tissues were compared with normal tissue, a statistically significant increase was observed in the expression levels of AXIN and CTNNB1 genes (3.5-fold, p=0.042; 8.2-fold, p=0.001, respectively; Figure 1A, B).

Expression levels of SHH-related GLI, SMO, SUFU, and PTCH1 genes were examined. When tumor tissues were compared with normal tissues, a statistically increased PTCH1 and GLI gene expression and a statistically decreased SMO and SUFU gene expression were observed (6.1-fold, p=0.024; 4.3-fold,

Table I: Clinicopathological Features of Medulloblastoma Patients

Variables, Total (N=45)	n (%)
Gender	
Woman	19 (42.2)
Male	26 (57.7)
Age	
0–3 years	7 (15.5)
3–10 years	21 (46.6)
10–16 years old	15 (33.3)
16–18 years old	2 (4.4)
Residential area	
Vermian midline IV. located in the ventricle	19 (42.2)
Extends from midline to basal cisterna/foramen luschkaya / cerebellopontine angle	11 (24.4)
Located in the cerebellar hemisphere	15 (33.3)
Treatment protocol	
Vincristine (VINC) (+) RT (+)	11 (24.4)
Vincristine Etoposide Carboplatin (VEC) (+) RT (+)	18 (40)
VINC / VEC (+) RT (+) Temozolamide (+)	6 (13.3)
VINC (+) RT (-)	0
VEC (+) RT (-)	5 (11.1)
No additional treatment (refused / exitus)	5 (11.1)
KSRT (+)	8 (17.7)
Survival time	
Survivors living above the average survival time of the survivors (101 months)	7 (41.1)
Survivors living below the average survival time of the inhabitants (101 months)	10 (58.9)
Patients who have lived over the average survival time of the deceased (29.6 months)	10 (35.7)
Patients who lived below the average survival time of the deceased (29.6 months)	18 (64.3)
All patients who lived above the average survival time (56.5 months) of all patients	16 (28.5)
All patients who lived below the average survival time of all patients (56.5 months)	29 (64.5)
KI-67 index value	
Above average (>453.8 / 1000)	25 (55.6)
Below average (<453.8 / 1000)	20 (44.4)
Molecular subgroup selection based on clinical data	
WNT	11 (24.4)
SHH	15 (33.3)
Group 3	11 (24.4)
Group 4	6 (13.3)
Undecided	2 (4.4)

p=0.041; -6.7-fold, p=0.001; -5.8 fold, p=0.027; respectively; Figure 2A-D). Comparison of genes between groups is shown in Figure 3A-F.

Comparison of Molecular and Clinically Determined Subgroups of Medulloblastoma Patients

Comparison of the molecular and clinically determined subgroups of the patients are shown in Table II–III. Clinically,

11 patients were defined as the Wnt subgroup. From the molecular analyses performed from these patients, 8 out of 11 clinically determined patients were molecularly defined as part of the Wnt subgroup (Clinical matching rate: 72.7%). Clinically, 15 patients were defined as SHH subgroup. From the molecular analyses performed from these patients, 10 of the 15 clinically determined patients were molecularly defined as part of the SHH subgroup (Clinical matching rate: 66.6%; Table IV).



Figure 1: Effects of normal tissues and tumor tissues on expression levels of Wnt related genes. A) AXIN, B) CTNNB1.



Figure 2. Effects of normal tissues and tumor tissues on expression levels of SHH-related genes. A) PTCH1, B) SMO, C) SUFU, D) GLI.



Figure 3. Comparison of related genes between groups. A) CTNNB1, B) AXIN, C) PTCH1, D) SMO. E) SUFU, F) GLI.

Overall Survival Relationships of Patients in Wnt and SHH Subgroups in Medulloblastoma Patients

Kaplan–Meier curves were applied to examine overall survival in patients in the Wnt and SHH subgroups. It was determined that only 1 (12.5%) of 8 cases in the Wnt group were in line with molecular level definitions, and 8 (80%) of 10 cases defined as SHH died within the follow-up period. It was determined that the patients in the Wnt subgroup showed a significantly longer survival compared to the patients in the SHH subgroup (p<0.001; Figure 4).

DISCUSSION

The existence of different molecular subgroups of medulloblastomas was first reported in 2002 by Pomeroy et al. (22). In the study, by examining the DNA microarray

Patients No P1 2													
P1 2	CTNNB1	AXIN	РТСН1	SMO	SUFU	GLI	Age years	Gender	Survival	Spinal Metastasis	CSF Diversion	Ki-67 index	Radiology
	9.92334938	31.89824677	31.31463814	30.90249825	30.23260498	31.35980797	2.5	Male	180 months (alive)	No	No	200/1000	CPA (WNT)
P4 3	3.34471893	37.13394547	35.29870224	33.70285797	32.99644852	Undetermined	13	Female	173 months (alive)	No	No	135/1000	CM-CPA (WNT)
P5 3	2.10314941	33.06726837	34.48836136	34.96702576	34.31785583	Undetermined	5	Female	170 months (alive)	No	No	150/1000	CM (WNT)
P6 U	ndetermined	35.20174408	37.42163467 1	Undetermined	37.13742065	Undetermined	14	Male	161 months (alive)	No	No	300/1000	CM-CPA (WNT)
P21 2	8.23941422	29.74406815	26.6630249	30.14196968	31.14177513	27.55165672	7.5	Male	123 months (alive)	No	No	500/1000	CPA-FL (WNT)
P22 3	2.77260208	33.56413269	37.89949417 (Undetermined	36.68754196	Undetermined	9	Female	63 months (death)	No	No	500/1000	CM-FL (WNT)
P31 3	0.79523277	36.13742447	34.22055054	35.57910156	34.27984619	Undetermined	80	Female	97 months (alive)	No	No	400/1000	CM (WNT)
P37 2	9.60584068	35.46809387	34.17284012	33.93776703	33.75271225	29.3282032	÷	Female	57 months (alive)	No	No	150/1000	CM (WNT)
P44 (30.4997673	35.952034	31.84303856	35.33149719	34.72510529	32.40512085	80	Male	33 months (alive)	No	No	350/1000	FL (WNT)
P45 2	6.50996399	31.40849113	34.38082504	33.33787537	32.26106644	Undetermined	œ	Female	41 months (alive)	No	No	250/1000	FL (WNT)

Table II: Gene Expression Results and Clinical Feagures of the WNT-Subgruops Medulloblastoma

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CPA: Cerebellopontin angle, CM: Cisterna magna, FI: Foramen Luschka.

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AXIN PTCH1 SMO SUFU GLI	SMO SUFU GLI	SUFU GLI	GLI		Age /ears	Gender	Survival	Spinal Metastasis	CSF Diversion	Ki-67 index	Radiology
4104729 34.63175583 31.60977364 31.91034126 Undetermin	31.60977364 31.91034126 Undetermin	31.91034126 Undetermin	Undetermin	eq	7	Female	77 months (Death)	No	Yes	300/1000	Cortical (SHH)
stermined 36.87014008 36.515522 36.13612366 37.289211	36.515522 36.13612366 37.289211	36.13612366 37.289211	37.289211	27	5.5	Male	29 months (Death)	No	Yes	620/1000	Cortical (SHH)
2849884 32.82820129 34.67991257 34.68025208 33.378154	34.67991257 34.68025208 33.378154	34.68025208 33.378154	33.378154	.75	15	Male	170 months (Alive)	N	No	150/1000	Cortical (SHH)
4301529 Undetermined Undetermined Undetermined Undetermi	Undetermined Undetermined Undetermi	Undetermined Undetermi	Undetermi	ned	o	Male	73 months (Death)	No	No	90/1000	Cortical (SHH)
7591019 32.82787704 34.5157547 34.45065689 33.23201	34.5157547 34.45065689 33.23201	34.45065689 33.23201	33.23201	37	16	Male	17 months (Death)	No	Yes	600/1000	Cortical (SHH)
7057915 32.37164307 30.24345207 35.00663757 29.78749	30.24345207 35.00663757 29.78749	35.00663757 29.787494	29.787494	166	15	Male	56 months (Death)	N	No	350/1000	Cortical (SHH)
stermined 37.06801987 37.04123688 Undetermined 35.27101	37.04123688 Undetermined 35.27101	Undetermined 35.27101	35.27101	517	1.5	Female	27 months (Death)	No	Yes	700/1000	Cortical (SHH)
1704483 30.22545242 32.9403801 32.46405029 30.48076	32.9403801 32.46405029 30.48076	32.46405029 30.48076	30.48076	439	12	Male	7 months (Death)	No	Yes	300/1000	Cortical (SHH)
2300415 32.78512573 32.87586212 30.57497978 Undeterm	32.87586212 30.57497978 Undetern	30.57497978 Undeterm	Undeterm	ined	5.5	Female	72 months (Death)	No	No	560/1000	Cortical (SHH)
9483757 30.32207298 28.4745636 30.95441246 32.39230	28.4745636 30.95441246 32.39230	30.95441246 32.39230	32.3923(347	14	Female	29 months (Death)	No	No	300/1000	Cortical (SHH)
stermined 36.83178711 Undetermined 35.13407898 Undetern	Undetermined 35.13407898 Undetern	35.13407898 Undetern	Undetern	nined	17	Male	22 months (Death)	N	Yes	500/1000	Cortical (SHH)
stermined 37.05261612 36.95272827 36.97740555 Undetern	36.95272827 36.97740555 Undetern	36.97740555 Undetern	Undetern	nined	14	Female	75 months (Alive)	No	No	500/1000	Cortical (SHH)
6117706 28.32009888 33.30418777 32.57322311 26.4735	33.30418777 32.57322311 26.4735	32.57322311 26.4735	26.4735	1646	13	Male	7 months (Death)	N	Yes	300/1000	Cortical (SHH)
9424973 27.39665985 32.96955872 32.22485352 28.8330	32.96955872 32.22485352 28.8330	32.22485352 28.8330	28.8330	2498	6.5	Male	44 months (Death)	N	No	600/1000	Cortical (SHH)
9854584 32.39166641 32.80643463 32.15068054 Undeter	32.80643463 32.15068054 Undeter	32.15068054 Undeter	Undeter	mined	;	Female	41 months (Alive)	No	Yes	300/1000	Cortical (SHH)

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Table III: Gene Expression Results and Clinical Feagures of the SHH-subgruops Medulloblastoma

P4	WNT	-	_
P5	WNT	WNT	+
P6	WNT	-	-
P7	SHH	SHH	+
P8	SHH	SHH	+
P9	SHH	-	-
P10	GROUP 4	_	_
P11	SHH	SHH	+
P12	SHH	SHH	+
P13	SHH	SHH	+
P14	GROUP 4	_	-
P15	WNT	SHH	_
P16	GROUP 3-WNT?	The decision could not be made	-
P17	GROUP 4	_	-
P18	SHH	SHH	+
P19	GROUP 4	_	_
P20	SHH	_	_
P21	WNT	WNT	+
P22	WNT	WNT	+
P23	GROUP 3	_	_
P24	GROUP 3	_	_
P25	GROUP 3	_	_
P26	GROUP 3	_	_
P27	GROUP 3	_	_
P28	SHH	_	_
P29	GROUP 3-4?	The decision could not be made	-
P30	GROUP 3	-	_
P31	WNT	WNT	+
P32	SHH	SHH	+
P33	GROUP 3	-	_
P34	SHH	-	_
P35	SHH	-	_
P36	GROUP 4	-	_
P37	WNT	WNT	+
P38	GROUP 4	-	-
P39	GROUP 3	-	-
P40	SHH	SHH	+
P41	GROUP 3		_
P42	SHH	SHH	+
P43	GROUP 3		_
P44	WNT	WNT	+
P45	WNT	WNT	+

Table IV: Clinical and Molecular Comparison of Patient Groups

WNT

GROUP 3

SHH

P1

P2

P3

Patient Clinical Diagnosis Molecular diagnosis Coupling

WNT

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+

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Figure 4: Kaplan–Meier curves on survival of patients in Wnt and SHH subgroups.

gene expression data, it was shown that PTCH, GLI, and MYCN are highly associated with desmoplastic/nodular type medulloblastoma. As a result of various studies on the same subject, the differences in medulloblastoma transcriptomes have been elucidated which led to close results being obtained. Furthermore, various subgroups were continued to be formed. In 2006, Thompson et al. (33) and in 2008, Kool et al. (11) explained that there are five distinct molecular subtypes: A-E. In 2011, Cho et al. showed six distinct molecular subgroups (C1-C6), while Northcott et al. explained that there are four separate molecular subgroups named SHH, Wnt, group C, and group D (2,19). In 2010, in Boston, the number, nature, and variations of these subgroups were discussed which led to the acceptance of four main transcriptional medulloblastoma subgroups named Wnt, SHH, group 3, and group 4. These subgroups have quite different demographic characteristics, histologies, and clinical results (32).

Among the subgroups, the Wnt subgroup is the rarest. Approximately 10% of all medulloblastomas are in this subgroup (23). Among the subgroups, it has the best prognosis, with average 5-year survival observed to be over 95% (10). This molecular subgroup is usually histopathologically associated with classical-type medulloblastoma. Wnt subgroup medulloblastoma occurs in children older than 3 years of age and young people, with a frequency that peaks between the ages of 10–12. It is very rare in infants. Its characteristic location is at the lateral side of the IV ventricle and may extend to the cisternae. In 85%–90% of cases, activation of the Wnt signaling pathway results from the activation of somatic mutations in CTNNB1 exon 3, leading to overexpression of β -catenin (2).

The SHH subgroup represents about 30% of all medulloblastomas with moderate prognosis. The 5-year average survival is about 70% (18). There is a strong relationship between desmoplastic/nodular variant histopathology and SHH subgroup tumors. SHH subgroup medulloblastomas show a bimodal distribution, most commonly seen in infants (<3 years) and adults (> 16 years of age), and less frequently in patients aged 4–15 years. The genetic events underlying SHH pathway activation are age-dependent: PTCH1 (Gorlin syndrome) mutations are common in infants. In addition, babies with SHH show a good prognosis even with only a chemotherapy regimen. TP53 (Li-Fraumeni syndrome) mutations are mostly seen in children aged 3 to 16 years. Therefore, all pediatric SHH tumors should be investigated with a pre-diagnosis of a potential Gorlin syndrome or Li-Fraumeni syndrome (24).

In the present study, Wnt and SHH molecular subgrouping of medulloblastoma patients was evaluated by looking at the expression profiles of specific genes in these pathways. When the expression of AXIN and CTNNB1 genes were used to determine the activity of the Wnt signaling pathway, and the expression of the GLI, SMO, SUFU and PTCH1 genes were used to determine the SHH signaling pathway activity, 17.8% of the single center medulloblastoma cases belonging to the Turkish population were found to be in the Wnt group molecularly, while 22% were determined to be in the SHH subgroup.

When the groups were compared according to clinical and molecular data, a matching rate of 72.7% in the Wnt group and 66.6% in the SHH group were observed. In the study conducted by Kool et al. in 62 cases with a diagnosis of medulloblastoma in 2008, they found that the subtumor group of Wnt had a rate of 14.51% and SHH had a rate of 24.19% (11). As a result of a meta-analysis involving data from 7 centers in 2012 that included 550 medulloblastoma cases belonging to a Russian population, the authors determined that Wnt tumors were 11% while SHH tumors were 28% (10). On the other hand, Ellison et al. in their study with 235 cases with medulloblastoma, stated that Wnt group tumors comprised 14%, while SHH group tumors comprised 31% (3).

In this study, it was determined that AXIN and CTNNB1 genes belonging to the Wnt signaling pathway showed statistically high expression by comparing normal tissue with tumor tissue. In the study conducted by Silva et al. using immunohistochemical, mutation, and expression methods in 61 medulloblastoma cases, it was observed that the expression levels of all genes belonging to the Wnt signaling pathway were highly parallel with the present study (30). When the expression levels of SHH-associated GLI, SMO, SUFU, and PTCH1 genes were examined, a statistically significant increase in the expression levels of the PTCH1 gene in the tumoral tissue compared to normal tissue, a statistically significant decrease in the expression levels of the GLI, SMO, and SUFU gene were observed in the present study. When the literature is reviewed, it is seen that immune staining is preferred in defining SHH subgroup in medulloblastoma cases. The current study is one of the pioneering studies with the use of gene expression analysis in determining this group.

Classification and risk classification based on biological subtypes of medulloblastomas can usually be made in a clinical setting. It is thought that the molecular determination of clinically determined subgroups may be important for reassembling patient groups that were stratified by clinical parameters in the past (28). In the last year, the results of four separate international collaborative genome-based studies on medulloblastoma have been published. Parsons et al.'s whole genome sequencing (20), Northcott et al. (19) and Cho et al.'s (2) gene expression and copy number analysis, and Remke et al. (25) performed a similar gene expression and copy number analysis to characterize a group of adult medulloblastoma patients (15). Considering these data, it has been revealed that molecular analysis and grouping of medulloblastoma patients can provide support for clinically determined subgroups.

Genome-level gene expression analysis has been used to analyze human cancers. This approach allows the identification of genes important in tumorigenesis and the identification of cancer molecular subgroups. At the same time, the discovery of gene expression signatures characteristic of clinicopathological features in various cancers has determined that expression profiles can be used for molecular classification of cancer (7). Studies have shown that medulloblastomas have different subgroups at the molecular level and patients with different subgroups show different prognosis (11). However, the frequencies of different subgroups determined may differ according to different ethnic patient origins. Depending on the genetic and environmental factors, the objective of our work is to determine the different clinical and molecular features of medulloblastoma patients in Turkey. Determining the proportions of different subgroups in different populations can be a tool to create more effective and specific cancer treatment models and targets for populations and individuals.

Mutation screening has been performed to determine the molecular subgroups of patients with medulloblastoma, and the differences between the related genes have been investigated. However, the gene expression levels of medulloblastoma patients belonging to the Turkish population were not determined in this study's population.

In the present study, 8 patients were molecularly identified to be part of the Wnt subgroup from 11 patients who were clinically defined as Wnt. On the other hand, 10 patients were molecularly identified to be part of the SHH subgroup from 15 patients who were clinically defined as SHH. Thus, a high similarity was detected in the molecular subgroups that were determined clinically and molecularly.

In this study involving 45 cases, 8 and 10 cases were determined to be in the Wnt and SHH subgroup, respectively. When five-year survival is considered, poor prognosis was observed in 12.5% of 8 cases in the Wnt subgroup and in 80% of 10 cases in the SHH subgroup. It was determined that the patients in the Wnt subgroup showed a significantly increased survival rate compared to the patients in the SHH subgroup.

CONCLUSION

Although there is a need to increase the number of patients and the parameters examined in the findings obtained in our study, it was determined that the incidence of the Wnt subgroup was 17.8% and the incidence of the SHH subgroup was 22.2% in the medulloblastoma cases obtained from a single center in the Turkish population. Additionally, it has been determined that the gene expression analysis method based on RT-PCR technique is suitable for routine practice. When the clinical and molecular results obtained were evaluated together, it was determined that the patients included in the Wnt subgroup had a better prognosis than the patients included in the SHH subgroup.

The findings obtained have the potential to lead the studies on group 3 and group 4. Thus, it is thought that the present study will contribute to the creation of new studies aimed at subdividing the medulloblastoma tumors, which are the most common malignant brain tumors in childhood, subsequently shaping the treatment protocols toward this direction.

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