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Genetic Grouping of Medulloblastomas by Representative Markers in Pathologic Diagnosis[1]

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Abstract

A recent analysis of the genetic features of medulloblastoma (MB) suggested classification into distinct subgroups according to gene expression profiles, including the Wingless signaling pathway–activated group (WNT group), the Sonic Hedgehog signaling pathway–activated group (SHH group), group 3, and group 4. To classify MB according to genetic features in practice, we analyzed 74 MBs using representative markers of each group. Based on immunohistochemistries (IHC), cytogenetic alterations, and a CTNNB1 mutation study, the patients were divided into the following three groups: cases showing nuclear β-catenin and/or CTNNB1 mutation and/or monosomy 6 were included in the WNT group (14/74, 18.9%); cases expressing GAB1 were included in the SHH group (15/74, 20.2%); cases that did not show positivity for markers of the WNT or SHH group were included in the non-WNT/SHH group (45/74, 60.6%). Immunoexpression of NPR3 seemed to lack sensitivity for classifying group 3, showing diffuse positivity in only two cases. KCNA1 was not specific to group 4 because it was expressed in all groups. Cases in the WNT group showed a slightly better survival than those in the SHH or non-WNT/SHH group, although additional cases are required for statistical significance. Isochromosome 17q ($P = .002$) and the large cell/anaplastic variant ($P = .002$) were demonstrated to be poor prognostic indicators in multivariate analysis. The representative IHC and cytogenetic data facilitated the division of MBs into the WNT and SHH groups; however, more specific markers should be added for the identification of group 3 and group 4 in practice.

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Introduction

Recent analyses of the genetic features of medulloblastoma (MB) have suggested classification of MB into four distinct subgroups according to gene expression profiles [1–4]. As MB exhibits highly variable biologic behavior and a wide histopathologic spectrum, these results could provide biologic risk stratification and tailored patient treatment.

Data produced by gene expression profiling revealed that MB can be subdivided into the Wingless signaling pathway–activated group (WNT group), the Sonic Hedgehog signaling pathway–activated group (SHH group), and additional two groups, both of which show neuronal/photoreceptor differentiation (group 3 and group 4) [5]. The molecular features of each group reflect the distinct developmental origin of MB and also correlate with clinicopathologic traits [3,5,6]. Briefly, the WNT group often comprises older children and exhibits a classic histology, nuclear β-catenin expression, *CTNNB1* mutation, and monosomy 6 with a relatively good prognosis. Meanwhile, the SHH group occurs in infants and young adults and frequently exhibits a desmoplastic/nodular (D/N) histology, GAB1 expression, *PTCH1*/*SUFU* mutation, and *MYCN*/*GLI2* amplification with an intermediate prognosis. Group 3 MBs show the worst prognosis, frequently occurring in infants and children. This group exhibits classic or large cell/anaplastic (LC/A) histology, NPR3 expression, chromosome 7 gain, chromosome 8 loss, i17q [i(17q)], and *MYC* amplification in many cases. Finally, group 4 MBs have a similar prognosis to the SHH group,

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having classic or LC/A histology frequently, KCNA1 expression, cytogenetic alterations similar to group 3 with chromosome X loss, and *MYCN*/*CDK6* amplification.

However, these genetic features were shown to be not specific for each group but overlapped between groups, except a few markers. Nuclear β-catenin expression, *CTNNB1* mutation, and monosomy 6 were found in the WNT group exclusively, and GAB1 immunoexpression and *PTCH1*/*SUFU* mutation were regarded as representative markers of the SHH group [1,3,7]. For groups 3 and 4, only expressions of NPR3 and KCNA1, respectively, were suggested to be specific markers. In addition, immunohistochemistries (IHC) of YAP1 and filamin A were useful for differentiation of both WNT and SHH groups from the non-WNT/SHH group [7], and CRX and GRM8 have been evaluated for classifying groups 3 and 4 [8].

To diagnose and classify MB according to genetic groups in practice, it is necessary to validate the genetic and IHC markers in formalin-fixed paraffin-embedded (FFPE) tumor tissue. We performed array-based comparative genomic hybridization (aCGH) with fluorescence *in situ* hybridization (FISH), IHC using markers representing each subgroup, and a *CTNNB1* mutation study in FFPE MB tissue. On the basis of these results, we attempted to divide MB cases into four genetic groups—WNT, SHH, group 3, and group 4—and to verify the usefulness of each marker for diagnostic practice.

Materials and Methods

Patients and Specimens

The FFPE tumor tissue samples of 74 MB patients who were admitted to the Seoul National University Hospital and Seoul National University Children's Hospital from 1999 to 2009 were retrieved from the pathology archive. Studies were performed with the approval of the Institutional Review Board, Seoul National University Hospital (H-1106-039-366). Table 1 summarizes patients' clinical information. The mean follow-up period was 45.2 months (range, 3–159 months). All patients except three were treated with surgical resection and adjuvant chemotherapy and radiotherapy. One patient did not receive both chemotherapy and radiotherapy after surgery because the patient died before the postoperative therapy, and two patients did not receive chemotherapy and radiotherapy, respectively.

Table 1. Summarization of Clinical and Histopathologic Features.

	No. of Cases (%; N = 74)
Age at diagnosis	
Median (range)	8 years (0–35 years)
≤3 years	15 (20.2)
>3 and ≤18 years	51 (68.9)
>18 years	8 (10.8)
Gender	
Male	55 (74.3)
Female	19 (25.6)
Histologic variant*	
Classic	47 (63.5)
D/N, MBEN (desmoplastic)	16 (21.6)
LC/A	11 (14.8)
Extent of surgery	
Gross total resection	36 (48.6)
Near total resection	20 (27.0)
Subtotal resection	15 (20.2)
Partial resection	1 (1.3)
NA†	2
Postoperative chemotherapy and radiotherapy	
Both	67 (90.5)
Chemotherapy only	3 (4.0)
Radiotherapy only	1 (1.3)
None	1 (1.3)
NA†	2
Recurrence	
Absence	50 (67.5)
Presence	21 (28.3)
NA†	3
Metastasis	
Absence	45 (60.8)
Presence	25 (33.7)
NA†	4
Follow-up duration, median (range)	45.2 months (3–159 months)

*D/N indicates desmoplastic/nodular; MBEN, medulloblastoma with extensive nodularity; LC/A, large cell/anaplastic.
†NA, not applicable.

Histopathologic Review and IHC

Two pathologists (S.-H.P. and H.S.M.) who were unaware of the clinical data reviewed all slides for histologic subtyping, and FFPE tissue microarrays (two 2-mm representative cores) were produced for IHC staining and FISH. MBs were classified as classic, D/N, MB with extensive nodularity (MBEN), or LC/A variants according to the 2007 World Health Organization (WHO) classification. Cases with a distinctive biphasic phenotype comprising a pale nondesmoplastic nodular area and an internodular desmoplastic cellular area were diagnosed as a D/N variant, and cases with predominant and extended lobular architecture with neurocytic differentiation were diagnosed as MBEN. A large cell variant displayed proliferation of discohesive large, round cells with prominent nucleoli, and an anaplastic variant exhibited marked nuclear pleomorphism and high mitotic activity. IHC staining of all 74 cases was performed automatically according to the manufacturer's protocol, based on a biotin-free polymer detection system. The primary antibodies used were YAP (1:50; sc-101199; Santa Cruz Biotechnology, Santa Cruz, CA), GAB1 (1:50; Abcam, Cambridge, MA), filamin A (1:300; PM6/317; Fitzgerald, Acton, MA), SFRP1 (1:300; Abcam), DKK1 (1:200; 2A5; Abnova, Taipei, Taiwan), β-catenin (1:200; BD Biosciences, Franklin Lakes, NJ), NPR3 (1:800; Abcam), and KCNA1 (1:500; Abcam). Positive immunolabeling was defined as uniform intense nuclear and cytoplasmic (β-catenin, filamin A), cytoplasm, or cytoplasmic membrane (YAP, GAB1, SFRP1, DKK1, NPR3, KCNA1) labeling in more than 10% of the tumor area.

Fluorescence In Situ *Hybridization*

FISH was performed on unstained FFPE array slides of all 74 cases as described previously using commercially available digoxigenin-labeled cosmid probes for *MYC* (8q24.12-q24.13; orange; Vysis, Downers Grove, IL), CEP8 (8p11.1-q11.1; green; Vysis), *MYCN* (2p24; orange; Vysis), CEP2 (2p11.1-q11.1; green; Vysis), *LIS1* (17p13.3; orange; Vysis), *RARA* (17q21; green; Vysis), and *MYB* (6q23; aqua; Abbott Molecular Inc, Des Plaines, IL). After performing aCGH, we confirmed the data by FISH on chromosomes 2, 6, 8, and 17. However, monosomy 6 and i17q were regarded as positive only by FISH in cases that aCGH was not feasible. Briefly, slides were deparaffinized and treated with proteinase K, then denatured and treated with prediluted probes and hybridized overnight. Values for each signal and the ratios of red/green signals were reported in at least 100 nonoverlapping nuclei per specimen. Specimens containing either more than 10 signals or innumerable tight clusters of signals in more than 10% of tumor cells were considered *MYC* or *MYCN* amplification. Evaluation of chromosome 6q loss and alteration of chromosome 17q were defined as described previously. Gains of 17q were diagnosed in cases of three or

more green (17q) signals in >10% of nuclei, and polyploidy cases were recorded separately. Loss of 17p was diagnosed as one red (17p) and two or more green (17q) signals in >50% of nuclei.

Gene Sequence Analysis

Slides stained with hematoxylin & eosin (H&E) were reviewed, and representative tumor areas were marked from which genomic DNA was extracted. *CTNNB1* (exon 3) was amplified by polymerase chain reaction using previously published primers and directly sequenced using the ABI Prism 3700 Analyzer (Applied Biosystems, Foster City, CA) [3].

Array-based Comparative Genomic Hybridization

aCGH was feasible in 38 of 74 (51.3%) MBs. After histologic subtyping, genomic DNA was extracted from FFPE tissues of 38 cases and purified using a standard protocol. We performed aCGH analysis consisting of 4363 human bacterial artificial chromosome clones (Macrogen, Seoul, South Korea). The experiments were conducted according to the manufacturer's protocol [9,10]. Briefly, 2 μg of test and reference DNA were digested and labeled by random priming (BioPrime Array CGH Genomic Labeling System; Invitrogen, Carlsbad, CA) using 3 μl of 1 mM Cy3 or Cy5 dCTP (GeneChem Inc, Daejeon, Korea). After incubation overnight at 37°C, non-incorporated fluorescent nucleotides were removed (Purification Module; Invitrogen), and the labeled samples were ethanol-precipitated. Hybridization was performed in slide chambers for 48 hours at 37°C, and the arrays were scanned (GenePix4200A two-color fluorescent scanner; Axon Instruments, Union City, CA) and quantitated using GenePix software (Axon Instruments).

Data Processing and Statistical Analysis

Frequency plots and tables showing the identified regions of the copy number errors were generated by analysis of copy number errors. To detect gains and losses in the aCGH data, the analysis of copy number error algorithm in *CGH-Explorer* was used with a *P* value less than .01 (false discovery rate of <0.0065) [11]. The threshold for homozygous deletion and amplification was set to the $\log_2$ ratio of ±1. Kaplan-Meier analysis with the log-rank test, Pearson chi-squared test, and Fisher exact test were used for univariate analysis, and logistic regression analysis and Cox proportional hazards model for the survival analysis were used for multivariate analysis (IBM SPSS Statistics 17.0; SPSS, Inc, Chicago, IL). Results with *P* values less than .05 were deemed significant.

Results

Genetic Grouping of MB

The MB series (n = 74) was composed of 47 classic (63.5%), 16 desmoplastic (21.6%; 2 D/N and 14 MBEN variants), and 11 LC/A (14.8%, 5 large cell and 6 anaplastic variants) variants (Table 1), of which proportions of histologic variants were slightly higher than those reported previously [12].

The overall aCGH data revealed several chromosomal aberrations (gain: 1p/1q, 2, 4p, 5, 7, 9p, 12, 17q, and 18; loss: 3p/3q, 6, 8p/8q, 10q, 11p, 13q, 16q, 17p, 19q, 20p, and X), showing heterogeneous cytogenetic features of MB. However, unsupervised random hierarchical clustering and clustering based on WNT and SHH signaling pathway–associated genes (KEGG GENES Database; http://www.genome.jp/kegg/genes.html) failed to divide MBs into the four known genetic groups (WNT, SHH, group 3, and group 4). Thus, on the basis of IHC and FISH results and the *CTNNB1* mutation study, we divided the 74 MBs into WNT, SHH, and non-WNT/SHH groups. A summary of the *CTNNB1* mutation study, IHC, and cytogenetic results for all six groups is shown in Table 2 and Figure 1.

WNT group (n = 12). Cases showing monosomy 6 (3/44 cases by FISH; 5%) and *CTNNB1* missense mutation (6/73 cases; 8%) primarily comprised the WNT group (n = 8). The *CTNNB1* mutations in four of six cases were located between codon 34 and codon 38. However, one case exhibited codon 53 mutation (c. G>A, p.E53K), and another exhibited codon 60 mutation (c. T>A, p.S60T), both of which have not been reported in MB previously [13]. The former case showed diffuse immunoexpression of NPR3. However, we could not further validate the coexistence of *CTNNB1* mutation and NPR3 expression because aCGH was not feasible in this case. Additionally, we added four cases to the WNT group based on diffuse nuclear β-catenin expression, of which one case showed neither monosomy 6 nor *CTNNB1* missense mutation, and *CTNNB1* sequencing and FISH were not feasible in three cases. Of eight cases harboring *CTNNB1* mutation and/or monosomy 6, only two showed diffuse nuclear β-catenin expression.

WNT group tumors exhibited robust immunopositivity for DKK1 (7/12), filamin A (8/12), and YAP (6/12; Figure 2). Foci of positivity for SFRP1 (3/12) and GAB1 (3/12) were observed, and KCNA1 (7/12) showed scattered positive cells in the tumor area (Figure 2*H*). Of three cases showing GAB1 positivity, one harbored monosomy 6, and the other two diffusely expressed nuclear β-catenin, comparing the weak and limited expression of GAB1, which accounted for more than 10% of the tumor. Eleven cases (11/12) were of the classic type, and only one case showed LC/A histology.

SHH group (n = 15). Fifteen cases showing GAB1 immunoexpression without monosomy 6, *CTNNB1* mutation, and nuclear β-catenin expression were included in the SHH group because a *PTCH1*/*SUFU* mutation study was not feasible. Of the IHC markers, filamin A (13/15), YAP (12/15), SFRP1 (11/15), and DKK1 (9/15) were expressed, and these four IHC markers and GAB1 were positive in the internodular

Table 2. IHC Results in Three Groups of MB.

Group	IHC Marker Expression (Case No., %)								Case No. (%)
	β-Catenin (*N*)*	GAB1	Filamin A	YAP	DKK1	SFRP1	NPR3	KCNA1	
WNT	6 (50)	3 (25)	8 (67)	6 (50)	7 (58)	3 (25)	1 (8)	7 (58)	12 (16)
SHH	0 (0)	15 (100)	13 (87)	12 (80)	9 (57.1)	11 (73)	0 (0)	12 (80)	15 (20)
Non-WNT/SHH	0 (0)	0 (0)	8 (17)	1 (2)	4 (9)	2 (4)	1 (2)	23 (49)	47 (64)
Case No. (%)	6 (8)	18 (24)	29 (39)	19 (26)	20 (27)	16 (22)	2 (3)	42 (57)	74 (100)

*Nuclear expression.

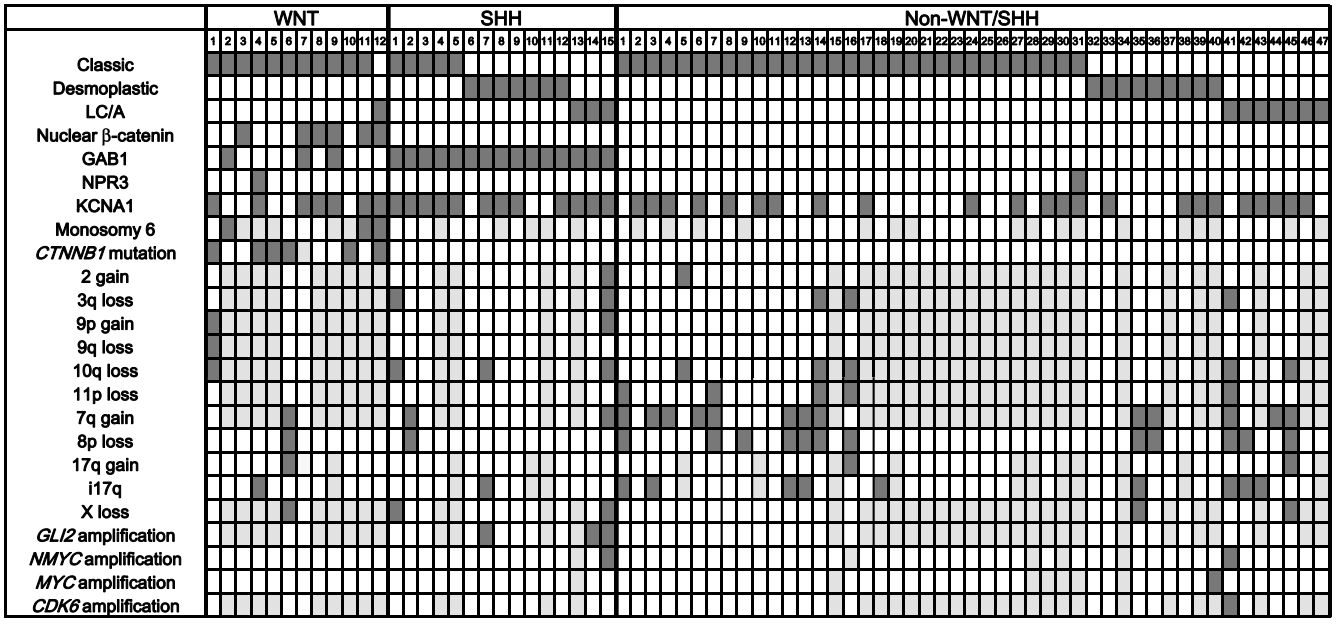

Figure 1. Summary of FISH and aCGH results. Charcoal gray, positive; empty, negative; soft gray, not available.

region in desmoplastic MB cases (Figure 3). Eleven cases were simultaneously positive for GAB1, filamin A, YAP, and SFRP1. KCNA1 was positive in 12 cases, in which KCNA1 was diffusely expressed in neuropil-like matrix regions of desmoplastic MBs (12/15; Figure 3*H*) or in some scattered cells, similar to that in WNT tumors. Nuclear β-catenin and NPR3 expression were negative. Regarding cytogenetic data, *GLI2* amplification (3/11), *MYCN* amplification (1/14), 10q loss (3/11), 3q loss (2/11), and X loss (2/11) were observed. Histologically, desmoplastic MB was observed in seven cases (7/15), the classic type in five, and the LC/A variant in three.

Non-WNT/SHH group (n = 47). Although only NPR3 and KCNA1 immunopositivity were used as specific markers for group 3 and group 4, respectively, these two markers did not clearly divide group 3 and group 4 in the present study. Therefore, the remaining 47 cases were included in the non-WNT/SHH group. Only one case in this group showed diffuse and strong NPR3 expression. The other case showing diffuse NPR3 expression was classified into the WNT group because it harbored the *CTNNB1* mutation (c. G>A, p.E53K). Moreover, KCNA1-expressing cases were found in the WNT and SHH group tumors as well as the non-WNT/SHH group tumors. The expression pattern of KCNA1 in the non-WNT/SHH group was diffuse or the same as that of the WNT and SHH group tumors. The neuropil-like matrix in desmoplastic MBs was diffusely positive for KCNA1, and in the other histologic subtypes, KCNA1-positive cells were aggregated or scattered among KCNA1-negative cells (Figures 2*H*, 3*H*, and 4*H*). KCNA1 was expressed in 49% (23/47) of cases, and other IHC markers including filamin A (8/47), YAP (1/47), DKK1

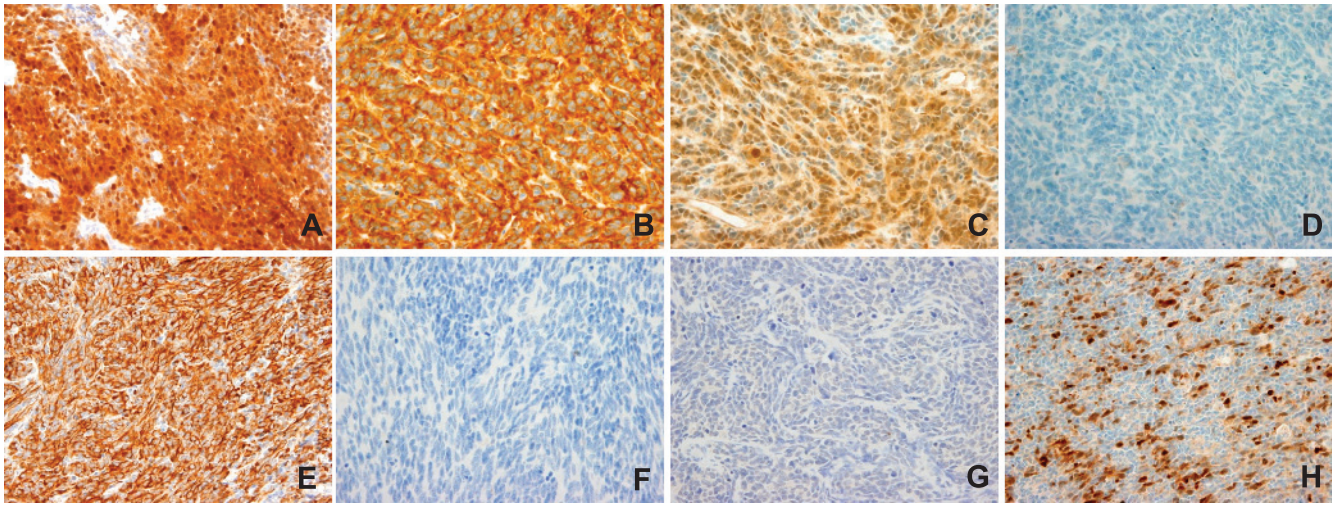

Figure 2. IHC expression in the WNT group. Diffuse nuclear β-catenin expression (A) and filamin A (B) and YAP (C) expression were observed. GAB1 expression (D) was negative, except in three cases. DKK1 expression was frequently positive (E), but SFRP1 (F) and NPR3 (G) expression were mostly negative. KCNA1 (H) showed a patchy infiltration of KCNA1(+) cells among KCNA1(−) cells.

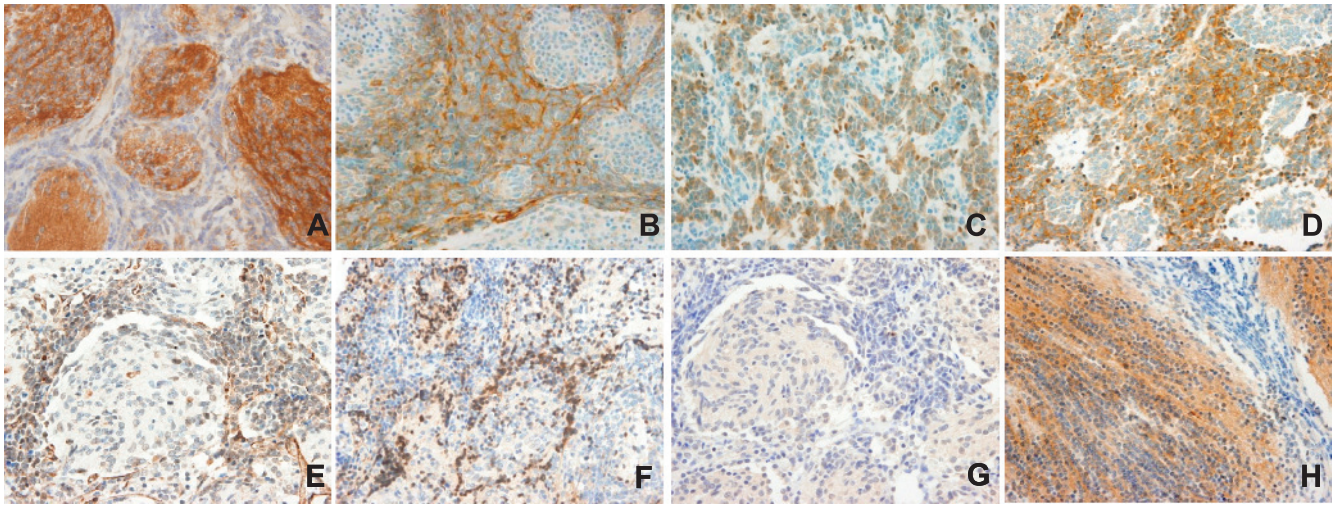

Figure 3. IHC expression in the SHH group. Cytoplasmic β-catenin expression was positive only in desmoplastic-type nodules (A), while filamin A (B), YAP (C), and GAB1 (D) expressions were positive in internodular tumor cells. Some cases exhibited DKK1 (E) and SFRP1 (F) positivity in internodular regions of desmoplastic MBs. NPR3 expression (G) was negative, and KCNA1 expression (H) was positive in desmoplastic-type nodules.

(4/47), and SFRP1 (2/47) were expressed in a few cases. Regarding chromosomal alterations, non-WNT/SHH tumors showed 10q loss (5/24), 11p loss (5/24), 7q gain (13/24), 8p loss (12/24), and X loss (2/24, all female cases), amplification of *CDK6* (1/24), *MYC* (1/24), and *MYCN* (1/24), and i17q (9/24). Histologically, 31 cases were classic (31/47, 66%), nine cases were desmoplastic (9/47, 19%), and seven were LC/A variant (7/47, 15%).

Survival of MB Patients

In univariate analysis, i17q (P = .003) and the LC/A variant (P = .011) were closely associated with survival. However, genetic subgroups failed to show statistically significant effects on survival. Children ($3 < \text{age} \leq 18$) were more frequent in the non-WNT/SHH group than in the WNT and SHH groups (P = .007). Classic histology was common in the WNT group, desmoplastic histology was common in the SHH group, and the LC/A variant was common in the non-WNT/SHH group (P = .011). In multivariate analysis, i17q (P = .006) and the LC/A variant (P = .027) were independently associated with poor survival.

In Kaplan-Meier survival analyses, patients with i17q had a worse prognosis than those without i17q ($P < .001$; Figure 5*A*), and the LC/A variant had a worse prognosis than either the classic or desmoplastic types (P = .004; Figure 5*B*). Although the WNT group showed a slightly better outcome than the SHH group and the non-WNT/SHH group regarding patients who had undergone gross total or near total resection (Figure 5*C*) and children ($3 < \text{age} \leq 18$) who had undergone gross total or near total resection (Figure 5*D*), the differences were not statistically significant. In a Cox proportional hazards

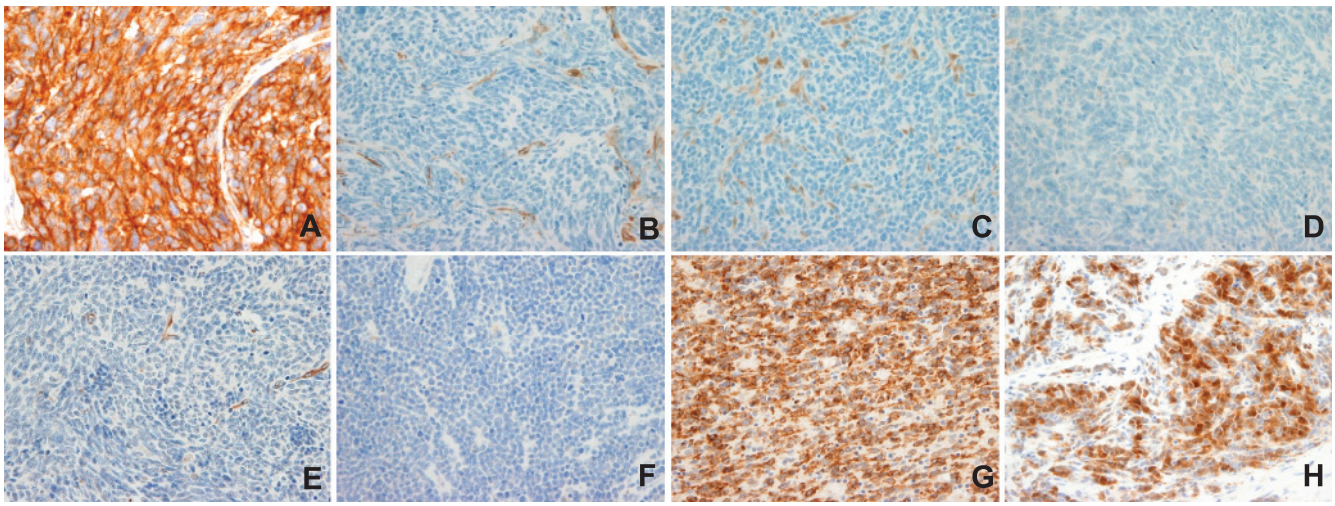

Figure 4. IHC expression in the non-WNT/SHH group. Expression of most of the IHC markers, including nuclear β-catenin (A), YAP (C), GAB1 (D), DKK1 (E), and SFRP1 (F), was negative, except in a few cases. Filamin A expression (B) was mostly negative, but eight cases (17%) were positive. NPR3 expression (G) was diffusely positive in one case, and KCNA1 expression (H) was positive in 23 cases (49%).

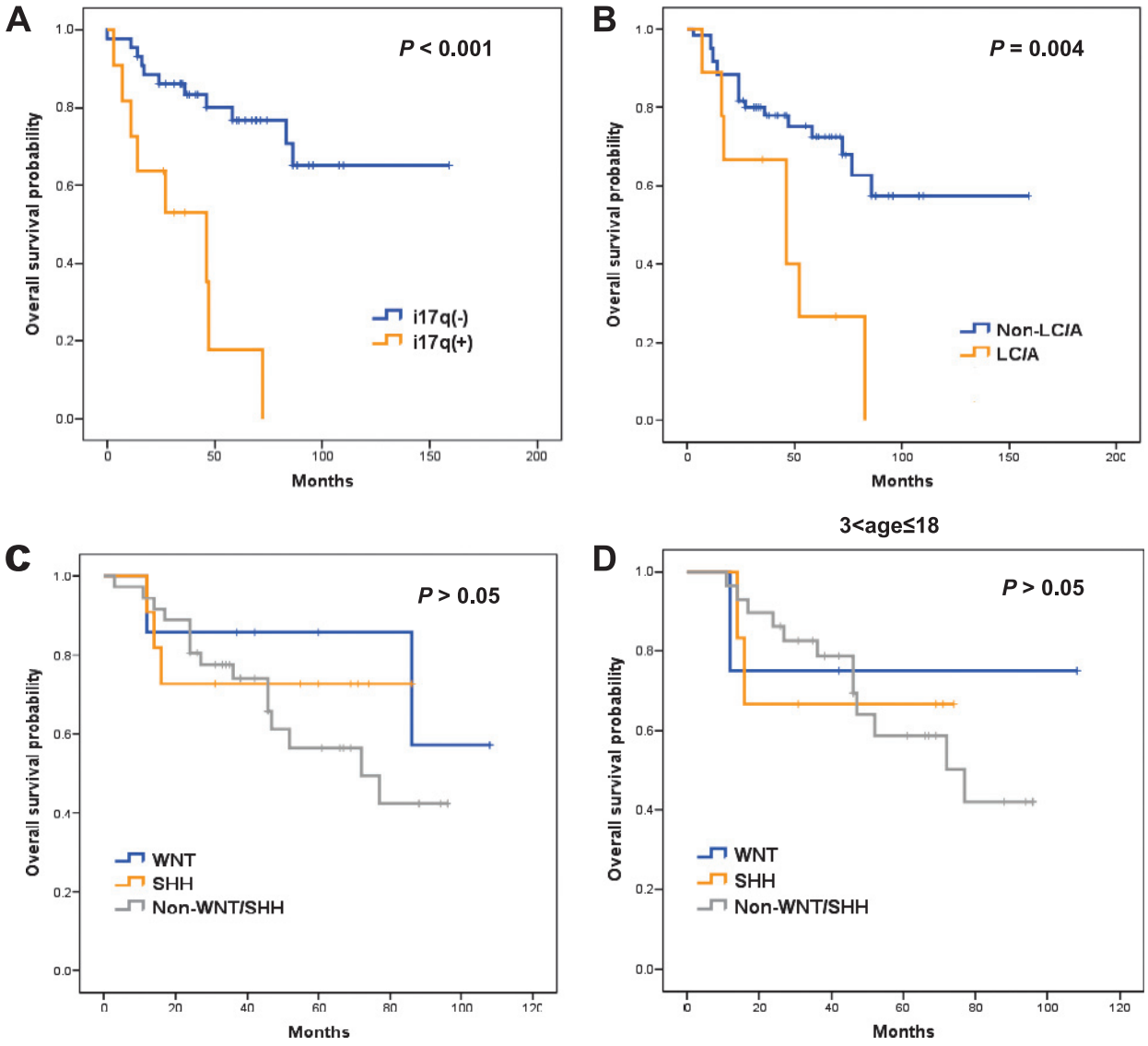

Figure 5. Kaplan-Meier survival curves of the i17q (+) and i17q (−) groups (A), histologic subtypes (B), genetic groups that had undergone gross total or near total resection (C), and genetic groups comprising only children (3 < age ≤ 18) (D), who had undergone gross total or near total resection.

model, i17q (P = .002, hazard ratio = 5.736) and the LC/A variant (P = .002, hazard ratio = 7.398) were all significant independent hazards to survival.

Discussion

Tumor classification by distinct gene expression profiling may provide optimal targeted therapy. However, for routine pathologic evaluation using FFPE tissue, it is essential to develop specific markers that are easily applicable, reproducible, and well correlated with each molecular subgroup [14]. The present study aimed to investigate the feasibility of some IHC and cytogenetic markers representing four genetic groups of MB. Because the results of aCGH could not provide the gold standard concerning genetic clustering of 74 MBs, we attempted to validate those markers and classify MBs with limitations. Moreover, several cytogenetic alterations overlapped between genetic groups, and IHC markers expected to be exclusive to one genetic group were sometimes expressed in other groups.

Regarding IHC markers, we used nuclear β-catenin and GAB1 in classifying the WNT and SHH groups, and these two groups were easily divided without significant overlapping, except three cases that showed both nuclear β-catenin and GAB1 expression. In one case, the existence of monosomy 6 was helpful for inclusion in the WNT group despite GAB1 expression. In the other two cases, β-catenin expression was intense and diffuse, in contrast to GAB1 expression, which was relatively weak and limited, although its expression accounted for more than 10% of the tumors. In addition, we found that DKK1, SFRP1, filamin A, and YAP immunoexpression could be relatively well controlled, but the expression levels lacked specificity for each genetic group. Moreover, each IHC expression profile depended on specific clones [7]. We used a few more clones for optimal immunoexpression of these markers; however, we failed to achieve specific expression, excluding the clones in current use. DKK1 and SFRP1 were expressed mostly in the WNT and SHH groups; however, SFRP1 was focally expressed in the WNT group, which harbored the *CTNNB1* mutation or expressed nuclear β-catenin. Likewise, DKK1 was

expressed in the internodular area of desmoplastic SHH group tumors, which showed GAB1 expression. Negative expression of YAP and filamin A has been reported to be a marker of non-WNT/SHH group [7]; however, some cases of non-WNT/SHH group tumors expressed YAP (2%) and filamin A (17%). We also tested GLI1, CRX, and GRM8 as markers of the SHH group, group 3, and group 4, respectively; however, we failed to optimally express these markers for grouping (data not shown).

For classifying groups 3 and 4, KCNA1 and NPR3 were neither specific nor sensitive markers in the present study. Although this result is in contrast to previous data [4,15], a recent report by Bien-Willner et al. supported the low specificity of KCNA1 and NPR3 [16]. KCNA1 lacked specificity as its expression was identified in all subgroups, and NPR3 lacked sensitivity and probably specificity, showing expression in only two cases, one of which in the WNT group harbored the *CTNNB1* mutation. Overall, the data suggest that, in the IHC study, only nuclear β-catenin and GAB1 expression are useful for differentiating the WNT and SHH groups, respectively, and no specific marker for classifying groups 3 and 4 is yet available.

In cytogenetic analyses, monosomy 6 was regarded as an indicator of the WNT group, and no other alterations are known to be specific to this genetic subgroup. Although 7 gain, 8 loss, and i17q were far more frequent in non-WNT/SHH tumors, these alterations were found in a few cases of WNT and SHH tumors. In cases in which aCGH was feasible, overall cytogenetic alterations, including 3p loss, 10q loss, 11p loss, and X loss, were identified more frequently in the non-WNT/SHH group than in the WNT and SHH groups.

Although the overall statistical analyses were more or less limited due to the small number of cases in each subgroup, a few well-known prognostic factors repeatedly showed their impact on clinical outcome. The patients showing i17q and the LC/A histology had a significantly poorer outcome in multivariate analysis, a finding that was consistent with previous data [12,17–20]. Regardless of the presence of residual tumor or disease stage, i17q was an independent negative prognostic factor in our study. i17q is the most common chromosomal alteration (25–35%) in childhood MB [19,21], and it is known to be associated with poor survival, independent of high-risk clinical factors [19]. Both isolated 17p loss [17,22,23] and isolated 17q gain [24] have shown a significant association with poor survival; however, these alterations did not show prognostic impact in our results. In addition, LC/A histology was shown to have prognostic significance. Although a close association with *MYC* or *MYCN* overexpression has been suggested [25,26], LC/A histology was reported to be an independent poor prognostic factor [25]. Because *MYC* or *MYCN* amplification was found in only one case each, the association with the LC/A phenotype was not validated in our series. Regarding genetic subgroups, the WNT group showed a better prognosis than the SHH and non-WNT/SHH groups in children who had undergone gross total or near total resection; however, a greater number of cases and longer follow-up duration are required to achieve statistical significance. Of the five patients in the WNT group who have died (5/12), two were young adults (age = 21 and 22 years, respectively) who died 7 years after the initial surgery. One pediatric patient who died 1 year after the surgery had i17q, and in the other two pediatric patients, FISH was not feasible.

Differentiation of tumors of the WNT group from the other groups is important because the WNT group shows a much more favorable outcome. On the basis of tests for the *CTNNB1* mutation, monosomy 6, and immunoexpression of nuclear β-catenin and GAB1, we classified cases into the WNT, SHH, and non-WNT/SHH groups as described previously [5]. However, the survival analysis failed to show a significant difference among genetic groups and demonstrated only the prognostic significance of i17q and LC/A histology. In addition, NPR3 and KCNA1 did not appear to be sufficiently sensitive or specific for genetic grouping, in contrast to previous data. Additional representative IHC markers are required for classifying groups 3 and 4 tumors in diagnostic practice.

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