The Cerebellum (2019) 18:469–488
https://doi.org/10.1007/s12311-019-1009-y

ORIGINAL PAPER

Differential Expression of Genes for Ubiquitin Ligases in Medulloblastoma Subtypes

Jerry Vriend[1] · Robert B. Tate[2]

Published online: 27 February 2019

Abstract

Using publically available datasets on gene expression in medulloblastoma (MB) subtypes, we selected genes for ubiquitin ligases and identified statistically those that best predicted each of the four major MB subgroups as separate disease entities. We identify a gene coding for an ubiquitin ligase, *ZNRF3*, whose overexpression alone can predict the WNT subgroup for 100% in the Pfister dataset. For the SHH subgroup, we identify a gene for a regulatory subunit of the protein phosphatase 2A (PP2A), *PPP2R2C*, as the major predictor among the E3 ligases genes. The ubiquitin and ubiquitin-like conjugation database (UUCD) lists PPP2R2C as coding for a Cullin Ring ubiquitin ligase adaptor. For group 3 MBs, the best ubiquitin ligase predictor was *PPP2R2B*, a gene which codes for another regulatory subunit of the PP2A holoenzyme. For group 4, the best E3 gene predictors were *MID2*, *ZBTB18*, and *PPP2R2A*, which codes for a third PP2A regulatory subunit. Heatmap analysis of the E3 gene data shows that expression of ten genes for ubiquitin ligases can be used to classify MBs into the four major consensus subgroups. This was illustrated by analysis of gene expression of ubiquitin ligases of the Pfister dataset and confirmed in the dataset of Cavalli. We conclude that genes for ubiquitin ligases can be used as genetic markers for MB subtypes and that the proteins coded for by these genes should be investigated as subtype specific therapeutic targets for MB.

Keywords Medulloblastoma subtypes · Ubiquitin ligase · Gene expression · *ZNRF3* · *RNF146* · *PPP2R2A* · *PPP2R2B* · *PPP2R2C* · *ABTB2* · *RCBTB2* · *TRIM58* · *ZBTB18* · *ZBTB20* · Logistic regression

Introduction

Medulloblastoma is described as a malignant tumor of the cerebellum, a tumor found most often in children. Four major molecular subtypes of medulloblastoma (MB) have been identified based on gene expression. By agreement, these four groups were termed the WNT group, the SHH group, group 3, and group 4 [1]. The molecular and clinical variation between subtypes led to the suggestion by Kool and others that MB should be considered as more than one disease [2]. This approach facilitates developing targeted therapy for each subtype considered as a unique disease.

Although expression of ubiquitin ligases was not considered as a factor in defining these subgroups, several publically available datasets include data on expression of genes coding for ubiquitin ligases.

Specific ubiquitin ligases, such as APC and SMURF, have been associated with the WNT signaling pathway [3] and SHH pathway respectively [4]. Group 3 MBs, which have the worst prognosis, have been associated with MYC overexpression [1, 5]. Several ubiquitin ligases associated with MYC activation and degradation have recently been reported [6]. Group 3 is also associated with overexpression of *GABRA5* [7]. Little is known concerning the role of ubiquitin ligases in group 3 MBs. Group 4 MBs, the most frequently occurring subtype, have been associated with a high frequency of isochromosome 17 [1, 8]. Molecular markers as therapeutic targets for group 4 MBs, however, are lacking.

Electronic supplementary material The online version of this article (https://doi.org/10.1007/s12311-019-1009-y) contains supplementary material, which is available to authorized users.

✉ Jerry Vriend
Jerry.Vriend@umanitoba.ca

[1] Department of Human Anatomy & Cell Science, Max Rady College of Medicine, Rady Faculty of Health Sciences, University of Manitoba, Rm134, BMSB, 745 Bannatyne Avenue, Winnipeg, Manitoba R3E 0J9, Canada

[2] Department of Community Health Sciences, Rady Faculty of Health Sciences, University of Manitoba, Winnipeg, Manitoba, Canada

In the present study, publically available datasets were used to determine the expression of ubiquitin ligases regulating signaling pathways associated with the four major types of MB. The goal was to document differential expression of genes for ubiquitin ligases and to determine whether expression of these genes could be used to predict individual MB subgroups. Of the E3 ligase genes whose expression could be used to predict MB subgroups we determined whether their expression was related to classical WNT and SHH signaling pathways. Finally, we determined which E3 ligase genes best predicted groups 3 and 4 MB. The statistical significance of these predictions raised the question of whether proteins coded for by these genes could serve as MB subtype-specific therapeutic targets.

Ubiquitin ligases have the ability to regulate protein stability, including those that function as transcription factors. E3 ligases are involved in regulating turnover of proteins involved in cell cycle, cell growth, cell death, and DNA repair [9]. As such, they could contribute to the abnormal growth and proliferation of cells in cancer. Thus, the objective of this research was to identify genes for E3 ligases whose expression could be used to predict subgroups of MBs and to identify proteins coded for by these genes as potential targets for development of therapeutic agents. While this is particularly important for the MB subgroup with the worst prognosis, the group 3 MBs, we investigate the expression of genes for ubiquitin ligases in each of the four "consensus" subgroups.

Methods

Differential gene expression for ubiquitin ligases associated with the four major subtypes of MBs was examined using publically available datasets. The R2 genomics analysis and visualization platform (R2 GAVP) website (http://r2.amc.nl) has available several datasets on gene expression in medulloblastoma. These include separate datasets contributed by Pfister, Northcott, Kool, Thompson, Gilbertson, and Cavalli. The ubiquitin and ubiquitin-like conjugation database (UUCD) (http://uucd.biocuckoo.org) was used to confirm the identity of various proteins as ubiquitin ligases. Gene expression data (Affymetrix gene expression) was downloaded and analyzed for differential gene expression across the four MB subgroups. A high level of statistical probability ($p < 0.00001$) by one way ANOVA (analysis of variance) was chosen for inclusion of candidate ubiquitin ligase genes. The FDR (false discovery rate) correction of p values was used when listing significance of differentially expressed genes. The uncorrected p values are slightly higher (see Supplementary Tables). Unless otherwise mentioned, expression data referred to is from the Pfister dataset, a training dataset to build a prediction model. The datasets of Northcott (Magic), Kool, Gilbertson, and Cavalli were used as test datasets to determine whether the major findings from the Pfister datasets were confirmed. Table S1 shows F values and p values for the top 100 differentially expressed genes for ubiquitin ligases. Table S2 shows F values and p values for other differentially expressed genes functionally related to pathways associated with ubiquitin ligases in MD subgroups.

In the Pfister dataset, using a cutoff point of $p < 0.0001$ in the ANOVA, we identified genes for 458 E3 ligases (including E3 ligase adapters) that were differentially expressed. The Pfister dataset included 223 tissue samples, of which 56 samples were classified as group 3 MBs, 91 as group 4 MBs, 17 as WNT MBs, and 59 as SHH MBs. Stepwise logistic regression analysis of the top 100 differentially expressed ubiquitin ligase genes of the Pfister dataset was used to determine which were most statistically significant in predicting the MB subtype tissues.

Since the range of gene expression values was substantially different for the various genes, the data were standardized by Z-score (x-mean/standard deviation). Logistic regression analysis was used to quantify separation of each MB subgroup from the other. Because of high correlation of some of the ubiquitin ligases associated with the WNT pathway, stepwise logistic regression of all four groups was followed by stepwise logistic regression of the three non-WNT groups to identify genes for ubiquitin ligases associated with the SHH subgroups and subgroups 3 and 4. Scatter plots were used to illustrate that the SHH group could be predicted from two genes. Further logistic regression analysis was used to identify genes for ubiquitin ligases that distinguished Pfister MB subgroups 3 and 4. Heatmap analysis of the ubiquitin ligases was used to illustrate gene expression of the top 100 differentially expressed ubiquitin ligases in the Pfister dataset. Heatmap analysis of the Cavalli dataset was used to confirm genes identified as highly significant predictors in the Pfister data.

The Gilbertson dataset included 73 samples in the 4 consensus groups of which 16 were in group 3, 39 in group 4, 10 in the SHH group, and 8 in the WNT group. The Kool dataset included 62 samples, of which 11 were classified as the equivalent of group 3 (Kool group E), 27 as the equivalent of group 4 (Kool groups C and D), 9 as WNT MBs, and 15 as SHH MBs. The large Cavalli dataset, recently made available through the R2 website, included 763 samples, of which 144 were in group 3, 326 in group 4, 223 in the SHH group, and 70 in the WNT group.

While the Kool and Gilbertson datasets illustrate the limitations of a small sample size, they did provide confirmation of differential expression at a high level of statistical significance for many E3 ligases. The Northcott (Magic) dataset, which contained no WNT data, was used to compare differential expression of ubiquitin ligases of non-WNT MBs. This dataset included 51 SHH samples, 46 group 3 samples, and 188 group 4 samples.

Results

The expression of the top 100 E3 ligase genes in the Pfister dataset is shown in the heatmap of Fig. 1 and in Table S1. The p values (corrected for multiple comparisons by FDR) of the ANOVA for the top 100 differently expressed genes for E3 ligases varied from $p = 2.68e\text{-}90$ (*ZNRF3*) to $p = 4.46e\text{-}17$ (*HERC3*) in the Pfister dataset (Table S1). Chromosome 17

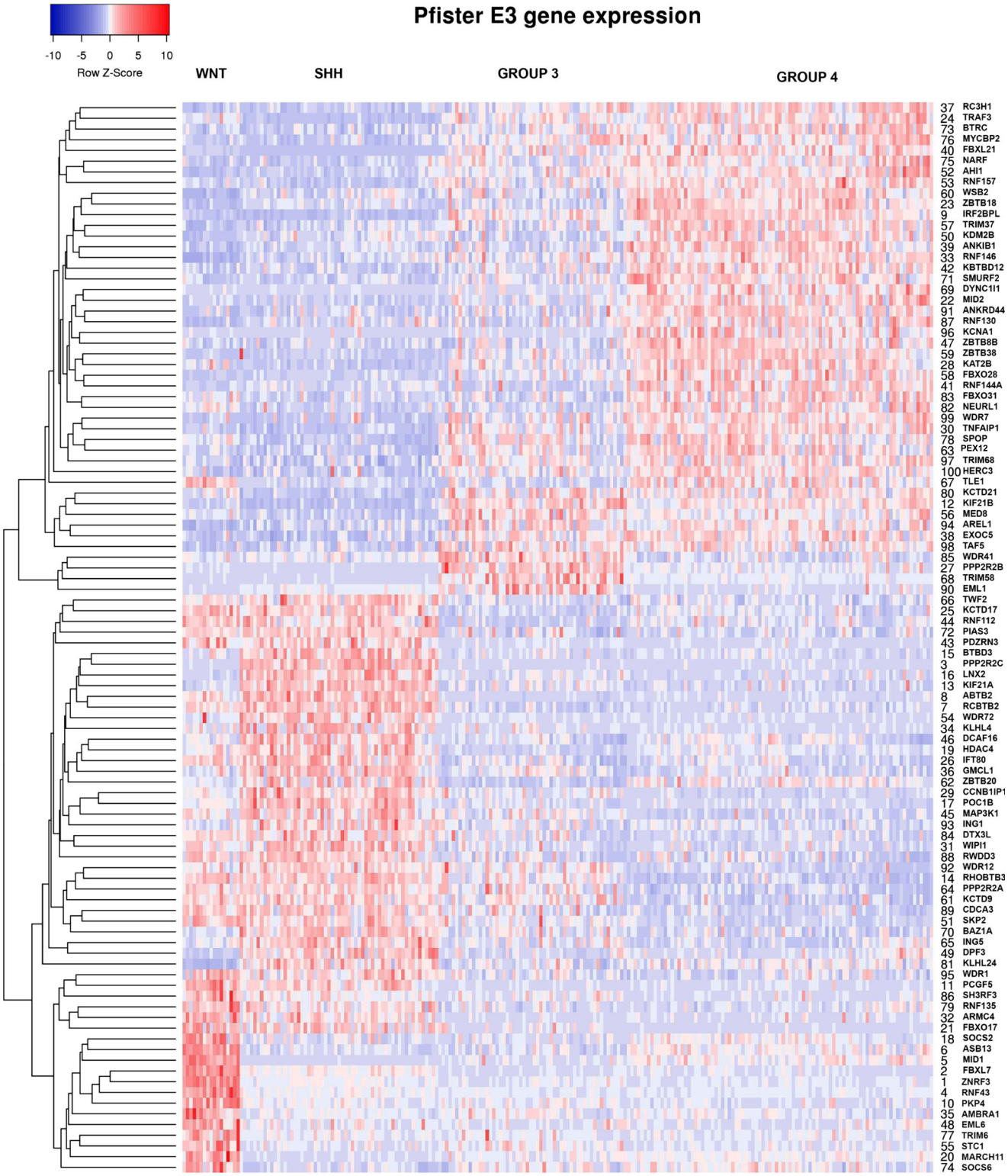

Fig. 1 Heatmap of 100 most significant ubiquitin ligases in the Pfister dataset. The clustering method used is the average linkage method

as a location for these genes was over-represented (12/100) compared to the other chromosomes (average of 4.26/100).

WNT Subgroup

The expression of two genes for ubiquitin ligases associated with regulation of WNT receptors, *ZNRF3*, and *RNF43* was significantly elevated in the WNT MBs. Differential expression of *ZNRF3* was highly significant ($F = 454.36$, $p = 2.68e\text{-}90$) (Fig. 2). This F value was the greatest of all the 458 genes for E3 ligases identified as differentially expressed in the Pfister data. A *ZNRF3* value of greater than 1.1 standard deviations above its mean identified all 17 WNT subgroup samples (100% accuracy, 100% sensitivity). This relationship also holds in the Kool dataset and in the Gilbertson dataset and in the Cavalli dataset (96% accuracy, 99.9% sensitivity). *RNF43* (a paralog of *ZNRF3* on chromosome 17) was also differentially expressed at a high level of significance ($F = 164.70$, $p = 1.97e\text{-}53$) (Fig. 2). In addition, the following were correlated with *ZNRF3* and could also be used as predictors for the WNT group: *FBXL7* ($R = 0.82$), *MID1* (R = 0.68), and *ASB13* ($R = 0.69$), all significantly overexpressed in the WNT subgroup. The interaction of ZNRF3 and RNF43 with the WNT receptor complex is shown in Fig. 3.

Based on the data of Koo et al. [3], increased RNF43 would be expected to be associated with decreased WNT membrane receptors, including FZD1, FZD3, and FZD5. In the Pfister dataset, expression of *FZD1* and *FZD3* was reduced in the WNT subgroup compared to the others, but not at a high level of significance. However, expression of two other FZD genes (*FZD6* and *FZD10*) was elevated compared to the others. According to the Pfister dataset, the expression of the *FZD10* gene was selectively increased more than 100-fold in the WNT subgroup of tumors compared to that of the other 3 subgroups ($F = 283.31$, $p = 4.42e\text{-}72$). Compared to normal cerebellar tissue (Roth dataset) expression of this gene was increased approximately 60-fold. Based on this evidence the *FZD10* gene could be considered to be overexpressed in WNT MBs. Statistically, the expression of FZD10 was correlated with expression of ZNRF3 ($R = 0.86$, $p = 1.8e\text{-}62$) and with expression of RNF43 ($R = 0.90$, $p = 2.2e\text{-}78$) in the Pfister dataset. The selective increase in *FZD10* expression in the WNT subgroup was confirmed in the datasets of Gilbertson ($F = 56.56$, 1.4e-18), Kool ($F = 98.77$, $p = 6.1e\text{-}25$), and Cavalli ($F = 430.22$, $p = 3.2e\text{-}163$). The very strong statistical association of the two ubiquitin ligases with *FZD10* expression is consistent with a significant functional relationship (see "Discussion").

The expression of *RNF146*, an E3 ligase gene located on chromosome 6, was substantially decreased in the WNT

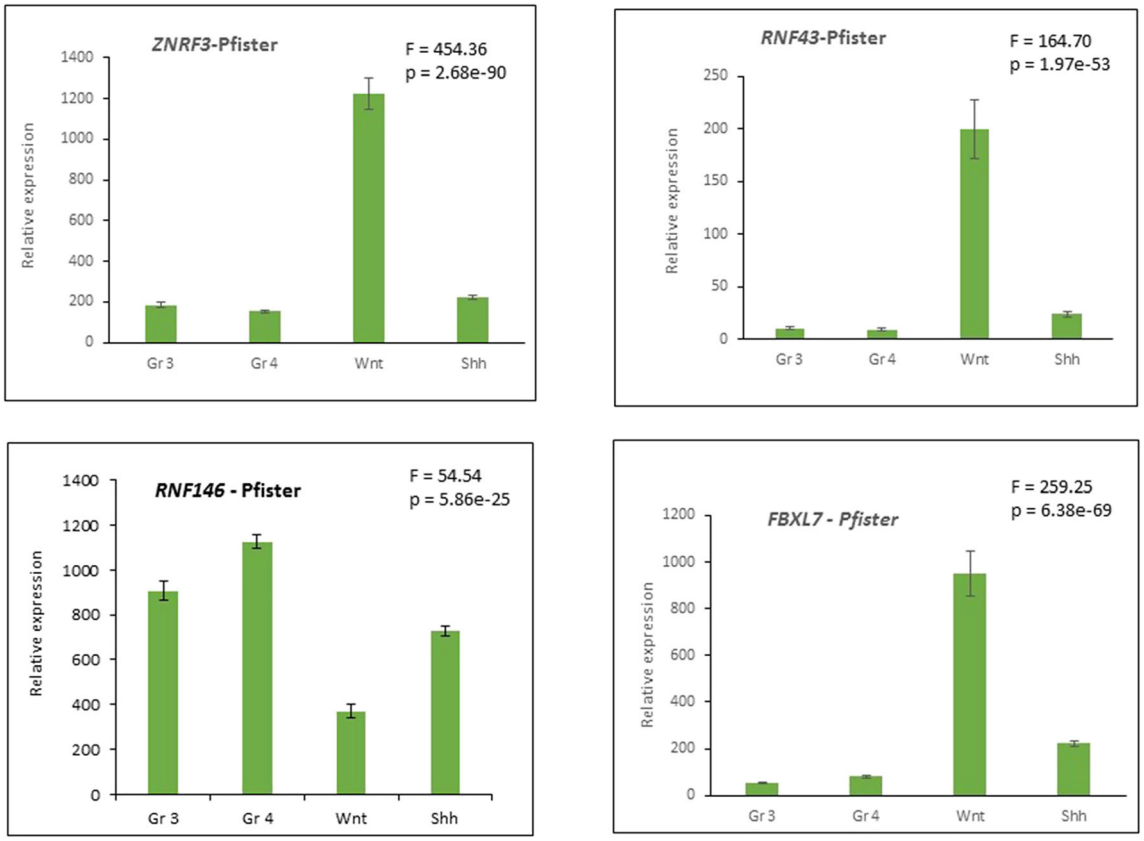

Fig. 2 Ubiquitin ligase gene expression as predictors of WNT MB. Bar charts shows means with standard error bars. By ANOVA for *ZNRF3* $F = 454.36$, $p = 2.27$ e-90; for *RNF43* $F = 164.70$, $p = 1.97e\text{-}53$; for *RNF146* $F = 54.54$, $p = 5.86e\text{-}25$; for *FBXL7* $F = 259.25$, $p = 6.38e\text{-}69$

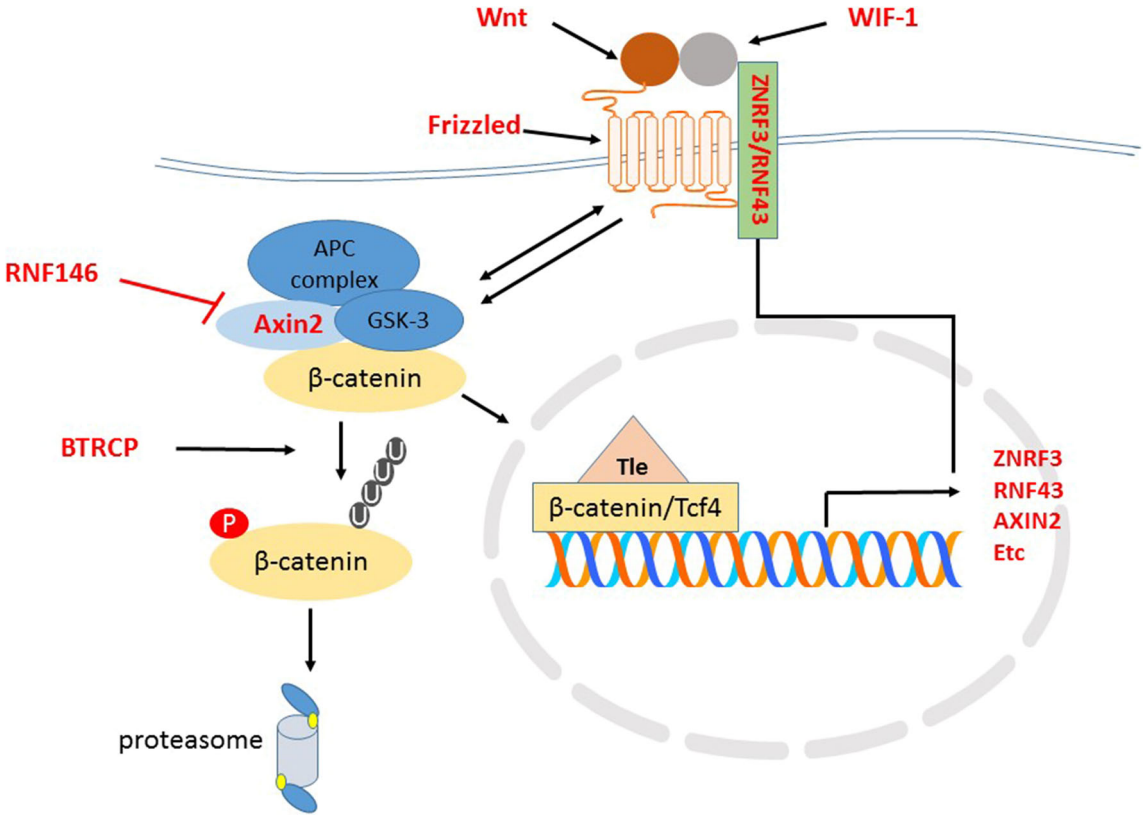

Fig. 3 Ubiquitin ligases and the WNT signaling pathway. ZNRF3, RNF43, RNF146, and APC are regulators of WNT receptors, AXIN2, and β-catenin

subgroup ($F = 54.55$, $p = 5.86\text{e-}25$) (Fig. 2), consistent with the high percentage of monosomy of chromosome 6 in the WNT subgroup [10]. A substrate for the ligase RNF146 is AXIN2, an inhibitor of WNT signaling (see "Discussion"). The expression of AXIN2 was increased approximately eight-fold in the WNT subgroup ($F = 934.59$, $p = 3.16\text{e-}120$). These findings were confirmed in the Cavalli dataset. The interaction of RNF146 with AXIN2 as part of the WNT pathway is shown in the model illustrated in Fig. 3.

SHH Subgroup

The 17 samples of WNT MBs were excluded from the ubiquitin ligase dataset for further analysis. With the remaining 206 (non-WNT) samples of MB, stepwise logistic regression modeling was used to identify the best E3 ligase predictors of the SHH subtype relative to the group 3 and group 4 subtypes. The best E3 ligase predictor of the 59 samples of SHH subtype was expression of the gene *PPP2R2C* (Fig. 4). Expression of the genes *RCBTB2* and *ABTB2*, genes for E3 ligase adaptors, was also found to distinguish the SHH subtype from groups 3 and 4 very well (Fig. 4). A scatterplot of the expression values of *PPP2R2C* and *RCBTB2*, separated the SHH subtype for 100% from the group 3 and group 4 subtypes (Fig. 5). The expression of both these genes was significantly increased ($F = 291.57$, $p = 4.24$ e-57; $F = 196.81$, $p = 2.50\text{e-}45$) in the SHH group. This was confirmed in the datasets of Northcott (Magic), Cavalli, Gilbertson, and Kool, supporting the findings in the Pfister dataset. In the Northcott (Magic) dataset, as well as in the Cavalli dataset, the top two differentially expressed E3 ligase genes in non-WNT MBs (according to ANOVA) were *PPP2R2C* and *RCBTB2*. Identification of all the 59 SHH samples of the Pfister dataset was also possible from expression of the three genes for *PPP2R2C*, *POC1B*, and *MAP3K1* by stepwise logistic regression (sensitivity of 100%, specificity of 97%).

Differential expressions of the ubiquitin ligase gene, *SMURF2*, which regulates degradation of the SHH receptor PTCH1, were also highly significant ($F = 53.03$, $p = 6.34\text{e-}18$) among the non-WNT subgroups. However, statistically, its expression was not as good as predictor of the SHH subtype as the expression of *PPP2R2C* and *RCBTB2*.

The ubiquitin ligase gene *FBXL21* appeared to be silenced (near zero expression) in SHH MBs compared to expression in groups 3 and 4 ($F = 66.15$, $p = 2.49\text{e-}21$). This was confirmed in the datasets of Northcott (Magic) and Cavalli ($F = 77.53$, $p = 1.5\text{e-}27$; $F = 360.21$, $p = 7.5\text{e-}108$).

Although we noted that expression of the gene *BOC*, which codes for a co-receptor for the SHH pathway was listed by UUCD as an ubiquitin ligase in one species, *Pteropus vampiris*, we did not include it in the logistic regression analysis since it has not been confirmed, in other species, that

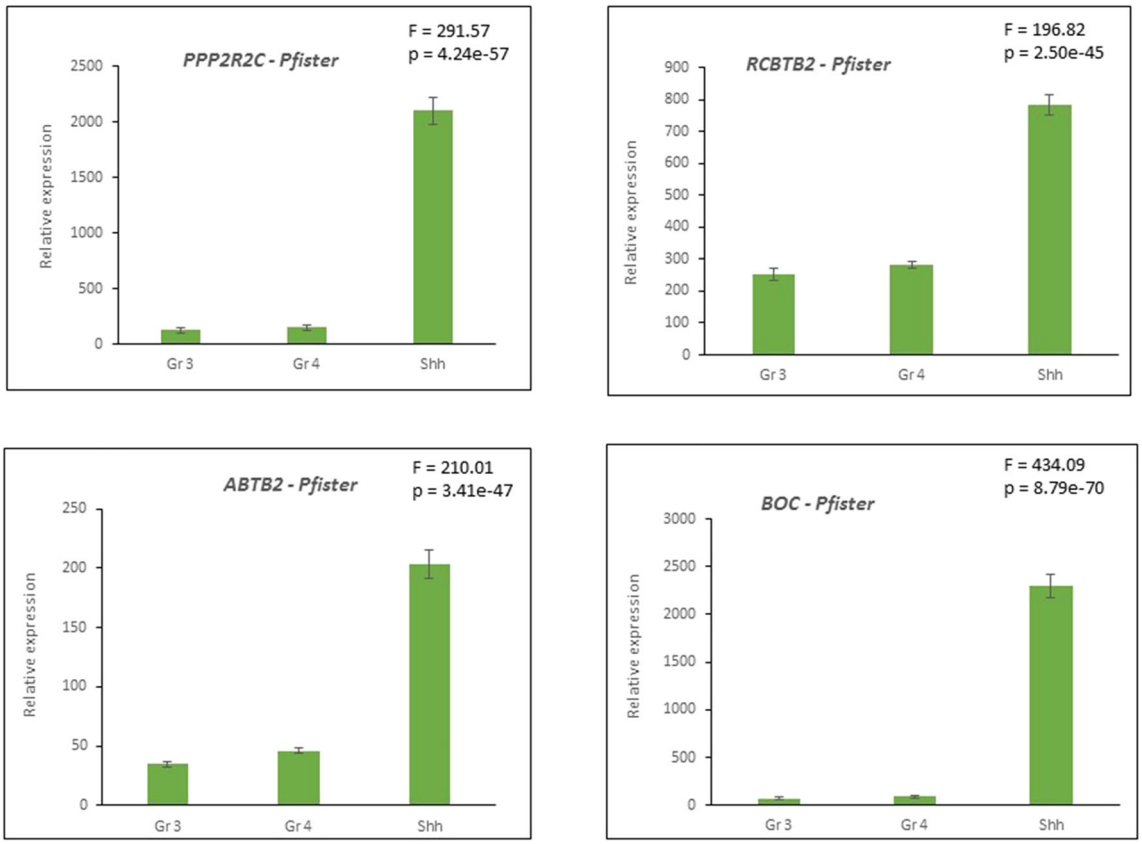

Fig. 4 Ubiquitin ligase gene expression predictors of SHH MB. Bar charts shows means with standard error bars. By ANOVA for *PPP2R2C* $F = 291.57$, $p = 4.24e\text{-}57$; for *RCBTB2* $F = 196.82$, $p = 2.50e\text{-}45$; for *ABTB2* $F = 210.01$, $p = 3.41e\text{-}47$; for *BOC* $F = 434.09$, $p = 8.79e\text{-}70$

BOC is a ubiquitin ligase. However, expression of *BOC* was a very good predictor of the SHH subgroup among the non-WNT MBs in the Pfister dataset (Fig. 4). In the Northcott Magic and Cavalli datasets, its increased expression in the SHH group was confirmed ($F = 225.36$, $p = 3.4e\text{-}59$; $F = 965.57$, $p = 1.1e\text{-}200$) among the non-WNT groups. A model illustrating the interaction of ubiquitin ligases with the SHH receptor complex is illustrated in Fig. 6.

Group 3

Logistic regression showed that the best non-WNT E3 ligase predictor for the 56 samples of group 3 in the Pfister data was expression of *PPP2R2B*. The heatmap (Fig. 1) and Fig. 7 illustrate that this gene is among the few E3 ligase genes selectively increased or decreased in group 3 MBs. *PPP2R2B*, like *PPP2R2C*, codes for a regulatory subunit of

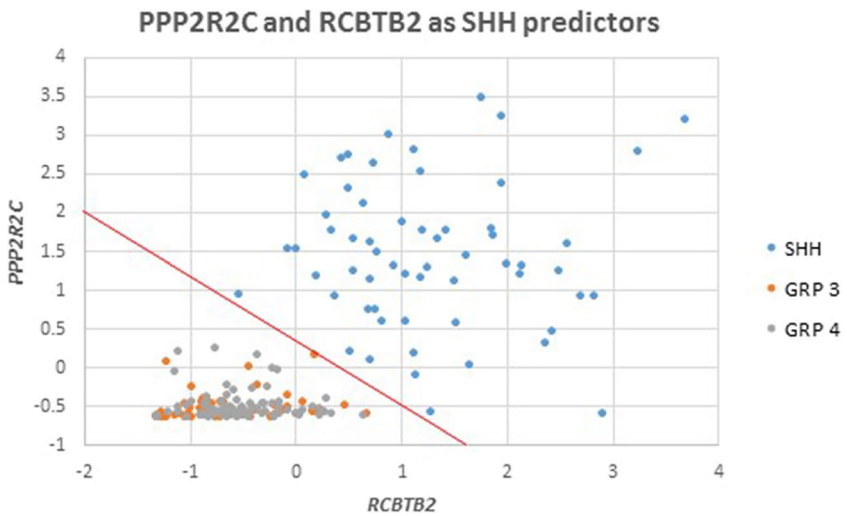

Fig. 5 Scatterplot of *PPP2R2C* and *RCBTB2*. The two genes together predict the SHH group for 100% in all MBs in the Pfister dataset (including WNT MBs). All SHH (blue) values are above the line separating SHH *Z*-score values from those of group 3 and group 4

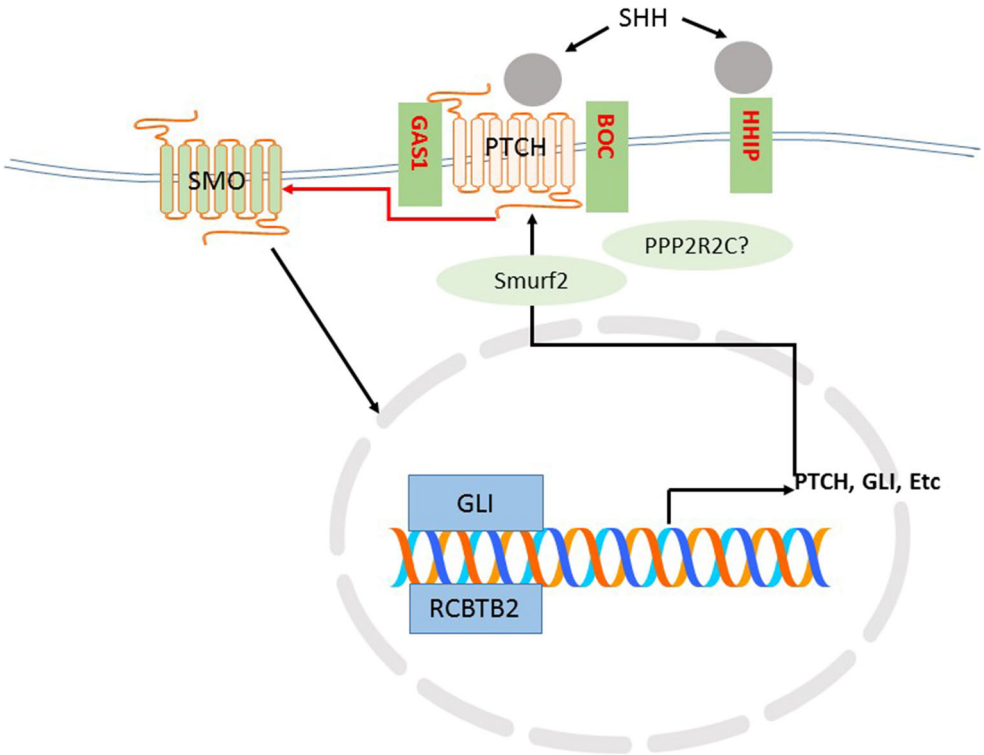

Fig. 6 Ubiquitin ligases and the SHH signaling pathway

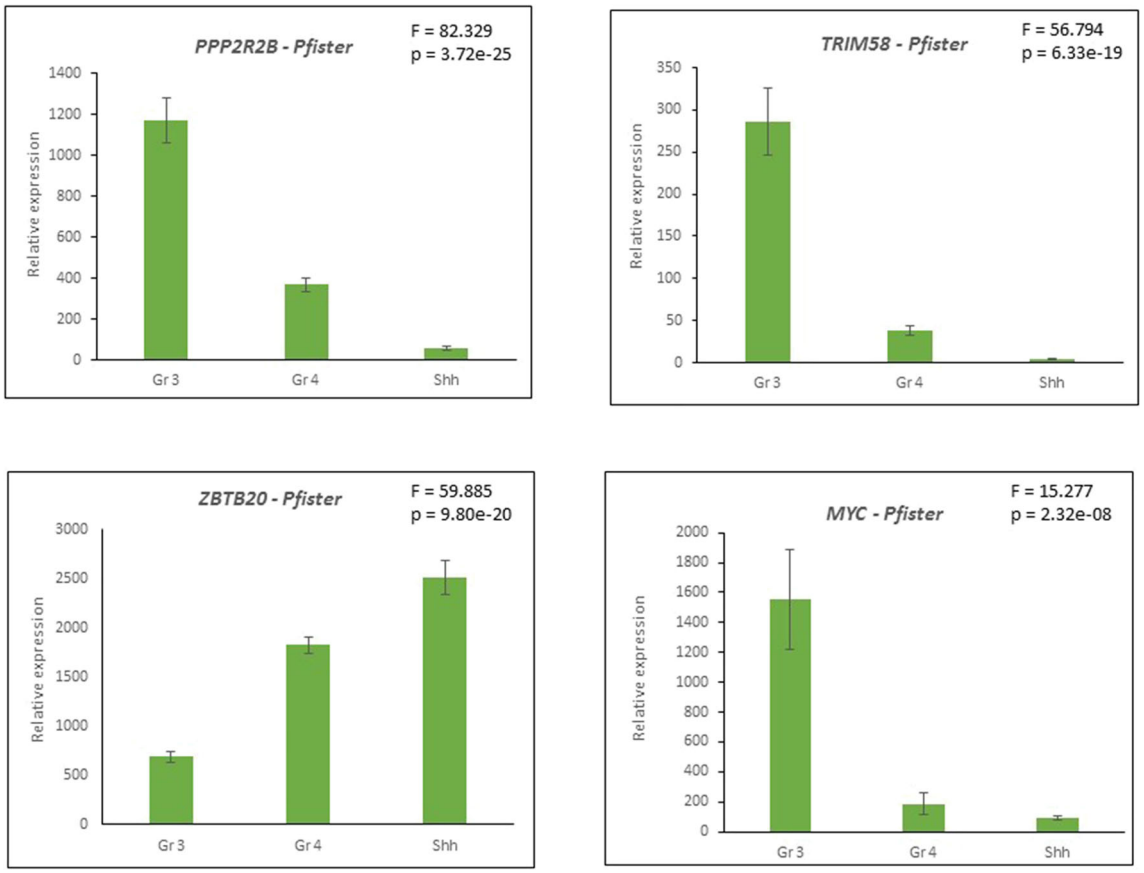

Fig. 7 Gene expression of ubiquitin ligases and *MYC* in group 3 MB. Bar charts shows means with standard error bars. By ANOVA for PPP2R2B $F = 82.329$, $p = 3.72e\text{-}25$; for *TRIM58* $F = 56.794$, $p = 6.44e\text{-}19$; for *ZBTB20* $F = 59.885$, $p = 9.80e\text{-}20$; for *MYC* $F = 15.277$, $p = 2.32e\text{-}08$

protein phosphatase 2. Its expression was increased in group 3 MBs compared to that of the other 2 groups at a high level of significance ($F = 82.33$, $p = 3.72e-25$). Thus, for both the SHH group and group 3 MBs, the best E3 ligase gene predictor, according to the results of stepwise logistic regression, is expression of a regulatory subunit of the phosphatase enzyme PP2A.

The expression of *TRIM58* was also increased in group 3 samples compared to that of the other MB subgroups ($F = 56.79$, $p = 6.33e-19$) (Fig. 7). TRIM58 is a ubiquitin ligase that controls degradation of two key proteins of the cytoplasmic dynein motor [11] (see "Discussion"). The expression of *TRIM58* was significantly correlated to the expression of *NPR3* a gene which codes for a protein marker of group 3 MBs. The comparative increases of *TRIM58* and *PPP2R2B* expression in group 3 were also found in the datasets of Northcott (Magic), Kool, Gilbertson, and Cavalli. The expression of *ZBTB20* (Fig. 7) in group 3 samples was significantly lower than that of the SHH and group 4 tissues. Figure 7 also illustrates the increased expression of the *MYC* gene associated with group 3 in the Pfister dataset.

Group 4

The best non-WNT predictors according to logistic regression analysis for group 4 MBs were *MID2* (an E3 ligase is associated with microtubules), *ZBTB18* (a transcriptional repressor), and *PPP2R2A* (which codes for another regulatory subunit of PP2A) (Fig. 8). Expression of *MID2* and *ZBTB18* was comparatively elevated in group 4 ($F = 89.69$, $p < 8.38e-27$; $F = 75.56$, $p < 1.33e-23$) in the Pfister dataset while expression of *PPP2R2A* was selectively depressed in group 4 MBs ($F = 62.82$, $p = 1.73e-20$). This was confirmed in the Magic dataset of Northcott ($F = 116.06$, $p < 7.05e-36$; $F = 189.53$, $p < 1.29e-50$; $F = 51.11$, $p = 9.74e-19$) and in the Cavalli dataset ($F = 362.29$, $p = 1.54e-106$; $F = 578.65$, $p = 6.9e-146$; $F = 155.55$, $p = 1.73e-55$) at a very high level of statistical significance.

The best separation of group 3 ($N = 56$) and group 4 ($N = 91$) by E3 gene expression (after excluding the WNT and SHH subgroups) was achieved by the expression of *ZBTB20*, *PPP2R2B*, *MID2*, *and PPP2R2A*. Expression of *ZBTB20* and *MID2* was comparatively elevated in group 4 ($F = 100.24$, $p = 8.76e-15$; $F = 65.95$, $p = 2.80e-11$) while expression of *PPP2R2A* was comparatively depressed in group 4 MBs ($F = 68.86$, $p = 1.21e-11$; $F = 54.46$, $p = 7.28e-10$). Expression of *PPP2R2D* was significantly increased in group 4 (Fig. 8) compared to that of the other groups, but was not included by ANOVA in the top 100 differentially expressed E3 ligases which we used as our training dataset.

A heatmap of E3 ligase gene expression data from the large Cavalli dataset (Fig. 9) illustrates that this dataset confirms the major findings noted in the Pfister dataset. *PPP2R2C*, *RCBTB*, and *ABTB2* gene expressions are elevated at a very high level of significance in the SHH subgroup. *PPP2R2B* and *TRIM58* expressions are elevated in group 3, while *MID2* and *ZBTB18* expressions are elevated in group 4 MBs.

In summary, in this project, we identified ubiquitin genes whose expression predicted at a high level of statistical probability, the four MB subgroups, as recognized in the consensus classification of Taylor et al. [1]. The data showing differential expression of genes for ubiquitin ligases suggest the possibility of developing ubiquitin ligase subtype-specific therapies designed to regulate stability of proteins important in diagnosis, prognosis, and treatment of MB.

Discussion

The present study illustrates that ubiquitin ligase expression can be used to classify MB subgroups (Fig. 1). Many ubiquitin ligases are associated with signaling pathways of the four consensus group MBs. The use of statistical methods identified the expression of 10 important ubiquitin ligases which could predict the MB subgroups. Furthermore, we showed the association of this gene expression with classical signaling pathways (e.g., WNT and SHH pathways). Our results using the Pfister dataset were validated with four other publically available datasets. Finally, our results enable us to make suggestions on several ubiquitin ligases that should be studied as potential prognostic markers and therapeutic targets for group-specific MBs. It has been suggested that the ubiquitin proteasome system will eventually provide many drug targets [12, 13].

For the WNT subgroup of MBs, we identify a gene for an ubiquitin ligase associated with the WNT receptor complex, *ZNRF3*. The expression alone of this one gene alone can predict the WNT subgroup. For the SHH subgroup, we identify a gene for a regulatory subunit of protein phosphatase 2A (*PP2A*), *PPP2R2C*, (which codes for B55γ), as the major statistical predictor among the ubiquitin E3 ligases genes in the Pfister dataset. For group 3 MBs the best E3 ligase predictor was *PPP2R2B*, a gene for another regulatory subunit of PP2A. For group 4 in the Pfister dataset, the best E3 gene predictors were *MID2*, a prognostic marker in breast cancer [14], *ZBTB18*, a transcription factor, and *PPP2R2A*, yet another PP2A regulatory subunit [15]. Thus, the genes for PP2A regulatory subunits, *PPP2R2A*, *PPP2R2B*, and *PPP2R2C*, which the UUCD database lists as coding for ubiquitin ligase adaptors, are very good predictors of MB subtypes. These proteins belong to a single subfamily of PP2A regulators. The high statistical significance of these data suggest investigating their role in the origin and maintenance of MB subtypes and their possible role as therapeutic targets. We discuss these findings in more detail as follows and indicate the extent to which the main findings are confirmed in other datasets.

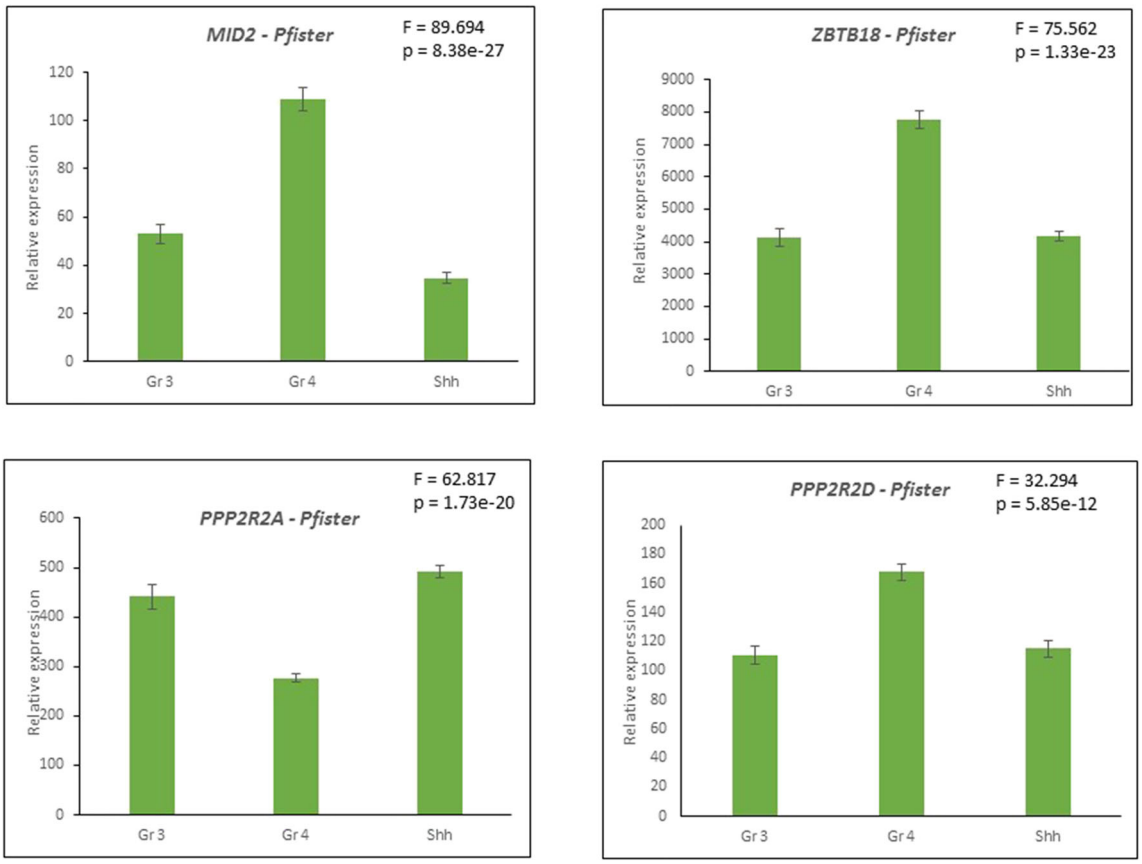

Fig. 8 Ubiquitin ligase gene expression as predictors of group 4 MB. Bar charts shows means with standard error bars. By ANOVA for *MID2* $F = 89.694$, $p = 8.38e\text{-}27$; for *ZBTB18* $F = 75.562$, $p = 1.33e\text{-}23$; for *PPP2R2A* $F = 62.817$, $p = 1.73e\text{-}20$; for PPP2R2D $F = 32.294$, $p = 5.85e\text{-}12$

Ubiquitin Ligases and the WNT Subgroup of MB

Our analysis of the ubiquitin ligases in the Pfister dataset shows that expression of ubiquitin ligases can be used to predict subgroups of MBs. The WNT and SHH MBs were predicted for 100% from expression of ubiquitin ligases in the Pfister dataset. These results were confirmed in other publically available datasets.

Ubiquitin Ligases and WNT Receptors

The WNT receptor is described and illustrated as the Frizzled (FZD) membrane receptor. However, there are at least 10 different genes of the human Frizzled family (*FZD1-FZD10*) [16]. Koo et al. [3] reported two ubiquitin ligases, RNF43 (ring finger protein 43, on chromosome 17) and ZNRF3 (zinc and ring finger 3, gene on chromosome 22), as selectively ubiquitinating Frizzled receptors. They concluded from their studies that RNF43 and its paralog ZNRF3 act as inhibitors of WNT signaling by stimulating the degradation of WNT receptors by ubiquitination of members of the Frizzled (FZD) family. Koo et al. [3] have described RNF43 as a tumor suppressor. However, prior to the current report, overexpression of genes for the two ubiquitin ligases ZNRF3 and RNR43 has not been previously discussed as being associated with the WNT subgroup of MBs. The data showing that WNT MBs can be predicted in the Pfister dataset for 100% by overexpression of the gene for the ZNRF3 ligase alone suggests that it may be a key protein in the development of WNT-type MBs.

Examination of the Pfister dataset revealed that gene expression for both of these ubiquitin ligases was increased in the WNT subgroup of MBs (Fig. 2) compared to that of the other MB subgroups, as well as compared to normal cerebellum (Roth dataset in the R2 site). *ZNRF3* expression was increased by more than sixfold ($F = 454.36$, $p = 2.68e\text{-}90$) whereas *RNF43* expression was increased by 14-fold ($F = 164.70$, $p = 1.97e\text{-}53$). In both the Pfister and Kool datasets, the F values for both these genes were among the top 6 of differentially expressed genes for E3 ligases, but not discussed as E3 ligases related to MB in the literature. Compared to normal cerebellum tissue (Roth dataset in the R2 site), the expression of *ZNRF3* in the WNT subgroup of the Pfister dataset was approximately fivefold. The selective increase in these two E3 ligases furthermore was confirmed in the smaller dataset of Gilbertson and in the larger dataset of Cavalli. Granted that comparing gene expression of MBs to that of normal cerebellar tissue is of limited value, this comparison does fit with the conclusion that these two genes are

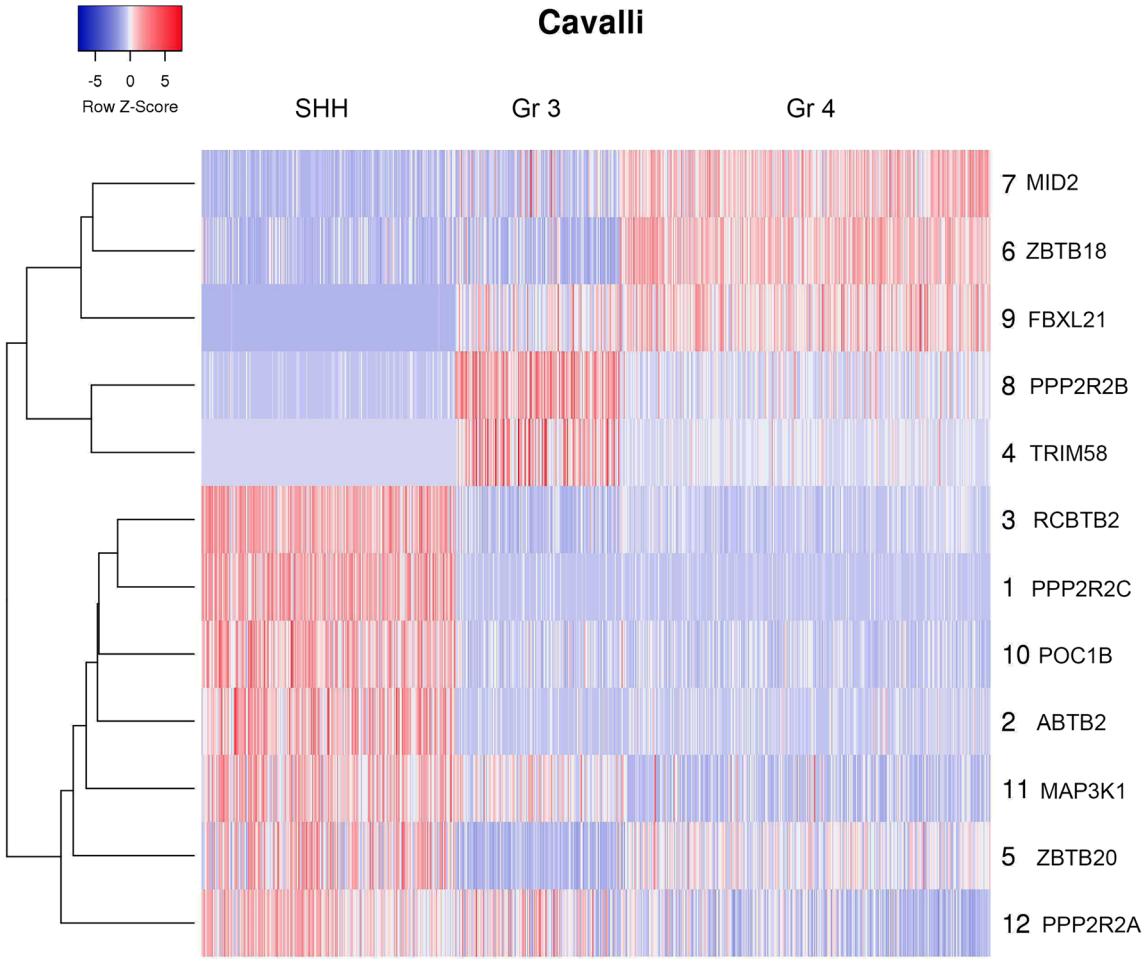

Fig. 9 Heatmap of E3 ligase gene expression in the Cavalli dataset

overexpressed in WNT-type MBs. These remarkable data indicate that these two E3 ligases, particularly *ZNRF3*, may have a key role in dysregulation of signaling in WNT MBs.

RNF43 exists as a complex together with two other proteins, LGR5 (leucine-rich repeat containing G protein-coupled receptor 5) and RSPO1 (R-Spondin 1) [17]. In a review on targeting the WNT pathway, Kahn has illustrated how the action of ZNFR3 and RNF43 depends on the presence or absence of R-Spondin [18]. Expression of the *LGR5* gene was selectively increased 20-fold in the Pfister dataset ($F = 83.48$, $p = 2.98e\text{-}34$) while the expression of *RSPO1* was elevated 11-fold ($F = 180.43$, $p = 2.03e\text{-}56$). Thus, expression of all three genes of this complex was selectively increased in the WNT subgroup of tumors. Regulation of WNT receptor turnover by the complex of R-Spondin, ZNFR3, and RNF43 has been recently reported by Hao et al. [19]. This complex reportedly regulates WNT receptors by facilitating endocytosis of WNT receptors, removal from the cell surface, and degradation by lysosomes [20, 21]. However, it is not clear which of the ten known FRZ receptors are regulated by this complex. The large (100-fold) increase in FZD10 gene expression in the WNT subgroup suggests dysregulation of this protein. The selective increase in *FZD10* expression in the WNT subgroup was confirmed in the datasets of Gilbertson, Kool, and Cavalli. The strong statistical association of the two ubiquitin ligases with *FZD10* expression is consistent with a significant functional relationship.

The ubiquitin ligase regulating FZD10 is unknown. One explanation for the differential expression of FZD receptors in MBs is selective dysregulation by one of the ubiquitin ligases regulating FZD receptors. In the model of Hao et al. [19], WNT signaling, through B-catenin, induces expression (at the level of transcription) of ZNFR3/RNF43 thereby contributing to a feedback loop. The FZD data in the Pfister dataset suggest that this feedback loop does not inhibit expression of genes for all FZD receptors in WNT MBs particularly not for FZD10, which was overexpressed (see above). A closer examination of the functional relationship of ZNRF3/RNF43 complex to the individual FZD proteins is warranted. Jin and Yoon (2012) have reviewed and illustrated the ZNRF3/RNF43/RSPO complex in activation and inhibition of WNT signaling [22].

The expression of genes for the Dickkopt (DKK) WNT signaling inhibitors was also increased in the WNT subgroup

of MBs in the Pfister dataset (greater than 25-fold for *DKK2* and *DKK4*). Furthermore, expression of the DKK receptor, *KREMEN1*, was also significantly increased (more than tenfold) in the WNT subgroup ($F = 452.93$, $p = 3.15e\text{-}90$). *KREMEN1* is a gene for a receptor for DKK1 [23]. Little or no information is available on the role of ubiquitin ligases in regulation of KREMEN1.

The expression of *WIF1* (WNT inhibitory factor 1), which codes for a well-established WNT inhibitor that binds to WNT ligands [24, 25], was dramatically increased in the WNT group in the Pfister dataset. *WIF1* was selectively increased 316-fold in the WNT subgroup compared to that of the data of the other combined groups. Differential expression by groups was significant at a very high level ($F = 277.85$, $p = 2.05e\text{-}71$). This large increase in *WIF1* expression is rather paradoxical. A decrease in *WIF1* expression would be expected with activation of the WNT signaling pathway [26]. One explanation for this is that in WNT MBs, some epigenetic factor (such as methylation) is interfering with normal silencing of *WIF1* gene expression. No information is available on regulation of WIF1 by ubiquitin ligases. Statistically, the ubiquitin ligase that is most highly correlated with *WIF1* expression in the Pfister data is *ZNRF3*. The correlation (*R* value) of *WIF1* and *ZNRF3* is 0.86 (*p* value = 2.9e-62). This strong statistical association suggests studies designed to determine the functional interaction of WIF1 and ZNRF3 in WNT signaling should be conducted.

The data thus document and illustrate dysregulation of WNT receptors in the WNT subgroup of MBs, a dysregulation associated with increased expression of the genes for the E3 ligases RNF43 and ZNRF3 (Fig. 2). The two E3 ubiquitin ligases, RNF43 and ZNRF3, appear to play a key role in regulating the expression of WNT receptor proteins in WNT-subtype MBs. The current study appears to be the first to note that expression of genes for these two ubiquitin ligases are selectively increased in WNT subtype of medulloblastomas and to relate this finding to current models of regulation of WNT signaling in medulloblastoma. Targeting these E3 ligases, or their transcription, could be an interesting experimental approach to therapy of WNT pathway-related tumors. A model illustrating the role of RNF43 and ZNRF3 in WNT MBs is shown in Fig. 3.

The Role of RNF146 and Chromosome 6 in WNT MBs

Another ubiquitin ligase shown in this model (Fig. 3) is RNF146, an ubiquitin ligase that regulates the degradation of AXIN2 [27] and is described as a negative regulator of the WNT pathway [28]. In the WNT group, *RNF146* expression was reduced by at least 50% ($F = 54.54$, $p = 5.85e\text{-}25$) (Fig. 2). The reduced expression of *RNF146* in WNT MBs could be predicted and explained by the fact that monosomy of chromosome 6 is associated with WNT MBs in more than 80% of cases [29]. Expression of *AXIN2*, which codes for a protein that binds to the APC complex, was increased approximately eightfold ($F = 934.59$, $p = 3.16e\text{-}120$). (It should be noted that according to the R2 genome analysis, this is the highest *F* value of all differentially expressed genes in the Pfister dataset). Decreased activity of RNF146 could account for the increase in AXIN2 due to decreased proteasomal degradation. Because of its role in degradation of AXIN2, the role of RNF146 as a key E3 ligase of the WNT pathway should be further investigated in WNT MBs. The interaction of the APC complex, RNF146, ZNRF3, and RNF43 in the WNT pathway is illustrated in Fig. 3.

The APC complex and the Groucho transcription factor in regulating WNT signaling have been described and illustrated by Yu & Virshup [30]. The ubiquitin ligase TLE2 (of the Groucho/TLE family) is a transcription repressor (and E3 ligase adaptor) in the WNT pathway [31]. In the Pfister dataset, expression of *TLE2* was selectively decreased (greater than fivefold) in the WNT subgroup of MBs ($F = 13.70$, $p = 1.06e\text{-}07$).

FBXL7: another Ubiquitin Ligase Overexpressed in WNT MBs

Statistically, another E3 ligase gene overexpressed in the WNT group MBs is *FBXL7*. In the Pfister data, differential expression of *FBXL7* statistically was second only to *ZNRF3* expression among the differentially expressed E3 ligases. Compared to normal cerebellum (Roth dataset), *FBXL7* expression was approximately ninefold higher in the Pfister WNT subgroup.

FBXL7 codes for an ubiquitin ligase that regulates the degradation of AURKA and induces mitotic arrest [32, 33]. The FBXL7 E3 ligase also ubiquitinates the protein survivin prior to degradation by the proteasome [33, 34]. As such, it has a regulatory role in mitosis and in apoptosis [34, 35]. FBXL7 has been reported to be proapoptotic [33]. Through its regulation of survivin, FBXL7 has been associated with various types of cancer [36, 37]. FBXL7 has been reported to associate with the centrosome during spindle formation [32].

The SHH Subgroup and Ubiquitin Ligases

Evidence for stimulation of SHH signaling in the SHH subgroup of MBs includes upregulation of the PTCH1 and PTCH2 receptors, upregulation of the co-receptors BOC (Brother of CDON; aka, cell adhesion associated, oncogene regulated 2) and GAS1, and, upregulation of GLI1 and GLI2 [38]. Their role in the SHH signaling pathway is illustrated in Fig. 6. In the Pfister dataset, expression of these genes are all significantly ($p < 0.00001$) increased in the SHH subgroup of MBs: *PTCH1* (5.62-fold compared to other groups), *PTCH2* (3.22-fold), *BOC* (13.09-fold), *GAS1* (5.76-fold), *GLI1* (16.99-fold), *GLI2* (8.17-fold). The most significant of these

differentially expressed genes was *BOC* ($F = 275.90$, $p = 4.3e-74$). Also, selectively upregulated (3.02-fold) ($F = 43.06$, $p = 1.07e-20$) in the SHH MBs is the gene for a proapoptotic protein DRAL (FHL2) that binds to the PTCH receptor [39, 40]. Frappart et al. [41] emphasized a tumor suppressor role for PTCH1 in cerebellar granule cells. BOC is reported to promote progression of MBs to advanced tumors [42]. Figure 6 illustrates a model including SHH receptors, co-receptors, and ubiquitin ligases.

A 2015 report by Hsia et al. [4] outlined regulation of Hedgehog signaling by ubiquitin ligases. They list E3 ligases for a number of SHH pathway components including PTCH, SMO, GLI1, and GLI2. The E3 ligases reported in this report are SMURF, ITCH, NEDD4, βTRCP, KAT2B, and SPOP. In the Pfister dataset, genes for several of these E3 ligases were also differentially expressed (see below). Statistically, however, we identified in the Pfister dataset genes for three ubiquitin ligases that were better predictors of the SHH subtype than those reviewed in the Hsia report.

Logistic Regression and the SHH Subgroup

Logistic regression analysis showed that the best E3 ligase predictor of SHH-type MBs was *PPP2R2C* (Fig. 4). In the Pfister dataset, this gene was differentially expressed at a higher level of significance than any of those in the Hsia report [4]. As noted above, the combined expression of *PPP2R2C* and *RCBTB2* can predict the SHH group for 100% in the Pfister dataset (Fig. 5).

PPP2R2C (protein phosphatase 2 regulatory subunit Bγ) (Fig. 3) codes for the regulatory subunit of a serine/threonine phosphatase, protein phosphatase 2, a target of anticancer therapy [43, 44], but has not previously been directly associated with medulloblastoma. PPP2R2C has been reported as a regulator of cell growth and division [45]. The enzyme coded for by the *PPP2R2C* gene regulates PP2A which dephosphorylates proteins involved in mitotic entry and exit [46, 47]. PP2A in cancer signaling has been described as a broken "off" switch [48]. Sablina and Hahn [49] concluded that impaired function of PP2A complexes regulate specific phosphorylation events necessary for cancer initiation.

The Pfister data show overexpression of the *PPP2R2C* gene (Fig. 4) in the SHH subgroup and under expression of *PPP2R2B* expression (Fig. 7). *PPP2R2C* belongs to a group of 4 genes (*PPP2R2A*, *PPP2R2B*, *PPP2R2C*, *PPP2R2D*) which code for PP2A regulatory subunits of the β55 subfamily, β55α, β55β, β55γ, and β55δ [50]. The gene expression data suggest the hypothesis of dysfunction of PP2A due to the abnormal amounts of the β55γ and B55β isoforms available as regulatory subunits for the holoenzyme in the SHH subgroup.

Although the β55 subfamily is not usually discussed in the MB literature in the context of E3 ligases, they are documented as Cullin Ring ubiquitin ligase adaptors in many species in the UUCD database. The expression of *PPP2R2C* definitely deserves further experimental attention for its potential role in the development of SHH MB. The data showing that another member of this subfamily of genes, *PPP2R2B*, is statistically the best E3 gene predictor of the group 3 subtype (see below), and that *PPP2R2A* is one of the top predictors of group 4 suggest that this subfamily of PP2A regulator genes is of considerable significance in more than one subgroup of MBs. As noted in the report of Wlodarchak et al. [50]. PP2A dephosphorylates over 300 substrates involved in the cell cycle. It has been estimated that up to 100 different PP2A holoenzyme complexes can be formed by combining combinations of the catalytic core with various regulatory subunits [51]. The extent to which each of the β55 E3 adaptor proteins confers specificity to the PP2A holoenzyme is, as far as we can determine, not known. A prediction that can be made from the overexpression of *PPP2R2C* in SHH MBs in the Pfister dataset (and confirmed in others) is that the B55γ protein may serve as a marker for SHH MBs.

Expression of *RCBTB2* and *ABTB2* in the SHH Subgroup

The expression of the gene for the E3 ligase RCBTB2 was elevated several fold in the SHH group compared to the group 3 and 4 groups in the Pfister dataset ($F = 196.18$, $p = 2.5e-45$). This was confirmed in the Northcott (Magic) dataset ($F = 217.13$, $p = 8.3e-58$) and in the Cavalli dataset ($F = 785.18$, $p = 1.68e-178$). Expression of RCBTB2 in the SHH group of MBs in the Pfister dataset was greater than 12-fold that of normal cerebellum (Roth dataset in the R2 genomics site).

The RCBTB2 protein (aka CHC1-L) is a regulator of chromosome condensation and a guanine nucleotide exchange factor [52]. The *RCBTB2* gene has been reported as a candidate gene for prostate carcinogenesis [53–55]. *RCBTB2* gene expression is positively correlated with *BOC* (a co-receptor of SHH) expression ($R = 0.73$) at a high level of significance ($p < 1.0e-20$). In the Pfister dataset, *ABTB2* expression is also strongly correlated with expression of the SHH co-receptor BOC ($R = 0.78$, $p = 1.3e-42$). These statistical associations suggest that it would be useful to examine further the roles of the RCBTB2 and ABTB2 proteins in the SHH pathway and as markers for SHH MBs. A recent study reports that a substrate of ABTB2 is a deoxynucleotidyl transferase [56] that may contribute to regulation of the cell cycle.

Expression of *POC1B* and *MAP3K1* in the SHH Subgroup

As noted together, the three genes *PPP2R2C*, *POC1B*, and *MAP3K1* could also predict the SHH for 100%. While *PPP2R2C* codes for a subunit of PP2A, a threonine serine phosphatase [57], *MAP3K1* codes for a serine/threonine kinase [58]. Regulation of MAPK signaling pathways by

PP2A has been reviewed by Junttila et al. [59]. POC1B codes for a protein involved in centriole assembly [60]. Since *MAP3K1* and *POC1B* expressions were significantly elevated in SHH MBs, it would be useful to determine whether the proteins coded for by these genes are markers for SHH MB.

Other E3 Ligases in the SHH Pathway

Evidence that the E3 ligases SMURF1 and SMURF2 regulate SHH signaling by tagging PTCH1 for degradation has been well demonstrated [4, 61]. The SMURF-related ubiquitination of SHH has been reported to be stimulated by SMO [4, 61]. In the Pfister dataset, differential expression of S*MURF1* and *SMURF2* between the four MB subtypes was highly significant ($F = 26.10$, $p = 1.28\text{e-}13$; $F = 41.37$, $p = 4.92\text{e-}20$), but the difference (decrease) was not specific to the SHH group. Comparatively, low levels were found in both the SHH and WNT subgroups. SMURF reportedly contributes to WNT signaling by regulating GSK3 through ubiquitination and degradation [62]. SMURF1 itself is regulated by an E3 ligase, FBXL15 [62, 63]. In the Pfister dataset, expression of *FBXL15* was selectively reduced in the SHH subgroup ($F = 12.50$, $p = 4.33\text{e-}07$). Also, reduced in the SHH subgroup was expression of *KAT2B* (K lysine acetyltransferase) (aka *PCAF*) ($F = 60.53$, $p = 4.85\text{e-}27$), a gene for an E3 ligase which functions as a circadian transcriptional co-repressor. According to Malatesta et al. [64] KAT2B is required for SHH MB cancer cell proliferation.

Expression of the E3 ligase gene *SPOP* was selectively depressed in the SHH subgroup of MBs in the Pfister dataset ($F = 39.12$, $p = 3.85\text{e-}19$). The *SPOP* gene is one of the E3 ligase genes located on chromosome 17 [65]. SPOP contributes to SHH signaling by targeting GLI2 and GLI3 for ubiquitination and degradation [66–68]. In prostate cancer SPOP can function as a tumor suppressor [69].

The E3 ubiquitin ligases KCTD21, KCTD6 [70], and KCTD11 (aka Kcash1, Kcash2, Kcash3) [71, 72] are ubiquitin ligases that act on post-receptor components of the SHH pathway. They act through ubiquitination and degradation of histone deacetylase (HDAC1) [70, 71]. HDAC1 inhibits deacetylation of GLI1 and GL2. Deacetylation of GLI1 and GLI2 promotes their action as transcriptional factors. GLI is regulated by ubiquitination-induced degradation, but its activation as a transcription factor also depends on HDAC1 deacetylation [72]. In the Pfister dataset *KCTD6*, *KCTD11*, and *KCTD21* were differential expressed ($F = 20.54$, $F = 12.67$, $F = 38.86$, $p < 0.00001$), all lower in the SHH MBs than in the other subgroups. Canettierei et al. have suggested that downregulation of KCTD11 contributes to proliferation and cell transformation in SHH MBs [73].

MYCN is overexpressed in some MBs including a subset of SHH MBs [74]. In the Pfister dataset, *MYCN* is overexpressed approximately twofold compared to the other MB subgroups ($F = 30.83$, $p = 1.08\text{e-}15$). The MYCN protein is degraded by the ubiquitin proteasome system [75]. MYCN is reported to be a substrate for the ligase FBXW7 [76] but also a substrate for the E3 ligase HUWE1 in embryonic stem cells [77, 78]. The ubiquitin ligase Aurora A (AURKA) has been reported to contribute to MYCN stabilization in neuroblastomas [76, 79]. The deubiquitinase USP7 has also been reported to contribute to MYCN stabilization [80].

Expression of *MID1* and the SHH Subgroup

MID1 is a gene that codes for an ubiquitin ligase (MIDLINE-1) of the TRIM family. The differential expression of *MID1* ($F = 154.42$ $p = 2.21\text{e-}51$) was highly significant in the Pfister dataset. This gene is overexpressed in the WNT subgroup (about 7.6-fold compared to the normal cerebellum of the Roth dataset). In the SHH group, expression of this gene was less than 50% of normal (Roth dataset). It has been recently suggested that the cells that develop into SHH MBs are themselves WNT dependent [81]. There is data reporting that WNT signaling activity attenuates the growth of SHH tumors [82].

MID1, while overexpressed in the WNT MBs, is reported as a regulator of SHH-dependent cerebellar development [83]. One of its substrates is FU, a protein involved in regulation of the SHH pathway [84]. In the model of Schweiger et al. [84], a protein complex of MID1 and PP2A regulates nuclear localization of GLI3 and thus regulates its target genes [83, 85]. MID1 has been reported to regulate the activity of GLI3 through the ubiquitination and cleavage of Fu kinase (aka STK36) [84].

TLE1, a transcriptional corepressor of the Groucho family, binds to GLI1 [86]. As such, it might be expected to influence GLI1-induced gene transcription. *GLI1* gene expression is also highly correlated with *BOC* expression in the Pfister dataset ($r = 0.89$, $p = 4.0\text{e-}73$). Indeed, the correlation of *GLI1* and *BOC* was the most significant of any other genes correlated with GLI1 expression. BOC is a co-receptor for SHH [87]; it is selectively elevated in the SSH subgroup of MBs ($F = 275.90$, $p = 3.6\text{e-}71$). Based on the statistics, BOC may be of more significance than PTCH receptors in SHH signaling in MBs. Indeed, among the non-WNT MBs in the Pfister dataset, differential expression of BOC was the most significant of all differentially expressed genes. Expression of *HHIP* (Hedgehog interacting protein) was also selectively elevated in the SHH subgroup ($F = 128.36$, $p = 9.51\text{e-}46$). The HHIP gene has been reported to be unexpressed (or low) in MB cell lines and primary tumors [88]. According to the Pfister dataset, this appears to be true only for non-SHH tumors. In SHH tumors, HHIP expression was elevated approximately 85-fold compared to the other 3 groups. Further studies on the role of ubiquitin ligases in regulation of *BOC* and *HHIP* expression are warranted. Based on our analysis of the

Pfister data (and confirmation in datasets of Gilbertson, Northcott, Kool, and Cavalli), we speculate that PPP2R2C and RCBTB2 function as regulators of cell cycle entry and exit in the SHH subgroup of MBs. Their role in SHH signaling and in development of the SHH subtype of medulloblastoma remains to be determined.

Group 3 MB, *PPP2R2B*, and E3 Ligase Predictors

Logistic Regression and Group 3 E3 Ligases

Logistic regression showed that the best non-WNT E3 ligase gene predictors for group 3 in the Pfister data was *PPP2R2B* (Fig. 7). This gene codes for the PP2A regulatory subunit B55β [50]. The PP2A-B55β holoenzyme has been associated with MYC phosphorylation [89] and has been reported to regulate phosphorylation of cyclin E1 [90]. Expression of *PPP2R2B* has not been previously associated with a subgroup of MB. While the UUCD database documents B55β as a Cullin Ring E3 adaptor in many species, this information has not been integrated with functional studies of PP2A in the literature. Oberg et al. [91], in 2012, however, suggested the possibility that each of the PP2A regulatory subunits is paired with a dedicated E3 ubiquitin ligase. Their hypothesis would contribute towards an explanation for the specificity that PP2A regulatory units provide for the holoenzyme. As far as we can determine, B55β has not been tested as a marker for group 3 MBs.

TRIM58 expression and *ZBTB20* (a transcription factor) expression were also significant predictors of group 3 MBs (Fig. 7). TRIM58 is an E3 ligase that regulates the degradation of the light-intermediate chain proteins of the dynein motor complex [11]. The dynein motor provides the forces required for centrosome separation prior to mitosis [92] and plays a role in spindle assembly [93]. Aberrant methylation of *TRIM58* has been reported in hepatocellular carcinoma [94] and in lung adenocarcinoma [95]; we found no previous reports of aberrant *TRIM58* expression (other than the Pfister data) nor reports of aberrant T*RIM58* gene methylation associated with medulloblastoma.

In the Pfister dataset, *TRIM58* was differentially expressed (Fig. 7). Group 3 gene expression values were 73.75-fold greater than SHH values, 110.8-fold greater than WNT values, 7.46-fold greater than group 4 values, and 35.91-fold greater than normal cerebellum (Roth dataset). This was confirmed in the smaller datasets of Gilbertson and Kool, in the large dataset of Cavalli, and in the Northcott (MAGIC) dataset which did not include the WNT MBs. One interpretation of these data is that *TRIM58* expression is silenced in most MB tissue samples other than in group 3 MB tissue samples.

ZBTB20 (aka *DPZF*) is an E3 ligase and transcription repressor [96, 97]. To the best of our knowledge, it has not been previously associated with MBs in the literature. Expression of this gene was decreased in group 3 MBs compared to those of the other groups. *ERCC8* expression was also increased in group 3 MBs ($F = 30.809$, $p = 1.1e\text{-}15$). This E3 ligase gene codes for a protein involved in a transcription-dependent DNA excision repair [98].

MYC is often overexpressed in MBs with the worst prognosis, including those of group 3 [5]. In the Pfister dataset, expression of the gene for MYC is elevated in group 3 MBs (Fig. 7). Felsher [99] and Hammond-Martel et al. [6] have described and illustrated the role of ubiquitin ligases in the regulation of MYC. They discuss five E3 ligases that target MYC and contribute to its regulation FBW7 (aka FBXW7), TRUSS (TRPC4AP), SKP2, HUWE1, and βTRCP. These ligases differ in the extent to which they use different lysine attachment sites on ubiquitin and hence the role of the ubiquitinated protein MYC. In the Hammond-Martel model [6], FBW7 tags MYC for proteasomal degradation. In the Pfister dataset, expression of FBW7 was not significantly different between groups; neither was it significant in the Kool, Gilbertson, Thompson, nor Northcott datasets. However, a role for FBW7 in the progression of group 3 MBs has been postulated based on its ubiquitination of SOX9 in MB cell lines [100].

SKP2 was differentially expressed in the Pfister dataset with lower expression levels in groups 3 and 4 ($F = 47.05$, $p = 3.18e\text{-}22$). In the Hammond-Martel model, SKP2 reportedly activates MYC as a transcription factor, but following polyubiquitination of MYC it then induces proteasomal degradation [101, 102]. This is consistent with the black widow spider model in which ubiquitinated transcription factors are first activated by monoubiquitination and then polyubiquitinated and destroyed by the proteasome when no longer needed [103]. *HUWE1* and *βTRCP* genes were also differentially expressed ($F = 8.62$, $p = 4.53e\text{-}05$; $F = 40.18$, $p = 1.48e\text{-}19$) in the Pfister dataset. In the Hammond-Martel model, HUWE1 is also associated with MYC activation while variations in βTRCP are necessary for the progression of the cell cycle.

According to the ANOVA results, the most significant E3 ligases in the Hammond-Martel model in the Pfister dataset are SKP2 and βTRCP (Table S2). However, our model shows that *PPP2R2B* is a better genetic marker than any in the Hammond-Martel model and suggests testing differential levels of the PP2A subunit protein, B55β, coded for by *PPP2R2B*.

Although group 3 is frequently associated with increased MYC expression, the overexpression of *GABRA5* in group 3 MBs [7] suggest *GABRA5* overexpression may characterize group 3 better than MYC expression. In the Pfister dataset, *GABRA5* expression was increased approximately 28-fold in the group 3 samples compared to expression in the other subgroups. The ubiquitin ligase that was most significantly correlated with GABRA5 expression in the Pfister dataset was

PPP2R2B ($r = 0.63$, $p = 1.7e-22$). This was confirmed in the large Cavalli dataset ($r = 0.60$, p = 1.4e-71). Overall, these data show a high statistical association of *PPP2R2B* with group 3 MBs and led to the prediction that B55β may be a marker for group 3 MBs. The data raise the question of dysregulation of PP2A by B55 subunits (B55β and B55γ) and dysregulation of PP2A modulation of mitotic kinases in the SHH and group 3 MB subgroups.

Differential Expression of E3 Ligases and MB Group 4

Logistic Regression and Group 4

The best non-WNT predictors among the E3 ligase genes according to logistic regression analysis for group 4 MBs were *MID2* (an E3 ligase is associated with microtubules), *ZBTB18* (a transcription factor), and *PPP2R2A* (which codes for another regulatory subunit of PP2A). Expression of *MID2* and *ZBTB18* was comparatively elevated in group 4, while expression of *PPP2R2A* was decreased in group 4 (Fig. 8). The best separation of groups 3 and 4 (after excluding the WNT and SHH subgroups) was achieved by *ZBTB20*, *PPP2R2B*, *MID2*, and *PPP2R2A* expression. The expression of *PPP2R2D*, although not among the top 100 differentially expressed E3 ligase genes in the Pfister dataset, was significantly increased in group 4 compared to group 3. Thus, these data again point to genes for the B55 family of regulatory factors of PP2A as statistical predictors of MB subgroups and suggest the possibility that one or more of the B55 family of regulatory proteins may play a significant role in initiation and/or maintenance of one or more subgroups of MB.

Although expression of the above E3 ligase genes significantly predict group 4 MBs, there is little information relating them functionally to medulloblastoma. There is no specific literature relating *PPP2R2A* or *PPP2R2D* to group 4 MB. Loss of PPP2R2A is reported to inhibit homologous recombination DNA repair [104]. ZBTB20 is a transcription factor [105] as well as an E3 ligase adaptor. ZBTB18 has been investigated as a tumor suppressor in glioblastoma and as a transcriptional repressor with a role in neuronal differentiation in the developing brain [106]. We could find no previous reports linking *ZBTB18* or *ZBTB20* expression to MB. Thus, the statistical predictions of gene expression of these three ligases suggest that testing of the corresponding proteins is necessary to determine their significance in prognosis and treatment of the MB groups 3 and 4.

Group 4 Ligases on Chromosome 17

Since MB subgroups 3 and 4 are associated with a high incidence of isochromosome 17 [1], the ubiquitin ligases that were differentially expressed were screened for their chromosome location. Of the ubiquitin ligases that were differentially expressed in the 4 MB subgroups (at $p < 0.00001$), 30 were located on chromosome 17; the expression of genes on chromosome 17 would be expected to be influenced by isochromosome 17.

Expression of the E3 ligase gene *CDC27*, also located on chromosome 17, was increased in groups 3 and 4 relative to the other two groups ($F = 15.35$, $p = 1.57e-08$). CDC27 is a protein component of the anaphase-promoting complex (APC/C), which functions as an E3 ligase [107]. This complex regulates the mitotic spindle assembly checkpoint [108–111] and chromosomal stability [107, 111]. Targeting CDC27 in group 4 MBs is a way of modulating the APC/C complex and could be a tool in decreasing resistance to therapy [111].

RHOT1 (ras homolog gene family member T1) (aka *MIRO1*) another E3 gene located on chromosome 17 was also expressed at elevated level in groups 3 and 4 ($F = 31.48$, $p = 5.67e-16$), and its expression was also increased compared to normal cerebellum. RHOT1 is a mitochondrial Rho GTPase. It is necessary for mitochondrial movement [112].

Group 4 and the APC/c Complex

The APC/C complex is activated by two ubiquitin ligases, CDC20 and CDH1, at different phases of the cell cycle. CDC20 activates APC/C during the early phases of mitosis, while CDH1 activates APC/C during the later phases of mitosis [113, 114]. The Pfister dataset provides evidence that both of these E3 coactivators are differentially expressed in MB subtypes at a high statistical level. There is at least one report relating PP2A-B55 to CDC20 and CDH1 dephosphorylation during the mitotic cycle [115]. Manchado et al. illustrated a role for PP2A-B55α and PP2A-B55δ in mitotic exit and suggested these isoforms were a factor in regulating mitotic exit in tumor cells [116]. Their conclusion is consistent with *PPP2R2A* and *PPP2R2D* expression in the Pfister medulloblastoma dataset (Fig. 8).

Gene expression of *CDC20* was reduced in group 4 compared to the other groups ($F = 16.90$, $p = 2.72e-09$). Furthermore, gene expression for two other proteins ubiquitinated by the APC/C complex, *BARD1* and *HMMR*, was substantially reduced in group 4 MBs ($F = 29.48$, $p = 4.14e-15$; $F = 23.61$, $p = 1.74e-12$). The HMMR protein is one of the proteins required for mitotic spindle assembly [109]. BARD1 is itself an E3 ligase. It is associated, together with BRCA1, with the cell response to DNA damage [117]. Expression of ubiquitin conjugase *UBE2D3*, which associates with the BRCA1/BARD1 E3 ubiquitin ligase complex during DNA damage, was also downregulated in group 4 MBs ($F = 23.52$, $p = 1.9e-12$). These data suggest that in group 4 MBs, there may be a reduced response to DNA damage.

Gene expression of *CDH1* in group 4 was elevated compared to that of the other 3 groups ($F = 16.63$, $p = 9.0e-10$). It

is not known whether targeting the CDH1 protein would be useful in group 4 MBs.

Expression of the gene, *UBE2C*, which codes for a ubiquitin conjugase that is a component of the APC/C complex [114] was significantly depressed in group 4 ($F = 14.53$, $p = 4.03e\text{-}08$). Although the role for UBE2C in other tumors has been documented [118], its role in MB is mostly unknown. *UBE2C* expression, however, has been associated with metastatic cancers [119].

The Pfister data showing differential expression of components of the APC/C complex, including UBE2C, suggest a physiological role for this complex in group 4 MB tumorigenesis. Because the APC/C complex has many substrates including the ubiquitin ligases SMURF1 and SKP2 [120, 121], dysregulation of the complex would have widespread physiological effects.

Group 4 and Potassium Channel Components

KCNA1 has recently been predicted as an E3 ligase [122, 123]. It has been previously reported as an immunocytochemical marker for group 4 MBs [1, 124–126]. In the Pfister dataset, expression of this gene was increased at least fivefold in group 4 MBs compared to the other groups. Expression of *KCNA5*, also classified as an E3 ligase, was increased in tissue of both groups 3 and 4. Both of these genes are located on the same cytogenetic region of chromosome 12, at 12p13.32. Huang and Jan [127] have suggested targeting potassium channels in cancer. The role of these two proteins should be further investigated for their role in group 4 tumorigenesis.

Summary and Perspectives

Our analysis of the Pfister dataset shows that gene expression of ten ubiquitin ligases can be used to predict MB subgroups (Figs. 1 and 10). The E3 ligase gene (*ZNRF3*) that can predict the WNT subtype has been documented as contributing to the regulation of WNT receptors. This report, however, is the first to note that expression of this gene is a molecular marker for the WNT subgroup of MBs. The protein coded for by this gene, and its paralog *RNF43*, should be examined as a markers for WNT MBs (Fig. 3) and as potential therapeutic targets. The large increase (approximately 100-fold) in expression of the gene for the WNT receptor protein, FZD10, in WNT MBs, is consistent with dysregulation of WNT receptors in the WNT subgroup of MBs.

The present study provides strong evidence that a subfamily of four genes coding for the B55 subfamily of regulatory factors for the serine/threonine phosphatase PP2A holoenzyme contains genes that are very strong predictors of SHH (*PPP2R2C*) and group 3 (*PPP2R2B*) MBs (Figs. 4 and 7). Another member of this subfamily, *PPP2R2A*, was a strong predictor of group 4 MBs (Fig. 8). In addition, significantly elevated expression of the fourth member of this subfamily, *PPP2R2D*, was noted in group 4 MBs. According to the UUCD database, these four genes code for Cullin Ring ubiquitin ligase adaptors. As such, the proteins coded for by these genes (the four B55 proteins) would regulate PP2A and provide some specificity for the PP2A holoenzyme. The B55 subfamily of regulatory proteins has been documented to be essential for normal mitotic progression [128]. Hein et al. [115] have documented the role of the PP2A-B55 holoenzyme in regulation of mitotic exit.

The role of the E3 ligase gene (*PPP2R2C*) that we identify as a major predictor of the SHH MB subtype has not been documented as a regulator of SHH receptor proteins. The current report is the first to identify this gene as a molecular marker for SHH MBs. A role for abnormal expression of PP2A subunits in lung, breast, and skin cancer has been reviewed by Seshacharyulu et al. [129]. As coding for one of the B55 regulatory proteins (B55γ), the overexpression of *PPP2R2C* could contribute to dysregulation of mitotic progression in SHH MBs.

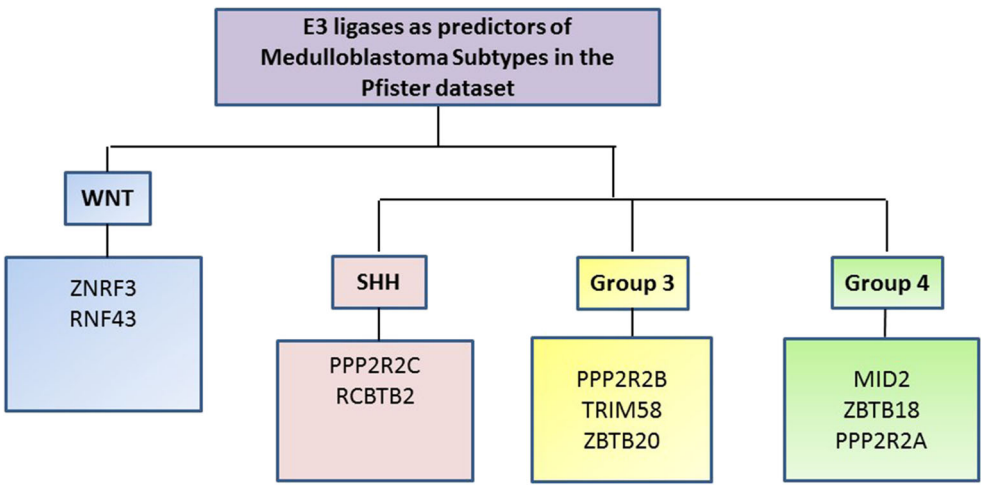

Fig. 10 Prediction of MB subgroups using logistic regression

For the SHH MBs, the E3 ligases SMURF1 and SMURF2 contribute to regulation of the SHH receptor PTCH. Logistic regression analysis, however, shows that expression of *PPP2R2C* and *RCBTB2* are better predictors of the SHH subtype than the genes for SMURF1 and SMURF2. As shown in the results, three genes coding for the E3 ligases: *PPP2R2C*, *POC1B*, and *MAP3K1* could predict the SHH subtype for 100% in the Pfister dataset. These results are consistent with a defect in mitotic progression in SHH MBs, a defect associated with serine/threonine kinases and serine/threonine phosphatases.

Since *PPP2R2B* expression was the best E3 predictor of group 3 MBs, the prediction can be made that B55β is a protein marker for group 3 MBs. Since group 3 MBs have the worst prognosis of the MBs, it is important to test this hypothesis and to determine whether B55β can be used as a therapeutic target. For group 4 MBs, *PPP2R2A* was one of the three major predictors of this subtype.

Based on the gene expression of *PPP2R2A* and *PPP2R2D*, a prediction could be made for increased B55α and decreased B55δ in group 4 MB tissue samples. MID2, the best E3 gene predictor of group 4, in the Pfister dataset, has been shown to interact with PP2A via a protein called alpha 4 (aka IGBp1). The MID2-alpha 4 complex has been described as an essential regulator of PP2A [130, 131]. Two additional genes (*ZBTB18* and *ZBTB20*) that are significant predictors of groups 3 and 4 MBs code for E3 ligases that serve as transcription factors. However, the model that emerges for group 4 MBs is one that includes dysregulation of PP2A by its regulatory subunits, dysfunction of the APC/C complex and abnormal progression of the cell cycle.

Targeting E3 ligases in MB subgroups may be a useful approach to future therapy for medulloblastomas. Our study is the first to document that gene expression for specific E3 ligases can serve as markers for MB subtypes. Further, our contribution comes from novel analysis of publicly accessible data, with predictive models developed using the largest available file at the time (Pfister). Our description of the relationship between E3 ligases and medulloblastoma was fully supported through testing of our models with more data files that became available during the time of our study (Cavalli). Our results shed new light on the pathways for potential intervention towards this cancer, specifically we suggest targeting specific E3 ligases as a therapeutic approach to MB subtypes. Prime candidates for this approach would be the E3 regulatory factors for PP2A that control dephosphorylation of mitotic proteins during progression of the cell cycle (Fig. 10).

Compliance with Ethical Standards

Conflict of Interest The authors declare that they have no of conflict of interest

Publisher's Note Springer Nature remains neutral with regard to jurisdictional claims in published maps and institutional affiliations.

References

1. Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, et al. Molecular subgroups of medulloblastoma: the current consensus. Acta Neuropathol. 2012;123(4):465–72.
2. Kool M, Korshunov A, Remke M, Jones DT, Schlanstein M, Northcott PA, et al. Molecular subgroups of medulloblastoma: an international meta-analysis of transcriptome, genetic aberrations, and clinical data of WNT, SHH, group 3, and group 4 medulloblastomas. Acta Neuropathol. 2012;123(4):473–84.
3. Koo BK, Spit M, Jordens I, Low TY, Stange DE, van de Wetering M, et al. Tumour suppressor RNF43 is a stem-cell E3 ligase that induces endocytosis of Wnt receptors. Nature. 2012;488(7413):665–9.
4. Hsia EY, Gui Y, Zheng X. Regulation of hedgehog signaling by ubiquitination. Front Biol (Beijing). 2015;10(3):203–20.
5. Roussel MF, Robinson GW. Role of MYC in Medulloblastoma. Cold Spring Harb Perspect Med. 2013;3(11). https://doi.org/10.1101/cshperspect.a014308.
6. Hammond-Martel I, Yu H, el Affar B. Roles of ubiquitin signaling in transcription regulation. Cell Signal. 2012;24(2):410–21.
7. Sengupta S, Pomeranz Krummel D, Pomeroy S. The evolution of medulloblastoma therapy to personalized medicine. F1000Res. 2017;6:490.
8. DeSouza RM, Jones BR, Lowis SP, Kurian KM. Pediatric medulloblastoma - update on molecular classification driving targeted therapies. Front Oncol. 2014;4:176.
9. Bielskiene K, Bagdoniene L, Mozuraitiene J, Kazbariene B, Janulionis E. E3 ubiquitin ligases as drug targets and prognostic biomarkers in melanoma. Medicina (Kaunas). 2015;51(1):1–9.
10. Clifford SC, Lusher ME, Lindsey JC, Langdon JA, Gilbertson RJ, Straughton D, et al. Wnt/wingless pathway activation and chromosome 6 loss characterize a distinct molecular sub-group of medulloblastomas associated with a favorable prognosis. Cell Cycle. 2006;5(22):2666–70.
11. Thom CS, Traxler EA, Khandros E, Nickas JM, Zhou OY, Lazarus JE, et al. Trim58 degrades dynein and regulates terminal erythropoiesis. Dev Cell. 2014;30(6):688–700.
12. Bedford L, Lowe J, Dick LR, Mayer RJ, Brownell JE. Ubiquitin-like protein conjugation and the ubiquitin-proteasome system as drug targets. Nat Rev Drug Discov. 2011;10(1):29–46.
13. Shen M, Schmitt S, Buac D, Dou QP. Targeting the ubiquitin-proteasome system for cancer therapy. Expert Opin Ther Targets. 2013;17(9):1091–108.
14. Wang L, Wu J, Yuan J, Zhu X, Wu H, Li M. Midline2 is overexpressed and a prognostic indicator in human breast cancer and promotes breast cancer cell proliferation in vitro and in vivo. Front Med. 2016;10(1):41–51.
15. Mayer RE, Hendrix P, Cron P, Matthies R, Stone SR, Goris J, et al. Structure of the 55-kDa regulatory subunit of protein phosphatase 2A: evidence for a neuronal-specific isoform. Biochemistry. 1991;30(15):3589–97.
16. Huang HC, Klein PS. The frizzled family: receptors for multiple signal transduction pathways. Genome Biol. 2004;5(7):234.
17. Chen PH, Chen X, Lin Z, Fang D, He X. The structural basis of R-spondin recognition by LGR5 and RNF43. Genes Dev. 2013;27(12):1345–50.
18. Kahn M. Can we safely target the WNT pathway? Nat Rev Drug Discov. 2014;13(7):513–32.
19. Hao HX, Jiang X, Cong F. Control of Wnt receptor turnover by R-spondin-ZNRF3/RNF43 signaling module and its dysregulation in cancer. Cancers (Basel). 2016;8(6). https://doi.org/10.3390/cancers8060054.
20. de Lau W, Peng WC, Gros P, Clevers H. The R-spondin/Lgr5/Rnf43 module: regulator of Wnt signal strength. Genes Dev. 2014;28(4):305–16.

21. Blitzer JT, Nusse R. A critical role for endocytosis in Wnt signaling. BMC Cell Biol. 2006;7:28.
22. Jin YR, Yoon JK. The R-spondin family of proteins: emerging regulators of WNT signaling. Int J Biochem Cell Biol. 2012;44(12):2278–87.
23. Mishra SK, Funair L, Cressley A, Gittes GK, Burns RC. High-affinity Dkk1 receptor Kremen1 is internalized by clathrin-mediated endocytosis. PLoS One. 2012;7(12):e52190.
24. Malinauskas T, Aricescu AR, Lu W, Siebold C, Jones EY. Modular mechanism of Wnt signaling inhibition by Wnt inhibitory factor 1. Nat Struct Mol Biol. 2011;18(8):886–93.
25. Surmann-Schmitt C, Widmann N, Dietz U, Saeger B, Eitzinger N, Nakamura Y, et al. Wif-1 is expressed at cartilage-mesenchyme interfaces and impedes Wnt3a-mediated inhibition of chondrogenesis. J Cell Sci. 2009;122(Pt 20):3627–37.
26. Mazieres J, He B, You L, Xu Z, Lee AY, Mikami I, et al. Wnt inhibitory factor-1 is silenced by promoter hypermethylation in human lung cancer. Cancer Res. 2004;64(14):4717–20.
27. Callow MG, Tran H, Phu L, Lau T, Lee J, Sandoval WN, et al. Ubiquitin ligase RNF146 regulates tankyrase and Axin to promote Wnt signaling. PLoS One. 2011;6(7):e22595.
28. Jho EH, Zhang T, Domon C, Joo CK, Freund JN, Costantini F. Wnt/beta-catenin/Tcf signaling induces the transcription of Axin2, a negative regulator of the signaling pathway. Mol Cell Biol. 2002;22(4):1172–83.
29. Northcott PA, Buchhalter I, Morrissy AS, Hovestadt V, Weischenfeldt J, Ehrenberger T, et al. The whole-genome landscape of medulloblastoma subtypes. Nature. 2017;547(7663):311–7.
30. Yu J, Virshup DM. Updating the Wnt pathways. Biosci Rep. 2014;34(5). https://doi.org/10.1042/BSR20140119.
31. Chen G, Courey AJ. Groucho/TLE family proteins and transcriptional repression. Gene. 2000;249(1–2):1–16.
32. Coon TA, Glasser JR, Mallampalli RK, Chen BB. Novel E3 ligase component FBXL7 ubiquitinates and degrades Aurora A, causing mitotic arrest. Cell Cycle. 2012;11(4):721–9.
33. Liu Y, Lear T, Zhao Y, Zhao J, Zou C, Chen BB, et al. F-box protein Fbxl18 mediates polyubiquitylation and proteasomal degradation of the pro-apoptotic SCF subunit Fbxl7. Cell Death Dis. 2015;6:e1630.
34. Liu Y, Lear T, Iannone O, Shiva S, Corey C, Rajbhandari S, et al. The proapoptotic F-box protein Fbxl7 regulates mitochondrial function by mediating the ubiquitylation and proteasomal degradation of survivin. J Biol Chem. 2015;290(19):11843–52.
35. Wheatley SP, McNeish IA. Survivin: a protein with dual roles in mitosis and apoptosis. Int Rev Cytol. 2005;247:35–88.
36. Kamran M, Long ZJ, Xu D, Lv SS, Liu B, Wang CL, et al. Aurora kinase a regulates survivin stability through targeting FBXL7 in gastric cancer drug resistance and prognosis. Oncogene. 2017;6(2):e298.
37. Kim S, Kon M, DeLisi C. Pathway-based classification of cancer subtypes. Biol Direct. 2012;7:21.
38. Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, et al. Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. PLoS One. 2008;3(8):e3088.
39. Lee RT, Zhao Z, Ingham PW. Hedgehog signalling. Development. 2016;143(3):367–72.
40. Brennan D, Chen X, Cheng L, Mahoney M, Riobo NA. Noncanonical hedgehog signaling. Vitam Horm. 2012;88:55–72.
41. Frappart PO, Lee Y, Russell HR, Chalhoub N, Wang YD, Orii KE, et al. Recurrent genomic alterations characterize medulloblastoma arising from DNA double-strand break repair deficiency. Proc Natl Acad Sci U S A. 2009;106(6):1880–5.
42. Mille F, Tamayo-Orrego L, Levesque M, Remke M, Korshunov A, Cardin J, et al. The Shh receptor Boc promotes progression of early medulloblastoma to advanced tumors. Dev Cell. 2014;31(1):34–47.
43. Chen W, Wang Z, Jiang C, Ding Y. PP2A-mediated anticancer therapy. Gastroenterol Res Pract. 2013;2013:675429.
44. Perrotti D, Neviani P. Protein phosphatase 2A: a target for anticancer therapy. Lancet Oncol. 2013;14(6):e229–38.
45. Hu P, Yu L, Zhang M, Zheng L, Zhao Y, Fu Q, et al. Molecular cloning and mapping of the brain-abundant B1gamma subunit of protein phosphatase 2A, PPP2R2C, to human chromosome 4p16. Genomics. 2000;67(1):83–6.
46. Cundell MJ, Hutter LH, Nunes Bastos R, Poser E, Holder J, Mohammed S, et al. A PP2A-B55 recognition signal controls substrate dephosphorylation kinetics during mitotic exit. J Cell Biol. 2016;214(5):539–54.
47. Pereira G, Schiebel E. Mitotic exit: determining the PP2A dephosphorylation program. J Cell Biol. 2016;214(5):499–501.
48. Ruvolo PP. The broken "off" switch in cancer signaling: PP2A as a regulator of tumorigenesis, drug resistance, and immune surveillance. BBA Clin. 2016;6:87–99.
49. Sablina AA, Hahn WC. The role of PP2A A subunits in tumor suppression. Cell Adhes Migr. 2007;1(3):140–1.
50. Wlodarchak N, Xing Y. PP2A as a master regulator of the cell cycle. Crit Rev Biochem Mol Biol. 2016;51(3):162–84.
51. Kaur A, Westermarck J. Regulation of protein phosphatase 2A (PP2A) tumor suppressor function by PME-1. Biochem Soc Trans. 2016;44(6):1683–93.
52. Devilder MC, Cadoret E, Cherel M, Moreau I, Rondeau G, Bezieau S, et al. cDNA cloning, gene characterization and 13q14.3 chromosomal assignment of CHC1-L, a chromosome condensation regulator-like guanine nucleotide exchange factor. Genomics. 1998;54(1):99–106.
53. Latil A, Morant P, Fournier G, Mangin P, Berthon P, Cussenot O. CHC1-L, a candidate gene for prostate carcinogenesis at 13q14.2, is frequently affected by loss of heterozygosity and underexpressed in human prostate cancer. Int J Cancer. 2002;99(5):689–96.
54. Latil A, Chene L, Mangin P, Fournier G, Berthon P, Cussenot O. Extensive analysis of the 13q14 region in human prostate tumors: DNA analysis and quantitative expression of genes lying in the interval of deletion. Prostate. 2003;57(1):39–50.
55. Ross-Adams H, Lamb AD, Dunning MJ, Halim S, Lindberg J, Massie CM, et al. Integration of copy number and transcriptomics provides risk stratification in prostate cancer: a discovery and validation cohort study. EBioMedicine. 2015;2(9):1133–44.
56. Maezawa S, Hayano T, Koiwai K, Fukushima R, Kouda K, Kubota T, et al. Bood POZ containing gene type 2 is a human counterpart of yeast Btb3p and promotes the degradation of terminal deoxynucleotidyltransferase. Genes Cells. 2008;13(5):439–57.
57. Janssens V, Goris J. Protein phosphatase 2A: a highly regulated family of serine/threonine phosphatases implicated in cell growth and signalling. Biochem J. 2001;353(Pt 3):417–39.
58. Pham TT, Angus SP, Johnson GL. MAP3K1: genomic alterations in cancer and function in promoting cell survival or apoptosis. Genes Cancer. 2013;4(11–12):419–26.
59. Junttila MR, Li SP, Westermarck J. Phosphatase-mediated crosstalk between MAPK signaling pathways in the regulation of cell survival. FASEB J. 2008;22(4):954–65.
60. Pearson CG, Osborn DP, Giddings TH Jr, Beales PL, Winey M. Basal body stability and ciliogenesis requires the conserved component Poc1. J Cell Biol. 2009;187(6):905–20.
61. Huang S, Zhang Z, Zhang C, Lv X, Zheng X, Chen Z, et al. Activation of Smurf E3 ligase promoted by smoothened regulates hedgehog signaling through targeting patched turnover. PLoS Biol. 2013;11(11):e1001721.

62. Farooqi AA, Waseem MS, Riaz AM, Bhatti S. SMURF and NEDD4: sharp shooters monitor the gate keepers and ion traffic controllers of lead astray cell. J Membr Biol. 2011;244(1):1–8.
63. Cui Y, He S, Xing C, Lu K, Wang J, Xing G, et al. SCFFBXL(1)(5) regulates BMP signalling by directing the degradation of HECT-type ubiquitin ligase Smurf1. EMBO J. 2011;30(13):2675–89.
64. Malatesta M, Steinhauer C, Mohammad F, Pandey DP, Squatrito M, Helin K. Histone acetyltransferase PCAF is required for hedgehog-Gli-dependent transcription and cancer cell proliferation. Cancer Res. 2013;73(20):6323–33.
65. Zuhlke KA, Johnson AM, Tomlins SA, Palanisamy N, Carpten JD, Lange EM, et al. Identification of a novel germline SPOP mutation in a family with hereditary prostate cancer. Prostate. 2014;74(9):983–90.
66. Cai H, Liu A. Spop regulates Gli3 activity and Shh signaling in dorsoventral patterning of the mouse spinal cord. Dev Biol. 2017;432:72–85.
67. Wen X, Lai CK, Evangelista M, Hongo JA, de Sauvage FJ, Scales SJ. Kinetics of hedgehog-dependent full-length Gli3 accumulation in primary cilia and subsequent degradation. Mol Cell Biol. 2010;30(8):1910–22.
68. Zhang Q, Shi Q, Chen Y, Yue T, Li S, Wang B, et al. Multiple Ser/Thr-rich degrons mediate the degradation of ci/Gli by the Cul3-HIB/SPOP E3 ubiquitin ligase. Proc Natl Acad Sci U S A. 2009;106(50):21191–6.
69. Gan W, Dai X, Lunardi A, Li Z, Inuzuka H, Liu P, et al. SPOP promotes ubiquitination and degradation of the ERG oncoprotein to suppress prostate cancer progression. Mol Cell. 2015;59(6):917–30.
70. De Smaele E, Di Marcotullio L, Moretti M, Pelloni M, Occhione MA, Infante P, et al. Identification and characterization of KCASH2 and KCASH3, 2 novel Cullin3 adaptors suppressing histone deacetylase and hedgehog activity in medulloblastoma. Neoplasia. 2011;13(4):374–85.
71. Canettieri G, Di Marcotullio L, Greco A, Coni S, Antonucci L, Infante P, et al. Histone deacetylase and Cullin3-REN(KCTD11) ubiquitin ligase interplay regulates hedgehog signalling through Gli acetylation. Nat Cell Biol. 2010;12(2):132–42.
72. Gulino A, Di Marcotullio L, Canettieri G, De Smaele E, Screpanti I. Hedgehog/Gli control by ubiquitination/acetylation interplay. Vitam Horm. 2012;88:211–27.
73. Canettieri G, Di Marcotullio L, Coni S, Greco A, Gulino A. Turning off the switch in medulloblastoma: the inhibitory acetylation of an oncogene. Cell Cycle. 2010;9(11):2047–8.
74. Korshunov A, Remke M, Kool M, Hielscher T, Northcott PA, Williamson D, et al. Biological and clinical heterogeneity of MYCN-amplified medulloblastoma. Acta Neuropathol. 2012;123(4):515–27.
75. Ciechanover A, DiGiuseppe JA, Schwartz AL, Brodeur GM. Degradation of MYCN oncoprotein by the ubiquitin system. Prog Clin Biol Res. 1991;366:37–43.
76. Otto T, Horn S, Brockmann M, Eilers U, Schuttrumpf L, Popov N, et al. Stabilization of N-Myc is a critical function of Aurora A in human neuroblastoma. Cancer Cell. 2009;15(1):67–78.
77. Zhao X, Heng JI, Guardavaccaro D, Jiang R, Pagano M, Guillemot F, et al. The HECT-domain ubiquitin ligase Huwe1 controls neural differentiation and proliferation by destabilizing the N-Myc oncoprotein. Nat Cell Biol. 2008;10(6):643–53.
78. King B, Boccalatte F, Moran-Crusio K, Wolf E, Wang J, Kayembe C, et al. The ubiquitin ligase Huwe1 regulates the maintenance and lymphoid commitment of hematopoietic stem cells. Nat Immunol. 2016;17(11):1312–21.
79. Faisal A, Vaughan L, Bavetsias V, Sun C, Atrash B, Avery S, et al. The aurora kinase inhibitor CCT137690 downregulates MYCN and sensitizes MYCN-amplified neuroblastoma in vivo. Mol Cancer Ther. 2011;10(11):2115–23.
80. Tavana O, Li D, Dai C, Lopez G, Banerjee D, Kon N, et al. HAUSP deubiquitinates and stabilizes N-Myc in neuroblastoma. Nat Med. 2016;22(10):1180–6.
81. Rodriguez-Blanco J, Pednekar L, Penas C, Li B, Martin V, Long J, et al. Inhibition of WNT signaling attenuates self-renewal of SHH-subgroup medulloblastoma. Oncogene. 2017;36(45):6306–14. https://doi.org/10.1038/onc.2017.232.
82. Poschl J, Bartels M, Ohli J, Bianchi E, Kuteykin-Teplyakov K, Grammel D, et al. Wnt/beta-catenin signaling inhibits the Shh pathway and impairs tumor growth in Shh-dependent medulloblastoma. Acta Neuropathol. 2014;127(4):605–7.
83. Blaess S, Stephen D, Joyner AL. Gli3 coordinates three-dimensional patterning and growth of the tectum and cerebellum by integrating Shh and Fgf8 signaling. Development. 2008;135(12):2093–103.
84. Schweiger S, Dorn S, Fuchs M, Kohler A, Matthes F, Muller EC, et al. The E3 ubiquitin ligase MID1 catalyzes ubiquitination and cleavage of Fu. J Biol Chem. 2014;289(46):31805–17.
85. Du H, Huang Y, Zaghlula M, Walters E, Cox TC, Massiah MA. The MID1 E3 ligase catalyzes the polyubiquitination of Alpha4 (alpha4), a regulatory subunit of protein phosphatase 2A (PP2A): novel insights into MID1-mediated regulation of PP2A. J Biol Chem. 2013;288(29):21341–50.
86. Lee EY, Ji H, Ouyang Z, Zhou B, Ma W, Vokes SA, et al. Hedgehog pathway-regulated gene networks in cerebellum development and tumorigenesis. Proc Natl Acad Sci U S A. 2010;107(21):9736–41.
87. Vuong TA, Leem YE, Kim BG, Cho H, Lee SJ, Bae GU, et al. A sonic hedgehog coreceptor, BOC regulates neuronal differentiation and neurite outgrowth via interaction with ABL and JNK activation. Cell Signal. 2017;30:30–40.
88. Shahi MH, Afzal M, Sinha S, Eberhart CG, Rey JA, Fan X, et al. Human hedgehog interacting protein expression and promoter methylation in medulloblastoma cell lines and primary tumor samples. J Neuro-Oncol. 2011;103(2):287–96.
89. Tan J, Lee PL, Li Z, Jiang X, Lim YC, Hooi SC, et al. B55beta-associated PP2A complex controls PDK1-directed myc signaling and modulates rapamycin sensitivity in colorectal cancer. Cancer Cell. 2010;18(5):459–71.
90. Tan Y, Sun D, Jiang W, Klotz-Noack K, Vashisht AA, Wohlschlegel J, et al. PP2A-B55beta antagonizes cyclin E1 proteolysis and promotes its dysregulation in cancer. Cancer Res. 2014;74(7):2006–14.
91. Oberg EA, Nifoussi SK, Gingras AC, Strack S. Selective proteasomal degradation of the B'beta subunit of protein phosphatase 2A by the E3 ubiquitin ligase adaptor Kelch-like 15. J Biol Chem. 2012;287(52):43378–89.
92. De Simone A, Gonczy P. Computer simulations reveal mechanisms that organize nuclear dynein forces to separate centrosomes. Mol Biol Cell. 2017;28(23):3165–70.
93. Mahale SP, Sharma A, Mylavarapu SV. Dynein light intermediate chain 2 facilitates the metaphase to anaphase transition by inactivating the spindle assembly checkpoint. PLoS One. 2016;11(7):e0159646.
94. Qiu X, Huang Y, Zhou Y, Zheng F. Aberrant methylation of TRIM58 in hepatocellular carcinoma and its potential clinical implication. Oncol Rep. 2016;36(2):811–8.
95. Kajiura K, Masuda K, Naruto T, Kohmoto T, Watabnabe M, Tsuboi M, et al. Frequent silencing of the candidate tumor suppressor TRIM58 by promoter methylation in early-stage lung adenocarcinoma. Oncotarget. 2017;8(2):2890–905.
96. Zhang W, Mi J, Li N, Sui L, Wan T, Zhang J, et al. Identification and characterization of DPZF, a novel human BTB/POZ zinc

finger protein sharing homology to BCL-6. Biochem Biophys Res Commun. 2001;282(4):1067–73.
97. Tonchev AB, Tuoc TC, Rosenthal EH, Studer M, Stoykova A. Zbtb20 modulates the sequential generation of neuronal layers in developing cortex. Mol Brain. 2016;9(1):65.
98. Groisman R, Kuraoka I, Chevallier O, Gaye N, Magnaldo T, Tanaka K, et al. CSA-dependent degradation of CSB by the ubiquitin-proteasome pathway establishes a link between complementation factors of the Cockayne syndrome. Genes Dev. 2006;20(11):1429–34.
99. Felsher DW. MYC inactivation elicits oncogene addiction through both tumor cell-intrinsic and host-dependent mechanisms. Genes Cancer. 2010;1(6):597–604.
100. Suryo Rahmanto A, Savov V, Brunner A, Bolin S, Weishaupt H, Malyukova A, et al. FBW7 suppression leads to SOX9 stabilization and increased malignancy in medulloblastoma. EMBO J. 2016;35(20):2192–212.
101. Kim SY, Herbst A, Tworkowski KA, Salghetti SE, Tansey WP. Skp2 regulates Myc protein stability and activity. Mol Cell. 2003;11(5):1177–88.
102. von der Lehr N, Johansson S, Wu S, Bahram F, Castell A, Cetinkaya C, et al. The F-box protein Skp2 participates in c-Myc proteosomal degradation and acts as a cofactor for c-Myc-regulated transcription. Mol Cell. 2003;11(5):1189–200.
103. Tansey WP. Transcriptional activation: risky business. Genes Dev. 2001;15(9):1045–50.
104. Kalev P, Simicek M, Vazquez I, Munck S, Chen L, Soin T, et al. Loss of PPP2R2A inhibits homologous recombination DNA repair and predicts tumor sensitivity to PARP inhibition. Cancer Res. 2012;72(24):6414–24.
105. Zhang H, Cao D, Zhou L, Zhang Y, Guo X, Li H, et al. ZBTB20 is a sequence-specific transcriptional repressor of alpha-fetoprotein gene. Sci Rep. 2015;5:11979.
106. Fedele V, Dai F, Masilamani AP, Heiland DH, Kling E, Gatjens-Sanchez AM, et al. Epigenetic regulation of ZBTB18 promotes glioblastoma progression. Mol Cancer Res. 2017;15(8):998–1011.
107. Link LA, Howley BV, Hussey GS, Howe PH. PCBP1/HNRNP E1 protects chromosomal integrity by translational regulation of CDC27. Mol Cancer Res. 2016;14(7):634–46.
108. Stewart S, Fang G. Anaphase-promoting complex/cyclosome controls the stability of TPX2 during mitotic exit. Mol Cell Biol. 2005;25(23):10516–27.
109. Song L, Rape M. Regulated degradation of spindle assembly factors by the anaphase-promoting complex. Mol Cell. 2010;38(3):369–82.
110. Manic G, Corradi F, Sistigu A, Siteni S, Vitale I. Molecular regulation of the spindle assembly checkpoint by kinases and phosphatases. Int Rev Cell Mol Biol. 2017;328:105–61.
111. Sansregret L, Patterson JO, Dewhurst S, Lopez-Garcia C, Koch A, McGranahan N, et al. APC/C dysfunction limits excessive cancer chromosomal instability. Cancer Discov. 2017;7(2):218–33.
112. Schwarz TL. Mitochondrial trafficking in neurons. Cold Spring Harb Perspect Biol. 2013;5(6). https://doi.org/10.1101/cshperspect.a011304.
113. Hagting A, Den Elzen N, Vodermaier HC, Waizenegger IC, Peters JM, Pines J. Human securin proteolysis is controlled by the spindle checkpoint and reveals when the APC/C switches from activation by Cdc20 to Cdh1. J Cell Biol. 2002;157(7):1125–37.
114. Sivakumar S, Gorbsky GJ. Spatiotemporal regulation of the anaphase-promoting complex in mitosis. Nat Rev Mol Cell Biol. 2015;16(2):82–94.
115. Hein JB, Hertz EPT, Garvanska DH, Kruse T, Nilsson J. Distinct kinetics of serine and threonine dephosphorylation are essential for mitosis. Nat Cell Biol. 2017;19(12):1433–40.
116. Manchado E, Guillamot M, de Carcer G, Eguren M, Trickey M, Garcia-Higuera I, et al. Targeting mitotic exit leads to tumor regression in vivo: modulation by Cdk1, Mastl, and the PP2A/B55alpha,delta phosphatase. Cancer Cell. 2010;18(6):641–54.
117. Densham RM, Garvin AJ, Stone HR, Strachan J, Baldock RA, Daza-Martin M, et al. Human BRCA1-BARD1 ubiquitin ligase activity counteracts chromatin barriers to DNA resection. Nat Struct Mol Biol. 2016;23(7):647–55.
118. Hao Z, Zhang H, Cowell J. Ubiquitin-conjugating enzyme UBE2C: molecular biology, role in tumorigenesis, and potential as a biomarker. Tumour Biol. 2012;33(3):723–30.
119. Alshalalfa M, Schliekelman M, Shin H, Erho N, Davicioni E. Evolving transcriptomic fingerprint based on genome-wide data as prognostic tools in prostate cancer. Biol Cell. 2015;107(7):232–44.
120. Liu Z, Yuan F, Ren J, Cao J, Zhou Y, Yang Q, et al. GPS-ARM: computational analysis of the APC/C recognition motif by predicting D-boxes and KEN-boxes. PLoS One. 2012;7(3):e34370.
121. Fuchsberger T, Lloret A, Vina J. New functions of APC/C ubiquitin ligase in the nervous system and its role in Alzheimer's disease. Int J Mol Sci. 2017;18(5). https://doi.org/10.3390/ijms18051057.
122. Li W, Bengtson MH, Ulbrich A, Matsuda A, Reddy VA, Orth A, et al. Genome-wide and functional annotation of human E3 ubiquitin ligases identifies MULAN, a mitochondrial E3 that regulates the organelle's dynamics and signaling. PLoS One. 2008;3(1):e1487.
123. Lehmann G, Udasin RG, Ciechanover A. On the linkage between the ubiquitin-proteasome system and the mitochondria. Biochem Biophys Res Commun. 2016;473(1):80–6.
124. Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, et al. Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. J Clin Oncol. 2011;29(11):1424–30.
125. Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, et al. Medulloblastoma comprises four distinct molecular variants. J Clin Oncol. 2011;29(11):1408–14.
126. Remke M, Hielscher T, Northcott PA, Witt H, Ryzhova M, Wittmann A, et al. Adult medulloblastoma comprises three major molecular variants. J Clin Oncol. 2011;29(19):2717–23.
127. Huang X, Jan LY. Targeting potassium channels in cancer. J Cell Biol. 2014;206(2):151–62.
128. Mochida S, Ikeo S, Gannon J, Hunt T. Regulated activity of PP2A-B55 delta is crucial for controlling entry into and exit from mitosis in Xenopus egg extracts. EMBO J. 2009;28(18):2777–85.
129. Seshacharyulu P, Pandey P, Datta K, Batra SK. Phosphatase: PP2A structural importance, regulation and its aberrant expression in cancer. Cancer Lett. 2013;335(1):9–18.
130. Nanahoshi M, Tsujishita Y, Tokunaga C, Inui S, Sakaguchi N, Hara K, et al. Alpha4 protein as a common regulator of type 2A-related serine/threonine protein phosphatases. FEBS Lett. 1999;446(1):108–12.
131. Kong M, Ditsworth D, Lindsten T, Thompson CB. Alpha4 is an essential regulator of PP2A phosphatase activity. Mol Cell. 2009;36(1):51–60.