Polycomb genes expression as a predictor of poor clinical outcome in children with medulloblastoma

Magdalena Zakrzewska · Krzysztof Zakrzewski · Sylwia M. Grešner · Sylwester Piaskowski · Beata Zalewska-Szewczyk · Paweł P. Liberski

Received: 12 February 2010 / Accepted: 30 July 2010 / Published online: 18 August 2010 © The Author(s) 2010. This article is published with open access at Springerlink.com

Abstract

Introduction Medulloblastoma is the most frequent type of embryonal tumor in the pediatric population, accounting for 20–25% of all brain tumors in children. Recently, the suspected contribution of the Polycomb group (PcG) genes in medulloblastoma development was described. PcG genes play an important role in developmental processes; they are also involved in the self-renewal of hematopoietic and neural stem cells as well as in malignant transformation.

Purpose In this study, we evaluated the expression of BMI1 and PCGF2, members of family of PcG genes, and their potential target, MYC oncogene, and analyzed their association with demographic and clinical data.

Materials and methods Thirty-one children (18 males and 13 females, aged from 0.4 to 17 years) with medulloblastoma were included in this study. The gene’s expression level was measured by quantitative real-time PCR, obtained using the two-color multiplexing technique.

Results We found that the higher expression levels of BMI1 and PCGF2 genes were associated with significantly decreased patient survival (p=0.02 and p=0.012, respectively). Significant differences between gender were found, with a higher expression level of the PCGF2 gene observed among females (p=0.02).

Conclusion Our analysis showed correlation between BMI1 and PCGF2 gene’s expression and survival in children with medulloblastoma.

Keywords BMI1 · Clinical risk · Expression · Medulloblastoma · MYC · PCGF2

Introduction

Medulloblastoma (MB) is the most frequent type of highly malignant tumor occurring in the pediatric population, and despite significant advances in therapeutic modalities, it is still associated with a high mortality rate. Due to its frequency and aggressive behavior, a precise disease risk stratification of patients is essential. The current estimation of disease progression in patients with MB, based mainly on clinical factors, seems to be insufficient. Moreover, risk estimated on the neuropathological classification of this tumor is still controversial, inasmuch as no unambiguous criteria of characteristic morphological features are well-defined [11]. Until now, a number of molecular factors correlated with survival and predictors of outcome have
been proposed, but there is still a need to search for more appropriate classifiers for medulloblastoma.

Recently, the suspected contribution of the Polycomb group (PcG) of genes, BMI1 and PCGF2 (also known as MEL18 or ZNF144), was described in MB pathogenesis [36]. Human PcG complexes were classified into polycomb repressive complex 1 (PRC1) and polycomb repressive complex 2 subtypes. BMI1 and PCGF2 genes encode for highly homologous proteins of PRC1 and have overlapping functions in terms of development, self-renewal processes, and cancer growth, mostly through the modification of the chromatin structure [3, 36]. The significance of BMI1 as a potential molecular diagnostic marker was described previously for breast and prostate cancer [10, 31]. In invasive ductal breast cancer, its overexpression was defined as the prognostic factor related to metastases and signaled a high possibility of a poor outcome [23]. Recently, the significance of BMI1 in non-small cell lung cancer expansion was also reported, as well as its role in maintaining the function of bronchioalveolar stem cells [4, 8]. Similar mechanisms, including brain stem cells, were also well documented for glioblastoma [1]. In medulloblastoma, BMI1 was found to be overexpressed and their potential role in tumor pathogenesis via expansion of tumor stem cells was also strongly suggested, but studies into its probable link with patient outcomes have not yet been conducted thoroughly [13, 25, 27].

PCGF2 was described previously as the regulator of hematopoietic stem cell fate which downregulation intensify stem cell capability [21, 22]. The clinical value of that gene was confirmed in breast and prostate cancers, where negative expression of PCGF2 was correlated with the proliferation of neoplastic cells and a worse prognosis of patients [16, 24, 34, 35].

In addition, an inverse correlation between PCGF2 and BMI1 expression in invasive breast cancer and breast cancer cell lines was observed [16, 24]. Interestingly, such a link was not reported for medulloblastoma cell lines and overlapping functions for both genes was strongly suggested in MB tumorigenesis [36]. Moreover, the negative correlation between PCGF2 and MYC, as well as between PCGF2 and BMI1 expression and PCGF2 downregulation of BMI1 through MYC, was observed in human fibroblast cell lines [15]. The MYC gene was amplified in approximately 5–10% of medulloblastomas, while overexpressed in up to 50% of tumor samples. Such inconsistency could be caused by altered transcriptional processes dependent on transcriptional repressor proteins such as PcG family members BMI1 and PCGF2 [2, 14, 20].

In this work, we evaluated the expression of BMI1, PCGF2, and MYC genes to determine their potential connection with survival data of children with medulloblastoma.

Materials and methods

Patient samples

Thirty-one children with classic type medulloblastoma, operated on in the Department of Neurosurgery, Polish Mother’s Memorial Hospital Research Institute, were included in this study. The group was comprised of 18 males and 13 females. The median age of patients at the time of diagnosis was 8 years (range, 0.4–17 years). All specimens were diagnosed at the Department of Molecular Pathology and Neuropathology, Chair of Oncology, Medical University of Lodz, according to the World Health Organization criteria [26].

Based on clinical data, the patients were divided into standard risk (SR) and high risk (HR) groups consist of 16 and 15 children, respectively. Patients under 3 years of age at the time of diagnosis, with more than 1.5 cm3 of residual disease after surgery or with evidence of metastases, were classified to HR group, while children older than 3 years of age, with no evidence of metastatic disease and less than 1.5 cm3 of residual disease, were stratified as SR group.

Seven patients under 3 years of age received chemotherapy (no radiotherapy) that consisted of alternate courses of vincristine, etoposide, and cisplatin and vincristine, etoposide, and cyclophosphamide during 18 months. HR patients were treated by preirradiation chemotherapy (four alternate courses) with vincristine, etoposide and carboplatin and etoposide, ifosfamide, and cisplatin and maintenance chemotherapy with vincristine, lomustine, and cisplatin (six to eight courses). SR patients received preirradiation chemotherapy (four alternate courses) consisting of vincristine, etoposide and carboplatin and vincristine, etoposide, and cyclophosphamide and maintenance chemotherapy with vincristine, lomustine, and cisplatin (six to eight courses). Radiotherapy was given with total doses of 35 Gy for brain and spine and 50–55 Gy on tumor site.

Survival periods were collected for all children. The mean follow-up time was 58 months (in a range from 4 to 156 months). The Bioethics Medical University Committee approved all analyses performed (no. RNN/154/06/KE).

RNA isolation

RNA was extracted using a commercially available RNasy Mini Kit (cat. no. 74104; Qiagen) and treated with DNase (cat. no. 79254; Qiagen). RNA concentrations were measured spectrophotometrically. One microgram of total RNA from all specimens and reference RNA (Human Cerebellum Brain Total RNA cat. no. 6820; Ambion, USA) was reverse-transcribed into single-stranded cDNA according to standard protocol (cat. no. 205313; Qiagen) in two independent reactions for each sample.
Amplification analysis

The expression levels of target and reference genes were measured by quantitative real-time PCR (Q-PCR), using the 2-color multiplexing technique. The Q-PCR experiment was designed using the free web-based ProbeFinder software version 2.41 for Human, which specifies a set of primers and probes from the Universal ProbeLibrary (cat. no. 04688627001, BMI1; cat. no. 04687671001, MYC; cat. no. 04687973001, PCGF2; Roche Diagnostic GmbH, Germany) as well as reference gene (cat. no. 05 046 157 001, HPRT; Roche Diagnostic GmbH, Germany). The sequences of the set of primers and numbers of probes used are listed in Table 1. The optimized Q-PCR assay was performed in a 20 μl reaction mixture containing water, 5 μl of template (cDNA), 10 μl FastStart TaqMan Probe Master (cat. no. 04 673 417 001; Roche Diagnostic GmbH, Germany), 500 nM of each of the primers, and 250 nM of each of the probe. Reactions were performed on a Rotor Gene 6000 instrument (Qiagene-Corbett Life Science, Sydney, Australia) under the following conditions: initial cDNA polymerase activation at 95°C for 15 min, followed by 45 cycles of 95°C for 15 s (denaturation) and 60°C for 45 s (annealing) with multi-channel detection. All samples were analyzed in triplicate to obtain replicates for statistical analyses.

Statistical analysis

A normalized relative expression level for a given target gene was calculated using the method described by Pfaffl et al. with pooled cDNA from all tumor samples used as a control, according to the equation,

$$\text{Ratio} = \frac{(E_{\text{TARGET}})^{\Delta\text{CP}_{\text{TARGET}}(\text{control-sample})}}{(E_{\text{REF}})^{\Delta\text{CP}_{\text{REF}}(\text{control-sample})}}$$

where $E_{\text{TARGET}}$ and $E_{\text{REF}}$ stand for the Q-PCR efficiency of target and reference gene amplification, respectively, and $\Delta\text{CP}_{\text{TARGET}}(\text{control-sample})$ and $\Delta\text{CP}_{\text{REF}}(\text{control-sample})$ denote the difference in crossing points (CP) between unknown and control samples for a given target and reference gene, respectively [28]. The differences in gene expression levels were evaluated by Mann–Whitney U. Statistical significance was assumed for $p$ value ≤0.05. Kaplan–Meier curves were estimated from survival data for two groups based on the median expression level of each gene.

Results

The clinical data, including patient’s age, sex, clinical risk, and follow-up data, are given in Table 2. Median levels of BMI1, MYC, and PCGF2 mRNA expression were 1.94 (range, 1.21–3.10), 3.80 (range, 1.06–53.86), and 1.79 (range, 0.98–2.45), respectively (Table 2). Significant differences ($p=0.02$) between gender were found for the PCGF2 gene, with higher expression level observed among females (Fig. 1). The survival analysis performed following the group stratification based on the median expression level of each gene revealed an association between higher expression levels of BMI1 and PCGF2 and a significantly decreased patient survival rate ($p=0.02$ and $p=0.012$, respectively). Estimation of outcomes, using the Kaplan–Meier method, demonstrate a prediction of 5-year survival rates of 35% and 65% for high and low expression levels of the BMI1 gene, respectively (Fig. 2), and 37% and 70% for high and low expression levels of the PCGF2 gene, respectively (Fig. 3). Kaplan–Meier survival analysis performed for female and male patients independently showed better outcome for female patients with low expression levels of the PCGF2 gene (Figs. 4 and 5). Expression level of MYC gene was unrelated to clinical outcome (Fig. 6). Statistical analysis revealed significant differences in expression levels between two groups, obtained based on the clinical risk stratification (high risk vs. standard risk) for the MYC gene, but not for BMI1 and PCGF2 genes. There was no significant differences in

| Probe/primer name | Sequence (5’-3’) | Position | Amplicon size | Reference gene assay |
|-------------------|-----------------|----------|--------------|-----------------------|
| Probe#63          |                 |          |              |                       |
| BMI1-F            | TTCTTTGACCAGAACAGATTGG | 888–909 | 112 bp       | HPRT                  |
| BMI1-R            | GCACTCAGGTCTGCTGCT | 981–999 |              |                       |
| Probe#34          |                 |          |              |                       |
| MYC-F             | CACGAGCAGCGACTTCTGA | 741–758 | 102 bp       |                       |
| MYC-R             | GATCCAGACTCTGACCTTTC | 821–842 |              |                       |
| Probe#39          |                 |          |              |                       |
| PCGF2-F           | TTCTCCGCAACAAAGATGGAT | 740–759 | 67 bp        |                       |
| PCGF2-R           | AGTGCGTCTCGCTGTAC | 788–806 |              |                       |
expression levels of any of the genes studied between the two age groups (under 3 years vs. over 3 years). The analysis of correlation between genes reached the level of statistical significance, nevertheless it showed only low values of rank correlation coefficients obtained using Spearman’s correlation test: MYC vs. BMI1 ($r^2=0.19$, $p=0.02$), MYC vs. PCGF2 ($r^2=0.24$, $p=0.005$), BMI1 vs. PCGF2 ($r^2=0.26$, $p=0.003$; Fig. 7). As indicated by obtained coefficient of determination, the correlation between PCGF2 and BMI1 accounted for only about 26% of common variability of these genes’ expression (this correlation was excluded from consideration).

### Discussion

Members of the PcG gene family act as epigenetic gene silencers and control biological processes, including self-renewal of both normal and neoplastic stem cells. Their role in tumorigenesis is related to the modulation of tumor suppressor pathways, crucial for the cell cycle and oncogenesis. A connection was observed between PcG genes overexpression and the INK4a/ARF, Rb, and TP53 pathways silencing [10, 24]. There was also some evidence of correlation between PcG transcriptional repressor proteins and developmental pathways such as Hedgehog, Wnt/
beta-catenin, and Notch considered to be essential in medulloblastoma pathogenesis [27]. Via these pathways, PcG genes suppressed tumor development, progression, and expansion mainly by the limitation of putative cancer stem cells. Their expression and potential value as clinical biomarkers have been considered in selected types of cancers. The most often observed alteration has been upregulation of \( \text{BMI1} \), which was related to a poor prognosis in epithelial cancers and leukemias [4, 8, 23, 29, 34]. In medulloblastoma, altered expression of \( \text{BMI1} \) was reported as well as correlation between \( \text{BMI1} \) expression and the Hedgehog pathway activation [13, 27].

Leung et al. established the plausible significance of \( \text{BMI1} \) in medulloblastoma pathogenesis but its clinical value has not been well estimated until now [25]. Based on microarray analysis, Glinsky et al. suggested the usefulness of \( \text{BMI1} \) expression in prediction of outcomes in medulloblastoma [13]. In this work, we presented an association between a higher expression level of \( \text{BMI1} \) and decreased patient survival. Estimation of outcomes using the Kaplan–Meier method showed a trend towards a better outcome in the group with lower expression level of the \( \text{BMI1} \) gene. \( \text{BMI1} \) expression and its potential usefulness as a molecular factor of outcomes were analyzed in brain tumors but not in...
Tirabosco et al. and Häyry et al. found no correlation between BMI1 expression and outcome in astrocytomas, but such a link was observed in oligodendroglial tumors [18, 32]. Häyry et al., after reviewing analysis of BMI1 loss in glial tumors, pointed out the association of gene deletion with poor outcomes in grade II–IV astrocytomas and considered that plausible BMI1 gene function (oncogenic or suppressing) could vary according to the type of tumor, kind of molecular alteration, or transcriptional events [17].

A similar phenomenon was noted for the PCGF2 gene, which encodes a functional homologue of BMI1 and
 regulates self-renewal processes in hematopoietic cells, as does BMI1 [12, 21, 22, 36]. While in prostate and breast tissue PCGF2 functioned as a tumor suppressor gene, there were evidences of its oncogenic function in medulloblastoma [15, 16, 34, 36]. Here, we demonstrated a link between a higher expression level of PCGF2 and decreased patient survival especially among females. Our results indicated the oncogenic function of PCGF2 in human medulloblastoma and confirmed the observation of Wiederschan et al. obtained in experimental study of medulloblastoma cell lines [36]. The data presented here showed also sex-dependent gene expression of the PCGF2. The higher gene expression in female patients may be interpreted as the consequence of the mechanism of chromosome X inactivation, induced via Xist RNA (X-inactive-specific transcript), in which PcG genes are involved during development [12, 19].

In our analysis, we also included the MYC gene, which was associated with BMI1 in oncogenic processes and in breast cancer was repressed by PCGF2 [15, 16]. MYC oncogene overexpression was described in approximately 50% of medulloblastomas, but its connection with prognosis predictions is unclear [9, 14, 20]. Rutkowski et al. and Herms et al. revealed the relevance of MYC expression for predicting outcomes, however Das et al. showed no correlation between the MYC labeling index and progress-free survival, similarly as De Bortoli et al., which presented no such link either for expression analysis [5, 6, 20, 30]. These discrepancies may be caused by the different criteria used in data interpretation, or the specificity of groups analyzed, and still needs elucidation, especially as criteria used in data interpretation, or the specificity of target inducing apoptosis in medulloblastoma [7, 33]. Here, we observed correlation between MYC expression level and risk stratification and no such relationship for PcG genes. In our opinion, lack of relation with clinical risk of BMI1 and PCGF2 genes could be connected with imperfect clinical risk classification of this tumor. Development of well-chosen molecular markers used in multivariate risk estimation together with clinical data must be concerned in case of medulloblastoma.

In summary, our analysis suggest that BMI1 and PCGF2 genes, acting as the epigenetic silencers engaged in the self-renewal processes of neoplastic stem cells, could be engaged in medulloblastoma oncogenesis. Higher BMI1 and PCGF2 gene’s expression levels should be considered as the predictors of poor clinical outcome for children with medulloblastoma.

Acknowledgments This work was supported by Ministry of Science and Higher Education Grants no. N401 180 32/3580 and N401 196 32/4137. Prof. Janusz Szemraj, Department of Medical Biochemistry, Medical University of Łódź, Mazowiecka 6/8, 92-215 Lodz, Poland, is kindly acknowledged for his cooperation.

Open Access This article is distributed under the terms of the Creative Commons Attribution Noncommercial License which permits any noncommercial use, distribution, and reproduction in any medium, provided the original author(s) and source are credited.

References

1. Abdouh M, Facchinio S, Chatoo W, Balasingam V, Ferreira J, Bernier G (2009) BMI1 sustains human glioblastoma multiforme stem cell renewal. J Neurosci 29:8884–8896
2. Aldosari N, Bigner SH, Burger PC, Becker L, Kapner JL, Friedman HS, McLendon RE (2002) MYCC and MYCN oncogene amplification in medulloblastoma. A fluorescence in situ hybridization study on paraffin sections from the Children’s Oncology Group. Arch Pathol Lab Med 126:540–544
3. Beil S, Tort F, Pinyol M, Puig X, Hernández L, Hernández S, Fernandez PL, van Lohuizen M, Colomer D, Campo E (2001) BMI-1 gene amplification and overexpression in hematological malignancies occur mainly in mantle cell lymphomas. Cancer Res 61:2409–2412
4. Becker M, Korn C, Sieneth AR, Vorwinckel R, Luetkenhaus K, Ceteci F, Rapp UR (2009) Polycomb group protein Bmi1 is required for growth of RAF driven non-small-cell lung cancer. PLoS ONE 4:e4230
5. Das P, Puri T, Suri V, Sharma MC, Sharma BS, Sarkar C (2009) Medulloblastomas: a correlative study of MIB-1 proliferation index along with expression of c-Myc, ERBB2, and anti-apoptotic proteins along with histological typing and clinical outcome. Childs Nerv Syst 25:825–835
6. De Bortoli M, Castellino RC, Lu XY, Deyo J, Sturla LM, Adesina AM, Perlaky L, Pomery SL, Lau CC, Man TK, Rao PH, Kim JY (2006) Medulloblastoma outcome is adversely associated with overexpression of EEF1D, RPL30, and RPS20 on the long arm of chromosome 8. BMC Cancer 6:223
7. de Haas T, Hasselt N, Troost D, Caron H, Popovic M, Zadravec-Zaletel L, Grajkowska W, Perek M, Osterheld MC, Ellison D, Baas F, Versteeg R, Kool M (2008) Molecular risk stratification of medulloblastoma patients based on immunohistochemical analysis of MYC, LDHB, and CCNB1 expression. Clin Cancer Res 14:4154–4160
8. Dovey JS, Zacharek SJ, Kim CF, Lees JA (2008) Bmi1 is critical for lung tumorigenesis and bronchioalveolar stem cell expansion. Proc Natl Acad Sci USA 105:11857–11862
9. Eberhart CG, Kratz J, Wang Y, Summers K, Stearns D, Cohen K, Ohgaki H (2006) Stratification of medulloblastoma on the basis of histopathological grading, immunohistochemical analysis of MYCC, NMYC, TrkC, and anaplasia. J Neuropathol Exp Neurol 65:440–449
10. Fan C, He L, Kapoor A, Gillis A, Rybak AP, Cutz JC, Tang D (2008) Bmi1 promotes prostate tumorigenesis via inhibiting p16 (INK4A) and p14(ARF) expression. Biochem Biophys Acta 1782:642–648
11. Giangaspero F, Welles K, Masuoka J, Gessi M, Kleihues P, Ohgaki H (2006) Stratification of medulloblastoma on the basis of histopathological grading. Acta Neuropathol 112:5–12
12. Gietzen RS, Hendzel MJ (2009) Polycomb group protein gene silencing, non-coding RNA, stem cells, and cancer. Biochim Cell Biol 87:711–746
13. Glinsky GV, Berezovska O, Glinskii AB (2005) Microarray analysis identifies a death-from-cancer signature predicting therapy failure in patients with multiple types of cancer. J Clin Invest 111(6):1503–1521
14. Grotzer MA, Hogarty MD, Janss AJ, Liu X, Zhao H, Eggert A, Sutton LN, Rorke LB, Brodeur GM, Phillips PC (2001)
messenger RNA expression predicts survival outcome in childhood primitive neuroectodermal tumor/medulloblastoma. Clin Cancer Res 7:2425–2433
15. Guo WJ, Datta S, Band V, Dimri GP (2007) Mel-18, a polycomb group protein, regulates cell proliferation and senescence via transcriptional repression of Bmi-1 and c-Myc oncoproteins. Mol Biol Cell 8:536–546
16. Guo WJ, Zeng MS, Yadav A, Song LB, Guo BH, Band V, Dimri GP (2007) Mel-18 acts as a tumor suppressor by repressing Bmi-1 expression and down-regulating Akt activity in breast cancer cells. Cancer Res 67:5083–5089
17. Häyry V, Tanner M, Blom T, Tyninnien O, Roselli A, Ollikainen M, Sariola H, Wartiovaara K, Nupponen NN (2008) Copy number alterations of the polycomb gene BMI1 in gliomas. Acta Neuro-pathol 116:97–102
18. Häyry V, Tyninnien O, Haapasalo HK, Wölfer J, Paulus W, Hasselblatt M, Sariola H, Paetau A, Sarna S, Niemeli M, Wartiovaara K, Nupponen NN (2008) Stem cell protein BMI-1 is an independent marker for poor prognosis in oligodendrogial tumours. Neuropathol Appl Neurobiol 34:555–563
19. Heard E (2004) Recent advances in X-chromosome inactivation. Curr Opin Cell Biol 16:247–255
20. Herms J, Neidt I, Lüscher B, Sommer A, Schürmann P, Schröder T, Bergmann M, Wilken B, Probst-Cousin S, Hernáiz-Driever P, Behnek J, Hanefeld F, Pietsch T, Kretzschmar HA (2000) C-MYC expression in medulloblastoma and its prognostic value. Int J Cancer 89:395–402
21. Kajiume T, Ninomiya Y, Ishihara H, Kanno R, Kanno M (2004) Bmi1 is essential for Hedgehog pathway-driven medulloblastoma. Nature 428:337–341
22. Kajiume T, Ohno N, Sera Y, Kawahara Y, Yuge L, Kobayashi M (2004) Alterations of the polycomb gene BMI1 in gliomas. Acta Neuro-pathol 116:97–102
23. Kim JH, Yoon SY, Jeong SH, Kim SY, Moon SK, Joo JH, Lee Y, Choe IS, Kim JW (2004) Overexpression of Bmi-1 oncoprotein correlates with axillary lymph node metastases in invasive ductal breast cancer. Breast 13:383–388
24. Lee JY, Jang KS, Shin DH, Oh MY, Kim HJ, Kim Y, Kong G (2008) Mel-18 negatively regulates INK4a/ARF-independent cell cycle progression via Akt inactivation in breast cancer. Cancer Res 68:4201–4209
25. Leung C, Lingbeek M, Shakhova O, Liu J, Tanger E, Sarembslani P, Van Lohuizen M, Marino S (2004) Bmi1 is essential for cerebellar development and is overexpressed in human medulloblastomas. Nature 428:337–341
26. Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2007) WHO classification of tumors of the central nervous system. International Agency for Research on Cancer 2007, Lyon
27. Michael LE, Westerman BA, Ermiolov AN, Wang A, Ferris J, Liu J, Blom M, Ellison DW, van Lohuizen M, Dlugosz AA (2008) Bmi1 is required for Hedgehog pathway-driven medulloblastoma expansion. Neoplasia 10:1343–1349
28. Pfaffl MW, Horgan GW, Dempfle L (2002) Relative expression software tool (REST) for group-wise comparison and statistical analysis of relative expression results in real-time PCR. Nucleic Acids Res 30:e36
29. Raaphorst FM (2005) Deregulated expression of Polycomb-group oncogenes in human malignant lymphomas and epithelial tumors. Hum Mol Genet Spec No 1:R93–R100
30. Rutkowski S, von Bueren A, van Hoff K, Hartmann W, Shahaby T, Deinlein F, Warmuth-Metz M, Soerensen N, Emser A, Bode U, Mittler U, Urban C, Benesch M, Cortmann RD, Schlegel PG, Kuehl J, Pietsch T, Grotzer M (2007) Prognostic relevance of clinical and biological risk factors in childhood medulloblastoma: results of patients treated in the prospective multicenter trial HIT’91. Clin Cancer Res 13:2651–2657
31. Saeki M, Kobayashi D, Tsujii N, Kuribayashi K, Watanabe N (2009) Diagnostic importance of overexpression of Bmi-1 mRNA in early breast cancers. Int J Oncol 35:511–515
32. Tirabosco R, De Maglio G, Skrap M, Falconieri G, Pizzolitto S (2008) Expression of the Polycomb-Group protein BMI1 and correlation with p16 in astrocytomas an immunohistochemical study on 80 cases. Pathol Res Pract 204:625–631
33. von Bueren AO, Shahaly T, Oehler-Jänne C, Arnold L, Stearns D, Eberhart CG, Arcaro A, Pruschky M, Grotzer MA (2009) RNA interference-mediated c-MYC inhibition prevents cell growth and decreases sensitivity to radio- and chemotherapy in childhood medulloblastoma cells. BMC Cancer 9:10
34. Wang W, Lin T, Huang J, Hu W, Xu K, Liu J (2009) Analysis of Mel-18 expression in prostate cancer tissues and correlation with clinicopathologic features. Urol Oncol. doi:10.1016/j.urolonc.2009.02.004
35. Wang W, Yuasa T, Tsuchiya N, Ma Z, Maita S, Narita S, Kumazawa T, Inoue T, Tsutsumi H, Horikawa Y, Saito M, Hu W, Ogawa O, Habuchi T (2009) The novel tumor-suppressor Mel-18 in prostate cancer: its functional polymorphism, expression and clinical significance. Int J Cancer 125:2836–2843
36. Wiederschain D, Chen L, Johnson B, Bettano K, Jackson D, Taraszka J, Wang YK, Jones MD, Morrissey M, Deeds J, Mosher R, Fordjour P, Lengauer C, Benson JD (2007) Contribution of polycomb homologues Bmi-1 and Mel-18 to medulloblastoma pathogenesis. Mol Cell Biol 27:4968–4979