Chromosomal heterogeneity and instability characterize pediatric medulloblastoma cell lines and affect neoplastic phenotype

Angel Mauricio Castro-Gamero · Kleiton Silva Borges · Regia Caroline Lira ·
Augusto Faria Andrade · Paola Fernanda Fedatto · Gustavo Alencastro Veiga Cruzeiro ·
Ricardo Bonfim Silva · Aparecida Maria Fontes · Elvis Terci Valera ·
Michael Bobola · Carlos Alberto Scrideli · Luiz Gonzaga Tone

Received: 28 August 2012 / Accepted: 20 December 2012 / Published online: 17 January 2013
© Springer Science+Business Media Dordrecht 2013

Abstract Chromosomal heterogeneity is a hallmark of most tumors and it can drive critical events as growth advantages, survival advantages, progression and karyotypic evolution. Medulloblastoma (MB) is the most common malignant central nervous system tumor in children. This work attempted to investigate chromosomal heterogeneity and instability profiles of two MB pediatric cell lines and their relationship with cell phenotype. We performed GTG-banding and cytokinesis-block micronucleus cytome assays, as well as morphological characterization, cell population doubling time, colony-forming efficiency, and chemo-sensitivity assays in two pediatric MB cell lines (UW402 and UW473). Both MB cells showed a high chromosomal heterogeneity. UW473 cells

showed ~2 fold higher both clonal- and non-clonal chromosomal alterations than UW402 cells. Besides, UW473 showed two clonal-groups well-differentiated by ploidy level ($<2n$ and $<4n$) and also presented a significantly higher number of chromosomal instability biomarkers. These results were associated with high morphological heterogeneity and survival advantages for UW473 and proliferation advantages for UW402 cells. Moreover, UW473 was significantly more sensitive to methotrexate, temozolomide and cisplatin while UW402 cells were more sensitive to doxorubicin. These data suggest that distinct different degrees of karyotypic heterogeneity and instability may affect neoplastic phenotype of MB cells. These findings bring new insights into cell and tumor biology.

A. M. Castro-Gamero · K. S. Borges · A. F. Andrade
Department of Genetics, School of Medicine of Ribeirão
Preto, University of São Paulo, São Paulo, Brazil

A. M. Castro-Gamero (✉)
Laboratório de Pediatria, Faculdade de Medicina de
Ribeirão Preto, Hospital das Clínicas, Universidade de
São Paulo, Bloco G, Av. Bandeirantes 3900,
14048-900 Ribeirão Preto, SP, Brazil
e-mail: amcgen@gmail.com

R. C. Lira · P. F. Fedatto · G. A. V. Cruzeiro ·
E. T. Valera · C. A. Scrideli · L. G. Tone
Division of Pediatric Oncology, Department of Pediatrics,
School of Medicine of Ribeirão Preto, University of São
Paulo, São Paulo, Brazil

R. B. Silva · A. M. Fontes
Laboratory of Genic Transference, Hemocentro,
School of Medicine of Ribeirão Preto,
University of São Paulo, São Paulo, Brazil

M. Bobola
Departments of Neurosurgery, University of Washington,
Seattle, WA, USA

Keywords Medulloblastoma · Chromosomal heterogeneity · Chromosomal instability (CIN) · Non-clonal chromosomal alterations (NCCAs) · Neoplastic phenotype

Introduction

Medulloblastoma (MB) is an aggressive and invasive embryonal central nervous system (CNS) tumor (WHO grade IV) and mainly affects children (Pomeroy et al. 2002; Louis et al. 2007). This tumor arises from cerebellar stem and precursor cells and has a tendency to metastasize via cerebrospinal fluid (Read et al. 2006; Louis et al. 2007). Current management strategies have achieved a 5-year survival rate of 60–80 % (Packer and Vezina 2008). Nevertheless, the aggressiveness and response to therapy can vary considerably between cases, with approximately 30 % of patients dying within 2 years from diagnosis (Pfister et al. 2009). The quality of life in long-term survivors is another major issue, because most surviving children have devastating neurologic and cognitive sequelae (Finlay et al. 2007; Packer and Vezina 2008; Pfister et al. 2009).

Additionally, MB is characterized by high chromosomal heterogeneity showing aneuploid karyotypes with complex chromosomal aberrations (Bayani et al. 2000; Cohen et al. 2004). Some recurrent chromosomal changes have been recently reported to predict survival in MB patients including loss of 17p (Batra et al. 1995; Lamont et al. 2004), gain of 17q (Pfister et al. 2009), presence of an isodicentric chromosome 17 (idic(17)(p11.2)) (Lamont et al. 2004; Pan et al. 2005), gain of 1q (Lo et al. 2007) and monosomy 6 (Thompson et al. 2006).

Recently, molecular studies have demonstrated that MB is not a single disease, but comprises a collection of four distinct molecular subgroups (Cho et al. 2011; Kool et al. 2008; Northcott et al. 2011; Remke et al. 2011a, b; Thompson et al. 2006). Two of these subgroups are characterized by either activated WNT or SHH signaling, consistently show the most distinct genetic profiles, and remain Non-WNT/Non-SHH tumors named Group 3 and Group 4. These four subgroups are transcriptionally, genetically, cytogenetically, demographically, clinically, and prognostically distinct, demonstrating high tumor heterogeneity (Taylor et al. 2012).

Certainly, with the identification of these subgroups, new challenges appear for the scientific

community for instance to determinate the true extent and nature of inter-tumoral heterogeneity, identify and characterize more the biological models representative of these subgroups, as well as the development of targeted therapies using these models.

Nowadays, cell lines represent an effective tool to study tumor biology, including cytogenetic and molecular heterogeneity, neoplastic behavior, biological pathways of tumor maintenance, chemo- and radio-sensitivity, as well as testing new targeted therapies (Keles et al. 1995; Aldosari et al. 2002). However, there are relatively few pediatric MB cell lines reported in the literature.

Although the establishment of patterns for recurrent chromosomal alterations is difficult for most solid tumors due to the high level of chromosomal heterogeneity (Albertson et al. 2003; Heng et al. 2006a), cytogenetic analyses based on identification of recurrent chromosomal alterations have provided useful information in malignancy biology. However, attention has been focused primarily on recurrent chromosomal alterations, while random chromosomal alterations have been disregarded as background noise or incidental findings of clinical insignificance (Albertson et al. 2003; Mitelman 2000).

The chromosomal heterogeneity is a hallmark of most tumors (Selvarajah et al. 2006; Glanz et al. 2007) and can be visualized by the coexistence of cytogenetically related cell populations (sidelines) that share several common chromosome anomalies and exhibit unique karyotypic characteristics (Heim and Mitelman 1994). It is represented by clonal (CCA) and non-clonal (NCCA) chromosomal alterations, essential elements for evolution and development of cancer, driving critical events as growth and survival advantages and karyotypic evolution (Heng et al. 2006a, b; Shaffer and Tommerup 2005). Moreover, since this model could be applied from transition to metastatic disease and in treatment resistance evaluation, it has been recently suggested the integration of monitoring of both NCCAs and CCAs into the standard practice of clinical cytogenetic reporting (Heng et al. 2006b, 2010; Ye et al. 2007; Bayani et al. 2007).

This work attempted to investigate chromosomal heterogeneity and CIN profiles of two MB pediatric cell lines and their possible relationship with cell phenotype. Cytogenetic analysis and identification of chromosomal instability (CIN) biomarkers as well as evaluation of cell morphology, growth kinetics, clonogenic capacity and chemo-sensitivity were performed.

Materials and methods

Cell culture

Pediatric medulloblastoma cell lines UW402 and UW473 were kindly provided by Dr Michael S. Bobola (Department of Neurological Surgery, University of Washington, Seattle, WA, USA). Cells were cultured in HAM-F10 medium supplemented with 10 % fetal bovine serum (FBS, Gibco BRL, Life Technologies, Carlsbad, CA, USA), penicillin (100 U/mL) and streptomycin (100 µg/mL) at 37 °C in a humidified atmosphere of 5 % CO₂ incubator. For maintenance purposes, cell lines were passaged once or twice a week using 0.05 % trypsin in 0.02 % EDTA. The medium was changed twice a week under all culture conditions. Culture medium, antibiotics, trypsin, and EDTA were purchased from Sigma (Sigma-Aldrich, São Paulo, Brazil).

Chromosomal analysis

Metaphase cells were harvested from exponentially growing cultures and mitotic arrest was performed by addition of 25 µg/mL of colchicine for 10–14 h at 37 °C. After, cells were detached, exposed to hypotonic potassium chloride solution (0.075 M) for 15–30 min and fixed with three washes of Carnoy's fixative (3:1; methanol : glacial acetic acid). Metaphase spreads were prepared and artificially aged overnight at room temperature. The chromosomes were G-banded (GTG), stained with Giemsa and analyzed by following routine cytogenetic procedures (Brassescio et al. 2009). Twenty-one metaphases were karyotyped and the CCAs and NCCAs were described according to the International System for Human Cytogenetic Nomenclature ISCN 2005 (Shaffer and Tommerup 2005). For objective of our study, we defined the chromosomal complexity based on three different factors: ploidy, numerical abnormalities and structural rearrangements.

Cytokinesis-block micronucleus cytome (CBMN-Cyt) assay

To determine the proliferative status and quantify the CIN phenotype of each MB cell line, we performed the CBMN-Cyt assay using Cytochalasin-B (Cyt-B; Sigma-Aldrich, São Paulo, Brazil), an inhibitor of mitotic spindle that prevents cytokinesis, according to

Fenech's protocol (Fenech 2007) with some modifications. Briefly, 2.5×10^5 cells were cultured in 25 cm² tissue culture flasks and, after 20 h, fresh medium containing 5 µg/mL Cyt-B (1 mg/mL) was added. After incubation for 28 h at 37 °C, the cells were detached, centrifuged at 1,000 rpm for 5 min, washed once with PBS 1X and fixed with (5:1; methanol: glacial acetic acid) plus 3.7 % formaldehyde in PBS 1X. After centrifugation, cells were subsequently fixed with three washes of Carnoy's fixative (3:1; methanol: glacial acetic acid). Cell spreads were prepared and stained with Giemsa. Cyt-B-treated cells have bi-nucleated appearance, and depending on growth kinetics and division profile, they also can show tri- or tetra-nucleated appearance. The frequency of cells with one, two, three or four nuclei was used to determine the proliferative status by nuclear division index (NDI) using the formula: $NDI = (M_1 + 2M_2 + 3M_3 + 4M_4)/N$, where M_1 – M_4 represent the number of cells with 1–4 nuclei and N is the number of viable cells. To determine the CIN status, it was measured the frequency of micronuclei (MNi), nucleoplasmic bridges (NPBs) and nuclear buds (NBUDs) exclusively in cytokinesis-blocked binucleated cells (BN), according to Fenech's protocol. MNi are defined as chromosome fragments or whole chromosomes that lag behind at anaphase during nuclear division, NPBs are biomarkers of dicentric chromosomes resulting from telomere end-fusions or DNA misrepair, and NBUDs represent a biomarker of gene amplification and altered gene dosage events. MNi, NPBs, and NBUDs are observed in binucleated cells as smaller nuclei, continuous DNA-containing structure linking the nuclei in a binucleated cell, and micronucleus-like bodies attached to the nucleus, respectively (For more detail, see review Fenech 2007). For each experiment, two different scorers using identical microscopes counted 2,000 cells. Three independent experiments were performed in duplicate.

Morphological characterization, growth kinetics, cell population doubling time and colony forming efficiency

Sub-confluent cultures of each cell line were washed with PBS 1X, fixed with methanol and stained with Giemsa. The cells morphological features were determined by direct observation under inverted microscope and in photomicrographs. To determine the growth

kinetics and doubling time, exponentially growing cells were seeded in 24-well plates at a density of 2×10^4 and 6×10^4 cells per well. Cell counts were performed in 24-h intervals for 8 days and cell population doubling time (DT) was estimated from the linear phase of the growth curve previously obtained. This value represents the period of time required for a cell population double its size. The colony forming efficiency (CFE) was obtained performing the protocol of Franken et al. (2006) with some modifications. Briefly, 300 cells per well were seeded in six wells plates and incubated for 9 days, then the cells were washed once with PBS 1X, fixed with methanol, and stained with Giemsa. The colonies containing more than 50 cells were counted and the CFE was calculated by the $\text{Ratio} = (\text{number of colonies formed} / \text{number of cells initially seeded}) \times 100 \%$. All assays were performed independently for at least three times in triplicate.

Cell cycle analysis

To calculate the cell cycle distribution of UW402 and UW473 cells, 1.5×10^5 cells were seeded in 25 cm² tissue culture flasks and cultured for 48 h. Then the cells were detached, suspended in PBS 1× and stained with propidium iodide (PI) using the CycleTEST™ PLUS DNA reagent kit (Becton–Dickinson Immunocytometry Systems, San Jose, CA, USA). The DNA content of the stained cells was immediately analyzed using a FACSCalibur (Becton–Dickinson, Franklin Lakes, NJ, USA). At least 10,000 events were acquired and the percentages of cells in G0/G1, S and G2/M phases were calculated using ModFit LT software (Verity Software House, Topsham, ME, USA). Values represent the mean $\pm$ standard deviation (SD) of three independent experiments performed in triplicate.

Drugs and chemo-sensitivity assay

Cisplatin powder (CDDP) was dissolved in saline solution and sonicated for 1 h, according to Fischer et al. (2008). Doxorubicin (DX), Methotrexate (MTX), and Temozolomide (TMZ) were diluted in dimethylsulfoxide (DMSO; Mallinckrodt Chemical Works, St. Louis, MO, USA). After preparation, all stock solutions were kept at -20°C and dissolved in culture medium immediately before the experiments. All reagents were purchased from Sigma (Sigma-Aldrich, São Paulo, Brazil).

To investigate cell chemosensitivity toward MTX, DX, TMZ and CDDP, proliferation assays were conducted. Cells were seeded in 96-well plates at 2×10^3 cells per well and maintained in culture for 24 h. After this period, fresh medium containing 0–24 mmol/L CDDP, 0–5 mmol/L DX, 0–50 mmol/L MTX, or 0–2,000 mmol/L TMZ was added. After 72 h of treatment, the culture medium was exchanged for a culture medium containing XTT dye (3 mg/mL) per well (XTT II; Roche Molecular Biochemicals, Indianapolis, IN, USA). The plates were incubated for 2 h at 37°C and formazan product was measured at 450 nm/655 nm using an iMark microplate reader (Bio-Rad Laboratories, Hercules, CA, USA). Values represent the mean $\pm$ standard deviation (SD) of at least three independent experiments performed in triplicate. The IC₅₀ value was defined as the concentration required for a reduction of 50 % in the cell proliferation and was calculated using the Calcsyn software (Biosoft, Ferguson, MO, USA).

Statistical analysis

To assess differences between means, statistical analyses was carried out using Student's *t* test (paired) or analysis of variance (ANOVA), followed by the Bonferroni-Dunn post hoc test. A value of $p < 0.05$ was considered statistically significant.

Results

UW473 cells show to have more karyotypic heterogeneity than UW402 cells

Based on ploidy, numerical abnormalities and structural rearrangements, it was observed that UW402 cell line have a clonal population cytogenetically well established, composed of hyperdiploid karyotypes with modal number 55 (50–57) (Fig. 1a), while UW473 cell line presented two clonal populations well distinguished by ploidy level, a group with hyperdiploid modal number 54 (52–58) (Fig. 1b) and another group with near-tetraploid modal number 95 (88–104) (Fig. 1c).

For UW402, the main numerical abnormalities were trisomy of chromosomes 9, 19 and 22; while for UW473 were +1, +5, and –15 (Table 1).

Fig. 1 Karyotypic analysis of MB cell lines. **a** G-banded karyotype of the hyperdiploid MB cell line UW402, showing both complex structural and numerical changes. **b–c** Representative karyotypes of the MB cell line UW473 showing **b** hyperdiploid and **c** near-tetraploid karyotypic populations. *Arrows* point to main chromosomal alterations. *M* marker chromosome

Both cell lines presented many clonal structural chromosomal abnormalities. All UW402 cells analyzed showed four common structural aberrations: +del(1)(p34), t(1;?)(q43;?), add(7)(p21) and del(7)(q22;q31). Isochromosome 17q was observed in 86 % of the cells, as well as four chromosome markers (mar 1–4) in all analyzed cell. On the other hand, the most common structural rearrangements in UW473 cells were: del(4)(p13), +del(7)(q22q32), der(7)t(7;?;?)(q22;q32;?), and i(13)(q10). Additionally, we found six chromosome markers (mar 1–6) and *double minutes* (dm) in 53 % of the analyzed UW473 cells (Table 1).

Considering numerical and structural alterations, we identified more CCAs in UW473 than in UW402 cells (26 vs. 14). Among the structural CCAs, we identified rearrangements mainly on the chromosomes 1 and 7 in both cell lines. Deletions of chromosome 1 were found in 100 % of UW402 and in 23 % of UW473 cells, while structural rearrangements in chromosome 7 were observed in 100 % of the analyzed cells for both cell lines. We also documented several non-clonal chromosomal alterations, mainly in UW473 cell line (23 vs. 12). Considering the origin of these NCCAs, they were mainly numerical in both cell lines (100 % for UW402 and 87 % for UW473) (Table 2).

Therefore, we consider that UW402 cell line has a chromosomal composition relatively more stable than UW473 due to the presence of four types of CCAs in all analyzed cells and the presence of fewer NCCAs compared to UW473 cells. Moreover, UW473 has a notable karyotypic complexity observed at different levels (ploidy, numerical and structural).

Evidence and levels of MNi, NPBs and NBUDs indicate a dominant CIN phenotype in MB cells

We choose the CBMN-Cyt assay to validate the proliferative status and quantify CIN phenotype of

both cell lines. We found significant differences between the numbers of mono-, bi-, tri- and tetra-nucleated cells in both cell lines and, consequently, significant differences between NDI values (Fig. 2a). As result, we noticed that UW402 cells have a proliferative potential significantly higher than UW473 cells ($NDI = 2.02 \pm 0.13$ vs. 1.74 ± 0.06 , respectively; $p < 0.001$). We also identified the presence of all CIN biomarkers in both MB cell lines: MNi, NPBs and NBUDs. The MNi was the most common biomarker and it was observed in 11.58 and 11.87 % of UW402 and UW473 BN cells, respectively (Figs. 2b, 3). Besides that, most MB cells showed only one MN, but others also presented two, three, four or five MNi (Fig. 3). Consequently, the total number of MNi identified in 4000 cells evaluated for each cell line was 159 ± 26.96 and 153.7 ± 30.7 for UW402 and UW473, respectively. On the other hand, we found that UW473 has a significantly higher number of NPBs (36 ± 7.12 vs. 23.5 ± 4.24 ; $p = 0.037$) and NBUDs (61.8 ± 7.14 vs. 29.25 ± 17.01 ; $p = 0.013$) when compared to UW402 cell line, respectively (Figs. 2b, 3).

Despite the MNi, NPBs and NBUDs found in mono-, bi-, tri- or tetra-nucleated cells, we also identified some aberrant mitotic figures in both cell lines (Fig. 3). However, these observations were not included in the instability analysis because of our criteria of inclusion.

MB cell lines are morphologically heterogeneous

The two cell lines are adherent and grow in monolayers with no contact inhibition at high cell density, as described previously (Keles et al. 1995). Microscopically, both cell lines exhibited morphological heterogeneity, composed predominantly by populations with small rounded cells, spindle-shaped or with

Table 1 Karyotypic nomenclature for medulloblastoma cell lines

Cell line	Karyotype nomenclature
UW402	50–57, XX, +del(1)(p34) [21], t(1;?)(q43;?) [21], add(7)(p21) [21], del(7)(q22;q31) [21], +9[16], –11 [3], t(11;?)(q14;?) [18], der(15)t(15;?)(q10;?) [3], +i(17)(q10)[18], +i(17)(q10) (×2) [2] +19 [12], +19(×2)[4], +20 [15], +22 [2], +mar 1–4
UW473	52–58 <2n>, X, –Y [10], +1[4], +del(1)(p21)[5], del(4)(p13) [9], +5[10], +6[6], +7 [2], +del(7)(q22q32)[10], +der(7)t(7;?;?)(q22;q32;?) [10], +8 [2], +del(8)(q21)[7], –9 [4], –10 [9], +11 [3], +12 [2], –12 [3], –13[5], i(13)(q10) [10], –15[10], +16 [2], –16[3], –17(2X) [3], –18 [7], +i(18)(q10)[2], –19[5], +21(X2) [7], +mar1–6, +1–4 dm [cp11]/88–103 <4n>, XX, idem (X2), +22 [4] [cp10]

stellate morphology. The UW402 cells presented two populations: one spindle-shaped population with nervous long extensions and neural connections and, a second group of large pyramidal multinucleated cells with several nucleoli (1–3) (Fig. 4a). The UW473 cells were morphologically more complex than UW402 and presented heterogeneous groups of elongated smooth cells with round nuclei containing 1–3 nucleoli. Moreover, some cell populations with anaplastic phenotype and an important cell population characterized by pyramidal shape and evident cytoplasmic extensions, typical of mono- or multi-nucleated nerve cells, were also identified in the UW473 cell line (Fig. 4b).

MB cells have different proliferation profiles, clonogenic capacity and cell cycle

To evaluate the growth kinetic of MB cells, the doubling time (DT) of each cell line was determined. DT_{UW402} was significantly lower than DT_{UW473} , which means that UW402 cells have a higher proliferative capacity and need less time to duplicate their population than UW473 cells regardless of initial plating concentration (2×10^4 or 6×10^4 cells), observed in the (Table 3; Fig. 4c, d).

When the clonogenic capacity was investigated, the CFE was significantly and dramatically different between both cell lines, with UW473 having almost 3 fold higher CFE (UW473 33 % vs. UW402 12 %), as shown in Table 3.

Analyzing the distribution of cell cycle, we determined that the highest cell population was in the G1-G0 phase for both cell lines. Statistical differences were found in this cell cycle phase between UW473 (59.15 %) and UW402 (52.07 %) ($p = 0.014$). Moreover, the proportion of cells in S phase was significantly higher in UW402 than in UW473 cell line (40 vs. 30 %, respectively, $p = 0.038$). No statistical difference was found between cell populations in G2-M phases in both cell lines (Table 3; Fig. 4e, f).

UW473 cells are more sensitivity to TMZ, CDDP and MTX, except to DX

The proliferation assay at 72 h was used to determinate and compare the IC_{50} values of both cell lines for TMZ, CDDP, MTX and DX. We found differences between cell lines response of both cell lines to these drugs. The

IC_{50} values were significantly lower for UW473 than for UW402 cells in the treatments with TMZ ($600.0 \pm 24.4 \mu\text{M}$ vs. $2088.4 \pm 503.7 \mu\text{M}$; $p < 0.01$), CDDP ($2.3 \pm 0.06 \mu\text{M}$ vs. $3.6 \pm 0.08 \mu\text{M}$; $p < 0.05$) and MTX ($0.0012 \pm 0.001 \mu\text{M}$ vs. $2.03 \pm 0.4 \mu\text{M}$; $p < 0.001$). The opposite happened for DX ($0.436 \pm 0.46 \mu\text{M}$ vs. $0.043 \pm 0.04 \mu\text{M}$, $p < 0.05$) (Fig. 5), which indicate that UW473 cell line is more sensitive than UW402 towards TMZ, CDDP and MTX, except to DX treatment.

Discussion

Tumor heterogeneity is a hallmark of solid tumors and it has been described from different perspectives, including cell morphology variations, tumor cell kinetics, metabolism, gene profile, chemo-sensitivity, metastatic potential, ploidy level, immunophenotype, DNA content, and cancer stem cell populations (Könemann et al. 2000; Hanahan and Weinberg 2011; Binda et al. 2012). As many solid tumors, MB is a heterogeneous tumor and its distinct heterogeneity at cellular level has been associated with substantial differences in its biological behavior (Huse and Holland, 2010).

Chromosomal heterogeneity is a product of a dynamic relationship between NCCAs and CCAs within an evolutionary context, which can also explain why some tumor cell lines display relatively stable abnormal karyotypes, while others have a greater degree of variation (Heng et al. 2006a, b). Considering these concepts, we demonstrated that both MB cell lines evaluated in this study presented tumor heterogeneity at different levels.

Morphologically, both cell lines were highly heterogeneous. However, UW473 exhibited more cellular heterogeneity than UW402. This observation was the first trace of heterogeneity between the cell lines. Considering the principle where intra-tumor morphological differences are a result of functional and genetic differences among cell subpopulations within a tumor, we investigated other functional key features to better understand their relationship.

At the chromosomal level, many authors have described that two well established chromosomal profiles may be generated by the karyotypic evolution process: the first one with a high frequency of CCAs, a pattern which provides growth advantage, and the

Table 2 Chromosome presentation according to frequency of involvement in all analyzed cells

Chromosome ^a	UW402	UW473
1	+del(1)(p34) [21] t(1;?)(q43;?) [21]	+1 [7] +del(1)(p21) [5] -1 [2] i(1)(q10) [1]
2	-2 [1]	+2 [1] -2 [2]
3		-3 [2] del(3)(p21) [1]
4	+4 [1]	del(4)(p13) [19] +4 [1]
5	-5 [1]	+5 [10] +5(2X) [1]
6	-6 [1]	+6 [9] -6 [1]
7	add(7)(p21) [21] del(7)(q22;q31) [21]	+del(7)(q22q32) [21] +der(7)t(7;?;?)(q22;q32;?) [21] +7 [2] -7 [1]
8	-8 [1]	+del(8)(q21)(2x) [14] +8[2] +8 (2X) [1] -8 [1]
9	+9 [16]	-9[4] +9[1]
10	-10 [1]	-10[9] -10 (2X) [1]
11	t(11;?)(q14;?), [18] -11 [3]	+11[3] -11[2] -11 (2X) [1]
12		-12 [3] +12 [2]
13		i(13)(q10) [18] -13 [8] +i(13)(q10) [1] +13 [1]
14		-14 [2]
15	der(15)t(15;?)(q10;?) [3] -15 [1]	-15 [20]
16	+16 [1]	-16 [5] +16 [2]

Table 2 continued

Chromosome ^a	UW402	UW473
17	+i(17)(q10) [18] (2X) +i(17)(q10) [2]	-17(2X) [3] -17 [1]
18	-18 [1]	-18 [12] +i(18)(q10) [3]
19	+19 [12] +19 (2X) [4]	-19 [9]
20	+20 [15]	+20 [1] -20 [1]
21	+21 [1] -21 [1]	+21(X2) [11]
22	+22 [2] -22 [1]	+22 [4] -22 [2]

^a Sexual chromosomes were excluded of analysis criteria

second one characterized by a high frequency of NCCAs, a pattern which provides survival advantage (Albertson et al. 2003; Heng et al. 2006c; Bayani et al. 2007; Ye et al. 2007). Comparison of DT and NDI values showed that UW402 cells have higher proliferative capacity than UW473 cells ($DT_{UW402} < DT_{UW473}$ and $NDI_{UW402} > NDI_{UW473}$), on the other hand, UW402 had significantly less capacity to form colonies than UW473. After cytogenetic investigation, we considered that MB cell lines fitted to the previous concept due their distinct chromosomal status. Thus, UW402 cells presented a more stable chromosomal status associated with a higher proliferative capacity (growth advantage) than UW473, whereas UW473 was chromosomally less stable and demonstrated higher ability to form colonies (survival advantage).

Some CCAs have also been proposed as independent prognostic markers and determinants of new MB subtypes (Lamont et al. 2004; Gilbertson and Ellison 2008; McCabe et al. 2011). Here, we identified many numerical and structural alterations in both cell lines evaluated, mainly chromosomal gain and structural rearrangement of chromosome 1 and 7. Likewise, cytogenetic studies performed in other MB cell lines and short-time cultures also have reported a high frequency of structural rearrangements on chromosomes 1, 7 and 17 (Bayani et al. 2000; Aldosari et al. 2002).

Our study identified also the presence of isochromosome 17q only in UW402 cells. This chromosomal marker is by far, the most consistent cytogenetic

Fig. 2 Determination of proliferative status and chromosomal instability phenotype of MB cells by CBMN-Cyt assay. **a** Frequencies of mono-, bi-, tri-, tetra-nucleated and apoptotic MB cells (per 500 cells scored). **b** Frequencies of binucleated MB cells with at least an MN, NPB, or NBUD (per 1,000 cells scored). Data represent mean $\pm$ SD of three independent experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. MN micro-nucleus, NPB nucleoplasmic bridge, NBUD nuclear bud

observation in MB, being observed in approximately one-third of MBs and considered as negative prognostic factor (Biegel 1999; Pan et al. 2005). According to Bayani et al. (2000), the combination of 17q gain and 17p loss, as consequence of i(17q) formation, may provide a selective growth advantage to MBs, leading to tumor progression. These data could also explain the higher proliferative capacity and growth advantages observed in UW402 when compared to UW473 cells.

Moreover, we correlated the cytogenetic findings of both cell lines to chromosomal profiles described for each MB group of new molecular classification in the meta-analysis paper by Kool et al. (2012). Interestingly, this classification system differentiates among two subgroups well characterized in their molecular

pathways with few common chromosomal alterations (Group Wnt and Group Shh), and two subgroups molecularly less well characterized harboring a high number of chromosomal alterations (Group 3 and Group 4) (Kool et al. 2012). Group 3 and Group 4 are characterized by high prevalence of mechanism of genomic structural variation, including somatic copy number aberrations, chromothripsis, tetraploidy, and high genomic instability (Jones et al. 2012; Northcott et al. 2012), all these mechanisms are related to high cytogenetic heterogeneity. Additionally, survival analyses showed a clear and significant difference in overall survival between the four molecular subgroups, and of particular interest, the worst outcome was seen for patients within the Group 3 and/or Group 4 tumors, reinforcing the necessity of more studies and to obtain more representative models, mainly of these MB groups.

In our investigation, many chromosomal alterations found in both cell lines are common between Group 3 and Group 4, as for instance, the gain of chromosomes 1 and 7. However, certain rearrangements appear to be most prevalent in a determined group, or could be exclusive of a specific cell type. The chromosomal alterations del(4p), +5, -10, +13, -15, -16, and +18, found in the UW473 cell line, have been described as most prevalent in Group 3 tumors, suggesting us that this cell line could be representative of Group 3 tumors. Furthermore, the concomitant alterations -17p and +17q, both represented by isochromosome 17q and found exclusively in the UW402 cell line, are mainly found in the Group 3 (42 and 62 %, respectively) and Group 4 (63 and 73 %, respectively). These findings guide us to suggest that UW402 could be cytogenetically representative of either Groups 3 as well as Group 4. Therefore, these MB cell lines gain methodological relevance, not only by their distinct cytogenetic heterogeneity, but also by the possibility of using them as models of MB tumors of worst outcome, such as Group 3 or 4 tumors.

Besides the clonal alterations observed, we also documented many NCCAs, mainly in UW473 cells. This high chromosomal heterogeneity found in UW473 cell line at karyotypic level, ploidy level and the well-differentiated clonal groups ($<2n>$ and $<4n>$) suggests a polyploidization origin of this cell line, followed by acquisition of numerical and structural changes, a common mechanism that lead to intratumoral cytogenetic heterogeneity and CIN in

Fig. 3 Dominant presence of instability biomarkers in MB cells identified by CBMN-Cyt assay. **a, f** Binucleated cells presenting one MN, the main instability biomarker found in both MB cell lines. **b, g** Binucleated cells containing many NPBs. **c, h** Presence of NBUDs indicating extrusion of amplified DNA and/or DNA repair complex. **d, i** Simultaneous presence of MNi, NPBs, and NBUDs indicating a dominant chromosomal instability phenotype in a trinucleated UW402 cell and a

binucleated UW473 cell. **e, j** Aberrant mitotic figures with anaphase bridges are identified in an apparent tetranucleated UW402 cell and a multipolar mitosis of UW473. In addition to anaphase bridges, lagging chromosomes are shown in the panels **e** and **j**. In all cases an $\times 100$ oil objective and Giemsa stain were used. *CBMN-Cyt* cytokinesis-block micronucleus cytome assay, *MNi* micronuclei, *NPBs* nucleoplasmic bridges, *NBUDs* nuclear buds

MB cells, as described by Cohen et al. (2004). Moreover, Roschke et al. (2003), analyzing 59 cancer cell lines, have found association between structural complexity of karyotype and modal chromosome number, which can be strongly driven by CIN (Lee et al. 2011). In our study, we found that both MB cell lines showed a significant number of NCCAs and a high chromosomal heterogeneity. However, this observation was noteworthy for UW473 cells, which demonstrates its relationship with high intrinsic CIN levels.

Aneuploidy and CIN contribute for the chromosomal heterogeneity in tumor cells (Sheltzer and Amon 2011; Gordon et al. 2012). Here, we found not only highly complex aneuploidy, but also CIN phenotype investigated by CBMN-Cyt assay. In our study, the marked presence of MNi and NBUDs without any stimulus demonstrates evident traces of mitotic errors and reinforces the CIN phenotype in both cell lines evaluated. The MNi are the result of lagging or broken chromosomes (double strand breaks), chromatid cohesion defects, telomere fusions or fragmentation of anaphase bridges (NBUDs) (Shimizu et al. 2005; Fenech 2007). Although we did not find differences in MNi quantity between the cell lines, we found that UW473 showed significantly more NPBs and BUDs than UW402 cells, important biomarkers of a chromosomally unstable phenotype (Gisselsson et al. 2000; Gisselsson 2008).

It has been suggested that adverse outcome associated with CIN may be related to high tumor cell

heterogeneity driving the ability of tumors to adapt to environmental stress, including drug treatment (Chandhok and Pellman 2009; Nicholson and Duesberg 2009). Here we found different degrees of sensitivity to chemotherapeutic drugs between MB cell lines. UW473 cells were relatively more sensitive to CDDP, MTX, and TMZ treatments and more resistant to DX than UW402 cell line. Although it has been largely described that CIN confers intrinsic multidrug resistance in tumors (Sheltzer and Amon 2011; Lee et al. 2011) and consequently poorer prognosis in cancer patients (Carter et al. 2006), in our study, UW473 showed a greater CIN phenotype and it was more chemosensitivity than UW402, which could confirm the hypothesis that excessive genomic instability may surpass a threshold compatible with cell viability (Cahill et al. 1999). This concept has been recently corroborated in the clinical context through studies reporting a paradoxical relationship between CIN and survival outcome in cancer, being that tumors exhibiting extreme CIN displayed improved prognosis relative to tumors with intermediate levels of CIN (Birkbak et al. 2011; Roylance et al. 2011; McGranahan et al. 2012; Lee and Swanton 2012).

These results are partially explained by evidence that aneuploidy in eukaryotic cell systems negatively impacts upon cell biological fitness (Torres et al. 2007; Williams et al. 2008) and excessive CIN may induce autonomous cell lethality (Kops et al. 2004; Janssen

Fig. 4 Cell morphology, proliferation and cell cycle distribution of MB cells. **a** UW402 showed a *spindle-shaped* population with long nervous extensions. **b** UW473 presented an heterogeneous morphology with groups of anaplastic and elongated smooth cells. Both *cells lines* showed a population characterized

by multinucleated *pyramidal shape* cells. An $\times 100$ oil objective and Giemsa stain were used. **c, d** Cell growth curves. **e, f** *Histograms* showing the percentages of cells in G1, S or G2/M phases

et al. 2009; Birkbak et al. 2011). Therefore, it is possible that even in an in vitro environment, as investigated in our study, chromosomally unstable cancer cells could favorably respond to certain

chemotherapeutic drugs. We consider that these interesting results need to be viewed with caution due to the fact that only two MB cell lines were investigated and in some situations the cell response in

Table 3 Doubling time, cell cycle distribution and colony-forming efficiency of MB cell lines

Cell line	Doubling time ^a (h)		Cell cycle distribution (%)			Colony-forming efficiency (%)
	2×10^4	6×10^4	G1-G0	S	G2-M	
UW402	$45.52 \pm 3.2^*$	$72.03 \pm 5.15^*$	$52.07 \pm 1.8^*$	$40.25 \pm 1.52^*$	7.67 ± 0.57	$11.77 \pm 2.2^*$
UW473	57.17 ± 5.3	$115 \times 51 \pm 7.4$	59.15 ± 3.04	30.21 ± 0.86	9.61 ± 1.4	32.72 ± 1.45

^a To calculate doubling time, growth curves were obtained from two plating cell concentrations: 2×10^4 and 6×10^4 cells as indicated. * $p < 0.05$ compared with UW473

Fig. 5 Chemosensitivity differences between both MB cell lines. Cell lines were exposed to increasing concentrations of TMZ, CDDP, MTX and DX for 72 h and the proliferation was determined by XTT assay. The IC₅₀ value was defined as concentration required by each drug for a reduction of 50 % in the cell proliferation. Data represent mean $\pm$ SD of three independent experiments. * $p < 0.05$; ** $p < 0.01$; *** $p < 0.001$. TMZ temozolomide, CDDP cisplatin, MTX methotrexate, DOX doxorubicin

culture might not always mirror the clinical situation. Nevertheless, further investigation to determinate how CIN could provide no growth advantage and even be deleterious for cancer cell survival through an evolutionary scenario similar to “mutational meltdown” in bacteria (Lynch et al. 1993) or “error catastrophe” in viruses (Eigen 2002), is needed not only in MB cells, but also in many other cancer cell types. Additionally, we believe that understanding the mechanisms underlying CIN could provide insight into the cellular consequences and will reveal the possibility to explore it in MB therapy or other cancers.

In summary, these data display how the cells could be affected by their cytogenetics status and how this status could influence tumor biology. These findings reinforce the importance of the cytogenetic field in the investigation of tumor heterogeneity and cancer biology, including MB. Moreover, it enhances the

relevance of CIN phenotype as survival predictor and as an attractive therapeutic target in clinical and pre-clinical contexts along with appropriated MB in vitro models.

Acknowledgments The authors thank Patrícia Vianna Bonini Palma, Camila Cristina de Oliveira Menezes Bonaldo, and Daiane Fernanda dos Santos, Hemocentro-FMRP-USP, Ribeirão Preto, Brazil, Brazil, for assistance with the flow cytometry. The authors also thank Coordenação para o Aperfeiçoamento de Pessoal em Nível Superior-Brazil (CAPES) and Fundação de Apoio ao Ensino, Pesquisa e Assistência do Hospital das Clínicas da FMRPUSP (FAEPA) for financial support.

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

References

- Albertson DG, Collins C, McCormick F, Gray JW (2003) Chromosome aberrations in solid tumors. *Nat Genet* 34: 369–376
- Aldosari N, Wiltshire RN, Dutra A, Schrock E, McLendon RE, Friedman HS, Bigner DD, Bigner SH (2002) Comprehensive molecular cytogenetic investigation of chromosomal abnormalities in human medulloblastoma cell lines and xenograft. *Neuro Oncol* 4:75–85
- Batra SK, McLendon RE, Koo JS, Castelino-Prabhu S, Fuchs HE, Krischer JP, Friedman HS, Bigner DD, Bigner SH (1995) Prognostic implications of chromosome 17p deletions in human medulloblastomas. *J Neurooncol* 24:39–45
- Bayani J, Zielenska M, Marrano P, Kwan Y, Taylor MD, Jay V, Rutka JT, Squire JA (2000) Molecular cytogenetic analysis of medulloblastomas and supratentorial primitive neuroectodermal tumors by using conventional banding, comparative genomic hybridization, and spectral karyotyping. *J Neurosurg* 93:437–448
- Bayani J, Selvarajah S, Maire G, Vukovic B, Al-Romaih K, Zielenska M, Squire JA (2007) Genomic mechanisms and measurement of structural and numerical instability in cancer cells. *Semin Cancer Biol* 17:5–18
- Biegel JA (1999) Cytogenetics and molecular genetics of childhood brain tumors. *Neuro-oncol* 1:139–151
- Binda E, Visioli A, Reynolds B, Vescovi AL (2012) Heterogeneity of cancer-Initiating cells within glioblastoma. *Front Biosci (Schol Ed)* 4:1235–1248

- Birkbak NJ, Eklund AC, Li Q, McClelland SE, Endesfelder D, Tan P, Tan IB, Richardson AL, Szallasi Z, Swanton C (2011) Paradoxical relationship between chromosomal instability and survival outcome in cancer. *Cancer Res* 71:3447–3452
- Brascesco MS, Castro-Gamero AM, Valera ET, Neder L, Elias J Jr, Tone LG (2009) 3q27 aberrations in a childhood ovary teratoma with associated malignant germ cell component. *Pediatr Blood Cancer* 52:398–401
- Cahill DP, Kinzler KW, Vogelstein B, Lengauer C (1999) Genetic instability and darwinian selection in tumours. *Trends Cell Biol* 9:M57–M60
- Carter SL, Eklund AC, Kohane IS, Harris LN, Szallasi Z (2006) A signature of chromosomal instability inferred from gene expression profiles predicts clinical outcome in multiple human cancers. *Nat Genet* 38:1043–1048
- Chandhok NS, Pellman D (2009) A little CIN may cost a lot: revisiting aneuploidy and cancer. *Current Opin Genet Dev* 19:74–81
- Cho YJ, Tsherniak A, Tamayo P, Santagata S, Ligon A, Greulich H, Berhoukim R, Amani V, Goumnerova L, Eberhart CG, Lau CC, Olson JM, Gilbertson RJ, Gajjar A, Delattre O, Kool M, Ligon K, Meyerson M, Mesirov JP, Pomeroy SL (2011) Integrative genomic analysis of medulloblastoma identifies a molecular subgroup that drives poor clinical outcome. *J Clin Oncol* 29:1424–1430
- Cohen N, Betts DR, Tavori U, Toren A, Ram T, Constantini S, Grotzer MA, Amariglio N, Rechavi G, Trakhtenbrot L (2004) Karyotypic evolution pathways in medulloblastoma/primitive neuroectodermal tumor determined with a combination of spectral karyotyping, G-banding, and fluorescence in situ hybridization. *Cancer Genet Cytogenet* 149:44–52
- Eigen M (2002) Error catastrophe and antiviral strategy. *Proc Natl Acad Sci USA* 99:13374–13376
- Fenech M (2007) Cytokinesis-block micronucleus cytome assay. *Nat Protoc* 2:1084–1104
- Finlay JL, Erdreich-Epstein A, Packer RJ (2007) Progress in the treatment of childhood brain tumors: no room for complacency. *Pediatr Hematol Oncol* 24:79–84
- Fischer SJ, Benson LM, Fauq A, Naylor S, Windebank AJ (2008) Cisplatin and dimethyl sulfoxide react to form an adducted compound with reduced cytotoxicity and neurotoxicity. *Neurotoxicology* 29:444–452
- Franken NA, Rodermond HM, Stap J, Haveman J, van Bree C (2006) Clonogenic assay of cells in vitro. *Nat Protoc* 1:2315–2319
- Gilbertson RJ, Ellison DW (2008) The origins of medulloblastoma subtypes. *Annu Rev Pathol* 3:341–365
- Gisselsson D (2008) Classification of chromosome segregation errors in cancer. *Chromosoma* 117:511–519
- Gisselsson D, Pettersson L, Hoglund M, Heidenblad M, Gorunova L, Wiegant J, Mertens F, Dal Cin P, Mitelman F, Mandahl N (2000) Chromosomal breakage-fusion-bridge events cause genetic intratumor heterogeneity. *Proc Natl Acad Sci USA* 97:5357–5362
- Glanz C, Rebetz J, Stewenius Y, Persson A, Englund E, Mandahl N, Mertens F, Salford LG, Widegren B, Fan X, Gisselsson D (2007) Genetic intratumour heterogeneity in high-grade brain tumours is associated with telomere-dependent mitotic instability. *Neuropathol Appl Neurobiol* 33:440–454
- Gordon DJ, Resio B, Pellman D (2012) Causes and consequences of aneuploidy in cancer. *Nat Rev Genet* 13:189–203
- Hanahan D, Weinberg RA (2011) Hallmarks of cancer: the next generation. *Cell* 144:646–674
- Heim S, Mitelman F (1994) *Cancer cytogenetics*. Alan R. Liss, Inc., New York, pp 23–37
- Heng HH, Bremer SW, Stevens J, Ye KJ, Miller F, Liu G, Ye CJ (2006a) Cancer progression by non-clonal chromosome aberrations. *J Cell Biochem* 98:1424–1435
- Heng HH, Liu G, Bremer SW, Ye KJ, Stevens J, Ye CJ (2006b) Clonal and nonclonal chromosome aberrations and genome variation/aberration. *Genome* 49:195–204
- Heng HH, Stevens JB, Liu G, Bremer SW, Ye KJ, Reddy PV, Wu GS, Wang YA, Tainsky MA, Ye CJ (2006c) Stochastic cancer progression driven by non-clonal chromosome aberrations. *J Cell Physiol* 208:461–472
- Heng HH, Stevens J, Bremer SW, Ye KJ, Liu G, Ye CJ (2010) The evolutionary mechanism of cancer. *J Cell Biochem* 109:1072–1084
- Huse JT, Holland EC (2010) Targeting brain cancer: advances in the molecular pathology of malignant glioma and medulloblastoma. *Nat Rev Cancer* 10:319–331
- Janssen A, Kops GJ, Medema RH (2009) Elevating the frequency of chromosome mis-segregation as a strategy to kill tumor cells. *Proc Natl Acad Sci USA* 106:19108–19113
- fJones DT, Jäger N, Kool M, Zichner T, Hutter B, Sultan M, Cho YJ, Pugh TJ, Hovestadt V, Stütz AM, Rausch T, Warnatz HJ, Ryzhova M, Bender S, Sturm D, Pleier S, Cin H, Pfaff E, Sieber L, Wittmann A, Remke M, Witt H, Hutter S, Tzaridis T, Weischenfeldt J, Raeder B, Avci M, Amstislavskiy V, Zapatka M, Weber UD, Wang Q, Lasitschka B, Bartholomae CC, Schmidt M, von Kalle C, Ast V, Lawrence C, Eils J, Kabbe R, Benes V, van Sluis P, Koster J, Volckmann R, Shih D, Betts MJ, Russell RB, Coco S, Tonini GP, Schüller U, Hans V, Graf N, Kim YJ, Monoranu C, Roggendorf W, Unterberg A, Herold-Mende C, Milde T, Kulozik AE, von Deimling A, Witt O, Maass E, Rössler J, Ebinger M, Schuhmann MU, Frühwald MC, Hasselblatt M, Jabado N, Rutkowski S, von Bueren AO, Williamson D, Clifford SC, McCabe MG, Collins VP, Wolf S, Wiemann S, Lehrach H, Brors B, Scheurle W, Felsberg J, Reifenberger G, Northcott PA, Taylor MD, Meyerson M, Pomeroy SL, Yaspo ML, Korbel JO, Korshunov A, Eils R, Pfister SM, Lichter P (2012) Dissecting the genomic complexity underlying medulloblastoma. *Nature* 488:100–105
- Keles GE, Berger MS, Srinivasan J, Kolstoe DD, Bobola MS, Silber JR (1995) Establishment and characterization of four human medulloblastoma-derived cell lines. *Oncol Res* 7:493–503
- Könemann S, Schuck A, Malath J et al (2000) Cell heterogeneity and subpopulations in solid tumors characterized by simultaneous immunophenotyping and DNA content analysis. *Cytometry* 41:172–177
- Kool M, Koster J, Bunt J, Hasselt NE, Lakeman A, van Sluis P, Troost D, Meeteren NS, Caron HN, Cloos J, Mrcic A, Ylstra B, Grajkowska W, Hartmann W, Pietsch T, Ellison D, Clifford SC, Versteeg R (2008) Integrated genomics identifies five medulloblastoma subtypes with distinct genetic profiles, pathway signatures and clinicopathological features. *PLoS ONE* 3:e3088

- Kool M, Korshunov A, Remke M, Jones D, Schlanstein M, Northcott P, Cho Y, Schouten-van Meeteren N, van Vuurden D, Clifford S, Pietsch T, von Bueren A, Rutkowski S, McCabe M, Collins P, Bäcklund M, Haberler C, Bourdeaut F, Delattre O, Doz F, Ellison D, Gilbertson R, Pomeroy S, Taylor M, Lichter P, Pfister S (2012) 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* 123:473–484
- Kops GJ, Foltz DR, Cleveland DW (2004) Lethality to human cancer cells through massive chromosome loss by inhibition of the mitotic checkpoint. *Proc Natl Acad Sci USA* 101:8699–8704
- Lamont JM, McManamy CS, Pearson AD, Clifford SC, Ellison DW (2004) Combined histopathological and molecular cytogenetic stratification of medulloblastoma patients. *Clin Cancer Res* 10:5482–5493
- Lee AJX, Swanton C (2012) Tumour heterogeneity and drug resistance: personalising cancer medicine through functional genomics. *Biochem Pharmacol* 83:1013–1020
- Lee AJX, Endesfelder D, Rowan AJ, Walther A, Birkbak NJ, Futreal PA, Downward J, Szallasi Z, Tomlinson IPM, Kschischo M, Swanton C (2011) Chromosomal Instability Confers Intrinsic Multi-Drug Resistance. *Cancer Res* 71:1858–1870
- Lo KC, Ma C, Bundy BN, Pomeroy SL, Eberhart CG, Cowell JK (2007) Gain of 1q is a potential univariate negative prognostic marker for survival in medulloblastoma. *Clin Cancer Res* 13:7022–7028
- Louis DN, Ohgaki H, Wiestler OD, Cavenee WK (2007) WHO classification of tumors of the central nervous system. International Agency for Research on Cancer (IARC), Lyon
- Lynch M, Burger R, Butcher D, Gabriel W (1993) The mutational meltdown in asexual populations. *J Hered* 84:339–344
- McCabe MG, Bäcklund LM, Leong HS, Ichimura K, Collins VP (2011) Chromosome 17 alterations identify good-risk and poor-risk tumors independently of clinical factors in medulloblastoma. *Neuro Oncol* 13:376–383
- McGranahan N, Burrell RA, Endesfelder D, Novelli MR, Swanton C (2012) Cancer chromosomal instability: therapeutic and diagnostic challenges. *EMBO Rep* 13:528–538
- Mitelman F (2000) Recurrent chromosome aberrations in cancer. *Mutat Res* 462:247–253
- Nicholson JM, Duesberg P (2009) On the karyotypic origin and evolution of cancer cells. *Cancer Genet Cytogenet* 194:96–110
- Northcott PA, Korshunov A, Witt H, Hielscher T, Eberhart CG, Mack S, Bouffet E, Clifford SC, Hawkins CE, French P, Rutka JT, Pfister S, Taylor MD (2011) Medulloblastoma comprises four distinct molecular variants. *J Clin Oncol* 29:1408–1414
- Northcott PA, Jones DT, Kool M, Robinson GW, Gilbertson RJ, Cho YJ, Pomeroy SL, Korshunov A, Lichter P, Taylor MD, Pfister SM (2012) Medulloblastomics: the end of the beginning. *Nat Rev Cancer* 12:818–834
- Packer R, Vezina G (2008) Management of and prognosis with medulloblastoma: therapy at a crossroads. *Arch Neurol* 65:1419–1424
- Pan E, Pellarin M, Holmes E, Smirnov I, Misra A, Eberhart CG, Burger PC, Biegel JA, Feuerstein BG (2005) Isochromosome 17q is a negative prognostic factor in poor-risk childhood medulloblastoma patients. *Clin Cancer Res* 11:4733–4740
- Pfister S, Remke M, Benner A, Menderzyk F, Toedt G, Felsberg J, Wittmann A, Devens F, Gerber NU, Joos S, Kulozik A, Reifenberger G, Rutkowski S, Wiestler OD, Radlwimmer B, Scheurlen W, Lichter P, Korshunov A (2009) Outcome prediction in pediatric medulloblastoma based on DNA copy-number aberrations of chromosomes 6q and 17q and the MYC and MYCN loci. *J Clin Oncol* 27:1627–1636
- Pomeroy SL, Tamayo P, Gaasenbeek M, Sturla LM, Angelo M, McLaughlin ME, Kim JY, Goumnerova LC, Black PM, Lau C, Allen JC, Zagzag D, Olson JM, Curran T, Wetmore C, Biegel JA, Poggio T, Mukherjee S, Rifkin R, Califano A, Stolovitzky G, Louis DN, Mesirov JP, Lander ES, Golub TR (2002) Prediction of central nervous system embryonal tumour outcome based on gene expression. *Nature* 415:436–442
- Read TA, Hegedus B, Wechsler-Reya R, Gutmann DH (2006) The neurobiology of neurooncology. *Ann Neurol* 60:3–11
- Remke M, Hielscher T, Korshunov A, Northcott PA, Bender S, Kool M, Westermann F, Benner A, Cin H, Ryzhova M, Sturm D, Witt H, Haag D, Toedt G, Wittmann A, Schottler A, von Bueren AO, von Deimling A, Rutkowski S, Scheurlen W, Kulozik AE, Taylor MD, Lichter P, Pfister SM (2011a) FSTL5 is a marker of poor prognosis in non-WNT/Non-SHH medulloblastoma. *J Clin Oncol* 29:3852–3861
- Remke M, Hielscher T, Northcott PA, Witt H, Ryzhova M, Wittmann A, Benner A, von Deimling A, Scheurlen W, Perry A, Croul S, Kulozik AE, Lichter P, Taylor MD, Pfister SM, Korshunov A (2011b) Adult medulloblastoma comprises three major molecular variants. *J Clin Oncol* 29:2717–2723
- Roschke AV, Tonon G, Gehlhaus KS, McTyre N, Bussey KJ, Lababidi S, Scudiero DA, Weinstein JN, Kirsch IR (2003) Karyotypic complexity of the NCI-60 drug-screening panel. *Cancer Res* 63:8634–8647
- Roylance R, Endesfelder D, Gorman P, Burrell RA, Sander J, Tomlinson I, Hanby AM, Speirs V, Richardson AL, Birkbak NJ, Eklund AC, Downward J, Kschischo M, Szallasi Z, Swanton C (2011) Relationship of extreme chromosomal instability with longterm survival in a retrospective analysis of primary breast cancer. *Cancer Epidemiol Biomarkers Prev* 20:2183–2194
- Selvarajah S, Yoshimoto M, Park PC, Maire G, Paderova J, Bayani J, Lim G, Al-Romaih K, Squire JA, Zielenska M (2006) The breakage-fusion-bridge (BFB) cycle as a mechanism for generating genetic heterogeneity in osteosarcoma. *Chromosoma* 115:459–467
- Shaffer LG, Tommerup N (2005) An international system for human cytogenetic nomenclature (2005). Karger Publishers, Basel
- Sheltzer JM, Amon A (2011) The aneuploidy paradox: costs and benefits of an incorrect karyotype. *Trends Genet* 27:446–453
- Shimizu N, Shingaki K, Kaneko-Sasaguri Y, Hashizume T, Kanda T (2005) When, where and how the bridge breaks: anaphase bridge breakage plays a crucial role in gene amplification and HSR generation. *Exp Cell Res* 302:233–243

- Taylor MD, Northcott PA, Korshunov A, Remke M, Cho YJ, Clifford SC, Eberhart CG, Parsons W, Rutkowski S, Gajjar A, Ellison DW, Lichter P, Gilbertson RJ, Pomeroy SL, Pfister SL, Kool M (2012) Molecular subgroups of medulloblastoma: the current consensus. *Acta Neuropathol* 123:465–472
- Thompson MC, Fuller C, Hogg TL, Dalton J, Finkelstein D, Lau CC, Chintagumpala M, Adesina A, Ashley DM, Kellie SJ, Taylor MD, Curran T, Gajjar A, Gilbertson RJ (2006) Genomics identifies medulloblastoma subgroups that are enriched for specific genetic alterations. *J Clin Oncol* 24: 1924–1931
- Torres EM, Sokolsky T, Tucker CM, Chan LY, Boselli M, Dunham MJ, Amon A (2007) Effects of aneuploidy on cellular physiology and cell division in haploid yeast. *Science* 317:916–924
- Williams BR, Prabhu VR, Hunter KE, Glazier CM, Whittaker CA, Housman DE, Amon A (2008) Aneuploidy affects proliferation and spontaneous immortalization in mammalian cells. *Science* 322:703–709
- Ye CJ, Liu G, Bremer SW, Heng HH (2007) The dynamics of cancer chromosomes and genomes. *Cytogenet Genome Res* 118:237–246