Characterization of drug-resistant neuroblastoma cell lines by comparative genomic hybridization*

J. BEDRNICEK[1], A. VICHA[1], M. JAROSOVA[2], M. HOLZEROVA[2], J. CINATL, JR.[3], M. MICHAELIS[3], J. CINATL[1], T. ECKSCHLAGER[1**]

[1]*Department of Pediatric Hematology and Oncology, e-mail: eckschlagertomas@yahoo.com, 2nd Medical School, Charles University and University Hospital Motol, Prague, Czech Republic;* [2]*Department of Hematological Oncology, Medical School, Palacky University and University Hospital, Olomouc, Czech Republic;* [3]*Institute for Medical Virology, J.W. Goethe University, Frankfurt a Main, Germany*

Received January 24, 2005

Three parental neuroblastoma cell lines and nine derived lines resistant to Vincristin, Doxorubicin and Cisplatin, respectively, using CGH were studied. CGH profiles of all three parental cell lines were obtained using DNA from a healthy volunteer as reference DNA. Labeled DNA from each of the drug resistant daughter cell lines and labeled DNA from their parental sensitive cell lines were hybridized to obtain a comparison of gains and losses that accompanied the development of resistance for that particular drug. All three parental cell lines were characterized by typical findings for high risk neuroblastoma: *N-myc* amplification, gain of 17q, and loss of 1p36.2-36.3. Acquired drug resistance in the neuroblastoma cell lines appeared to be accompanied by a large array of DNA sequence copy number changes. The regions frequently affected in chemoresistant cell lines included gains of 13q14.1-32, and 7q11.2-31.3, 4q. Amplifications were seen at 7q 21.1 consistent with MDR1 amplification in UKF-NB-2 VCR, UKF-NB-3 DOXO, UKF-NB-4 VCR, and UKF-NB-4 DOXO, but not in any Cisplatin resistant line. All Cisplatin and Doxorubicin and two Vincristin resistant line (UKF-NB-2 VCR and UKF-NB-4 VCR) had a deletion of part of 19q or the whole 19 chromosome. All lines resistant to Vincristin or Doxorubicin and two Cisplatin resistant lines (UKF-NB-2 CDDP and UKF-NB-4 CDDP) had a deletion of at least part of 17q, UKF-NB-4 DOXO had deletion of the whole chromosome 17. The loss of 17q may cause chemoresistance by deletion of topoisomerase IIα gene. Deletion of 19q in all but one chemoresistant lines may influence of cytochromes P450 genes which are located on 19q13.2. Also gains of 15q22, which were detected in UKF-NB-4 VCR, UKF-NB-2 DOXO and UKF-NB-4 DOXO, may affect other cytochromes P450 genes.

Key words: neuroblastoma, comparative genomic hybridization, chemoresistance

Neuroblastoma is the third most common pediatric cancer and is responsible for approximately 15% of all childhood cancer deaths [17]. It is a malignant tumor consisting of neuronal crest derived undifferentiated neuroectodermal cells [25]. The clinical hallmark of neuroblastoma is heterogeneity. Some of the tumors undergo spontaneous regression or differentiate into benign ganglioneuromas, some are curable with surgery and little or no adjuvant therapy and some progress despite intensive multimodal therapy. This clinical diversity is closely correlated with the molecular biological features of the tumor. Among the most prominent are N-myc amplification [14, 25], gain of 17q [8], loss of 1p36.2-36.3 [8, 30], loss of 11q23 [20] and less frequently, gains at 2p, 3q 4q and 6p [2] and losses at 14q [28], 3p [7], 4p [5], 5q [19], 9p [18] and 18q [24]. Tumors with 1p loss often have N-myc amplification and highly malignant clinical behavior [17, 25].

The development of resistance to cytostatic agents is a major cancer therapy problem and has been the focus of many research efforts [9]. Tolerance to one agent is often accompanied by cross-resistance to a variety of others, often unrelated compounds. The behaviour may be explained by a selection of subclones of cells within the original tumor that have the ability to survive the cytotoxic effects of anticancer drugs [9]. The description of non-random chromosomal aberrations in resistant tumors is a vital clue in the identification of genes participating in the development and progression of chemoresistance. In the past, one of the main problems in in-

*This work was supported by grants IGA No NC/7441-3 and MSMT No MSM 0021620813.

**Corresponding author

vestigating the drug resistance mechanism was that some prior information on the changes that occurred was required, thus separate reagents to screen each possible change were needed. When analyzing the genetic changes, screening was limited to the use of gene- or region-specific probes. Standard cytogenetic techniques are not very successful for the characterization of the chromosomal aberrations. One DNA-based technique that may be applicable to this problem is the molecular-cytogenetic technique comparative genomic hybridization (CGH) [13]. CGH is a technique for the analysis of DNA copy-number losses and gains across the genome in a single hybridization experiment. This method can identify regions of chromosomal deletions and/or amplifications/gains in genomic DNA. It is not able to detect balanced aberrations like translocations or inversions. When modified to comparatively hybridize DNA from a drug-resistant line to DNA from the non-resistant parental line, it provides unique results from the first examination [4, 27, 29].

At the cellular level, a number of resistance mechanisms can potentially operate. These mechanisms include drug efflux via membrane pumps, such as P-glycoprotein, drug metabolism, including inactivation or failure to activate a prodrug, alteration in the abundance of the target protein, for example the topoisomerase II enzyme, mutation of the target protein and/or inactivation of pathways leading to cell death, such as apoptotic signaling [9, 23].

There is considerable evidence that acquired resistance to multiple natural products in vitro is mediated primarily by P-glycoprotein, a cell surface glycoprotein [9, 12] that is encoded by the MDR1 gene located at chromosome band 7q21.1 [3]. P-glycoprotein expression was found significantly more frequently in soft tissue sarcomas, neuroblastomas, and hepatoblastomas, and generally in disseminated disease [16].

Material and methods

Cell lines. The neuroblastoma cell lines UKF-NB-2, UKF-NB-3 and UKF-NB-4 were established from bone marrow metastases of high risk neuroblastoma harvested at relapse in three patients. The Vincristin, Doxorubicin and Cisplatin resistant cell sublines designated UKF-NB-2 VCR, UKF-NB-2 DOX, UKF-NB-2 CDDP, UKF-NB-3 VCR, UKF-NB-3 DOX, UKF-NB-3 CDDP, UKF-NB-4 VCR, UKF-NB-4 DOX and UKF-NB-4 CDDP were established by incubation of parental cells with increasing concentrations of the respective drug [15, 30]. All cell lines were grown in Iscove's modified Dulbecco's medium supplemented with 10% fetal calf serum. Medium for chemoresistant lines contained appropriate cytostatic drug (20 ng/ml of Vincristine, 100 ng/ml of Doxorubicin or 100 ng/ml Cisplatin). The chemoresistance of cell lines to the respective druges was verified by MTT test [6, 15]. The resistant cell lines showed et least twenty fold increase in resistance to respective drug measured as IC_{50}.

DNA. Genomic DNA was extracted from all cell lines, using DNA extraction kit QIAGEN Genomic-tip 100/G (Qiagen, Valencia, CA, USA). The extracted DNA was treated with a RNase cocktail to exclude possible RNA interference.

CGH. Test DNA, isolated from drug sensitive parental cell lines or drug resistant cell lines, and reference DNA, from a healthy volunteer and a drug sensitive parental cell line, were differentially labeled by nick-translation using a Vysis nick translation kit according to the manufacturer's recommendation (Vysis, Downers Grove, IL, USA.). Test DNA (parental or chemoresistant cell lines) was labeled by SpectrumGreen (Vysis, Downers Grove, IL, USA) and reference DNA (healthy volunteer or parental cell lines) by SpectrumRed (Vysis, Downers Grove, IL, USA). Equal amounts of the differentially labeled test and reference DNAs were mixed with human Cot-1 DNA (Vysis, Downers Grove, IL, USA), used to inhibit binding of the labeled DNA to the centromeric and heterochromatic regions and salmon sperm DNA (Vysis, Downers Grove, IL, USA), used as a carrier for precipitation of the DNA [10, 13]. The slides were dehydrated in an ethanol series prior to denaturation at 73 °C for 5 min. by a denaturation solution, followed by dehydration in an ethanol series. The DNA mix was precipitated with 100% ethanol and dissolved in a hybridization buffer (Vysis, Downers Grove, IL, USA). After denaturation at 73 °C for 5 min, it was placed in 37 °C for 20 min to allow pre-annealing. The mix was then placed on the denaturized and dehydrated metaphase slide (Vysis, Downers Grove, IL, USA.), covered and sealed with rubber cement. After 3-day hybridization at 39 °C in a humid environment, the metaphase slide was washed and let to dry. A DAPI II counter stain was applied, which produced a banding pattern used to identify the chromosomes.

Digital image analysis. The hybridizations were evaluated as previously described [10] with minor modifications. Slides were examined through an Olympus BX50 fluorescent microscope equipped with filter sets appropriate for FITC, Texas-Red and DAPI. CGH images were captured by a CCD camera connected to a computer with the MetaSystem analysis system. Separate gray-level images of DAPI, FITC and TRITC were taken. Thirty digital images were collected from each hybridization and the chromosomes were identified based on the DAPI II banding pattern. Relative DNA sequence copy number changes were estimated by analyzing the signal intensities of the control and test hybridization along the length of all chromosomes [1, 13]. The chromosomal regions were interpreted as gains when the corresponding ratio exceeded 1.25 and as losses when the ratio was less than 0.85.

Results

We studied three parental cell lines and nine derived daughter lines that were resistant to Vincristin, Doxorubicin, and Cisplatin, respectively. CGH profiles of all three parental

cell lines were obtained using DNA from a healthy volunteer as reference DNA. All three cell lines were derived from a high risk neuroblastoma and were characterized by typical findings for that group: N-myc amplification, gain of 17q, and loss of 1p36.2-36.3 [25] (Tab. 1).

Labeled DNA from each of the drug resistant daughter cell lines and labeled DNA from their parental cell lines were hybridized to obtain a comparison of gains and losses that accompanied the development of resistance for that particular drug, thus excluding the changes that were present in the parental cell line. The CGH results identifying chromosomal gains and losses for all drug resistant cell lines are shown in Table 2.

Acquired drug resistance in the neuroblastoma cell lines UKF-NB-2 VCR, UKF-NB-2 DOXO, UKF-NB-2 CDDP, UKF-NB-3 VCR, UKF-NB-3 DOXO, UKF-NB-3 CDDP, UKF-NB-4 VCR, UKF-NB-4 DOXO and UKF-NB-4 CDDP appeared to be accompanied by a large array of DNA sequence copy number changes which affected most of the chromosomes. The regions affected in the significant part of chemoresistant cell lines included gains of 13q14.1-32, 7q11.2-31.3, 4q High-level amplifications or gains were seen at chromosome 7 which included 7q 21.1 consistent with *MDR1* amplification in UKF-NB-2 VCR, UKF-NB-3 DOXO, UKF-NB-4 VCR, and UKF-NB-4 DOXO, but not in any Cisplatin resistant line. All Cisplatin and Doxorubicin and two Vincristin resistant line (UKF-NB-2 VCR and UKF-NB-4 VCR) had a deletion of part of 19q or the whole 19 chromosome. All lines resistant to Vincristin or Doxorubicin and two Cisplatin resistant lines (UKF-NB-2 CDDP and UKF-NB-4 CDDP) had a deletion of at least part of 17q, UKF-NB-4 DOXO had deletion of the whole chromosome 17.

Discussion

Analysis of our results suggests that chemoresistance is a very complex phenomenon and those multiple gains and

Table 1. DNA copy number alterations detected by CGH in three neuroblastoma parental cell lines UKF-NB-2, UKF-NB-3 and UKF-NB-4

Cell line	gains	losses
UKF-NB-2	1p21-31.1; 2p22-25.3;ampl myc-n;2q36-ter; 4q31.3-34; 17q; 19p	1p35-ter; 4q13.1-24; 6q25.3-ter; 11q23.3; 13; 18p11.2-ter;18q22-ter; 19q13.2.-ter; 21; Y
UKF-NB-3	2p13-ter;amp myc-n;2q24.3-35; 10p12.1-14.1; 12q; 13; 17q21.1-ter; 20	1p34.2-ter; 2q35-ter; 4p15.3-ter; 5q32-ter; 6p2.3-ter; 16q23-ter; 17p11.2-ter; 18p11.2-ter; 19q13.1-ter; 21q22.1-ter; X
UKF-NB-4	1p31.3-1qter; 2;ampl n-myc; 3p14.1-3qter; 5; 7; ampl 7q21.3; 8pter-8q24.1; 11q13.4-ter; 12; 14q; 15q; 16p; 17q; 18q; X	1p32.2-ter; 3p21.1-ter; 4; 6; 9; 10; 11p; 13; 16q; 17p12-ter; 22; Y

Table 2. DNA copy number alterations detected by CGH in nine neuroblastoma chemoresistant cell lines UKF-NB-2 VCR, UKF-NB-2 DOXO, UKF-NB-2 CDDP, UKF-NB-3 VCR, UKF-NB-3 DOXO, UKF-NB-3 CDDP, UKF-NB-4 VCR, UKF-NB-4 DOXO and UKF-NB-4 CDDP

Cell line	gains	losses
	Vincristin	
UKF-NB-2	3q25.1-26.3; 4q11-24; 7pter-7q32; 11q21-23.1; 13q32-ter; 18p; Xq11-24; Y	1p31.3-ter; 6p21.1-21.3; 9q34.1-ter; 11q13.2-14.1; 12q13.2-14; 12q23-ter; 15q23-25; 16p; 17q; 19; 20q; 22
UKF-NB-3	1p31.1-31.2; 4p; 5q32-ter; X; Y	4q13.1-24; 4q28-31.1; 5p13.2-ter; 11q23.3-24; 13q31-34; 17q21.3-ter; 20q11.2-13.3; 22
UKF-NB-4	1p21-35;1q25-32.1; 3q24-26.2; 4p15.1-4q24; 5p; 6q12-24.1; 7q21.1-22.1;amp 21.2-3; 8q12-21.3; 8q22.3-23; 10; 12q15-21.3; 13q14.1-32; 15q21.3-22.3; 21	2; 5q12-ter; 7q32-36; 9q34.1-ter; 11q13.1-13.5; 15q24-ter ; 16p; 17q; 18pter-18q21.3; 19; 20q; 22
	Doxorubicin	
UKF-NB-2	4q12-21.3;4q31.3-ter; 8q22.1-23; 13; 15q25-ter; 21q22.1-ter; Xq11.1-21.3; Y	2q36-ter; 3p22-ter; 6q22.3-ter; 12q24.1-ter; 17q12-21.3; 19; 20q
UKF-NB-3	4p13-ter; 5q32-ter; amp7q11.1-22.1; Xq21.2-22.1; Y	1p32.3-ter; 4q12-24;4q27-31.1; 9q34.1-ter; 11q13.1-14; 16p12-ter; 17q12-ter; 19; 20q11.2-ter; 22
UKF-NB-4	1p31.2-1q23; 2q22-34 ; 3p22-24.1;3p14.1-3q13.3; 3q24-27; 4p15.3-4q34; 6p12-6q25.1; 7q11.2-22;ampl 21.2; 8q11.2-24.1; 9p22-9q22.1; 10q21.1-21.3; 11p11-15.2;11q14.2-23.2; 12p11-12.3;12q14-23;amp21.3; 13q14.1-31;amp21.2; 15q; 20q; 22; Y	1p32.3-ter;1q32.1-43; 2pter-2q21.3;2q35-ter; 5p14-ter;5q11.2-13.1; 5q23.3-ter; 7p21-ter; 8p; 9q34.1-ter; 11q13.3-13.5; 11q13.3-13.5; 12q24.1-ter; 13q32-ter; 16p; 17; 18; 19; 20p; Xq24-28
	Cisplatin	
UKF-NB-2	4q11-28; 6q11-21; 11q21-23.1; 13q21-1.31; Xq; Y	1p21-31.1;1p31.6-ter; 3p14.1-24.1; 11q13.3-14.1; 12q24.1-ter; 16p; 17q; 19; 20q; 22
UKF-NB-3	5q; 12p; 17; X; Y	2p; 11q13.4-ter; 12q23-ter; 13; 16p12-ter; 19q13.1-13.3; 20q
UKF-NB-4	2q32.1-35.1; 4q; 5p11.1-14; 6; 7p; 9p21-9q34.1; 10; 11q14.2-22.3; 12q13.3-ter; 13q13-ter;amp 13q21-22; 16q11.2-ter; 17p11.2-ter; 20; 22; Y	1p32-ter; 2p22-2q14.3; 3p13-14.3;3q24-ter; 5q23.1-ter; 7q21.1-ter; 8p; 15; 16p; 17q12-ter; 18; 19q13.1-ter

losses indicate that drug resistance is thought to be mediated through multiple complementary pathways [4]. Drug resistance to Doxorubicin and Vincristin was mediated not only by over expression of the MDR1 gene [9, 11, 15], but also by amplification of the gene. In the cell line UKF-NB-4 where amplification on chromosome 7 occurred in the parental cell line the drug resistant daughter cell line had even greater amplification in the region where the MDR1 gene is located. Cell lines resistant to Cisplatin did not show any amplification of the MDR1 gene, which corresponds with YASUNO et al findings [31], even though other authors have described cell lines resistant to cisplatin that were overexpressing the MDR1 gene [22]. Amplifications of the MDR1 gene in Doxorubicin and Vincristin resistant lines UKF-NB-2, UKF-NB-3 and UKF-NB-4 were accompanied by overexpression of P-glycoprotein and its function, which was evaluated by immunological detection and Rhodamine 123 efflux, respectively [6, 15]. Amplification of 7q21-q22 was also previously described in cell lines that were resistant to Vinblastine [27].

We found gains and losses of parts of the genome that accompany or even cause the development of drug resistance. Some of them can be more universal – the loss of 17q region. STRUSKI et al [27] found that the loss of genetic material in the region 17q is in connection to resistance to VP-16. Carlson with coworkers detected del 17q22-23 in leukemic cell line K562 resistant to idarubicin [4]. One may speculate that chemoresistance is caused by deletion of topoisomerase IIα gene. Deletion of 19 q in all but one chemoresistant lines may cause resistance by influencing of cytochromes P450 genes which are located on 19q13.2. Other members of cytochromes P450 family genes CYP1A1 and CYP1A2 are located on 15q22. This region was gained in UKF-NB-4 VCR, UKF-NB-2 DOXO and UKF-NB-4 DOXO. Cytochromes P450 are one of the most prominent group of drug-metabolizing enzymes [21].

Gains of 16q13-22 where the genes for methalothioneins are located was detected only in UKF-NB4 CDDP, other two cisplatin resistant lines have not gain of this region. Methalothioneins caused resistance to cisplatin because binds toxic metal ions including platinum derivatives. There were gained various parts of 13. chromosome with shortest region of overlap 13q21-22 in lines UKF-NB-4 VCR, UKF-NB-2 DOXO, UKF-NB-4 DOXO, UKF-NB-2 CDDP, and UKF-NB-4 CDDP. Possible gene located in this region which may caused chemoresistance seems ERCC5 (excision repair cross-complementing rodent repair deficiency gene). Product of this gene is involved in transcription-coupled repair (TCR) of oxidative DNA lesions [26].

Many genetic abnormalities in chemoresistant cell lines are not in relationship with chemoresistance because they are caused by multiple passages and by cytostatic drugs treatment. Therefore further studies and other methods including microarrays are to be performed in the aim to clarify the cytogenetic characteristics of resistant neuroblastoma cells and especially to find candidate genes that lead to the development of drug resistance in the cell lines as well as in the tumor in patients undergoing chemotherapy.

References

[1] BAYANI J, THORNER P, ZIELENSKA M, PANDITA A, BEATTY B et al. Application of a simplified comparative genomic hybridization technique to screen for gene amplification in pediatric solid tumors. Pediatr Pathol Lab Med 1995; 15: 831–844.

[2] BRINKSCHMIDT C, CHRISTIANSEN H, TERPE HJ, SIMON R, BOECKER W et al. Comparative genomic hybridization (CGH) analysis of neuroblastomas – an important methodological approach in paediatric tumour pathology. J Pathol 1997; 181: 394–400.

[3] CALLEN DF, BAKER E, SIMMERS RN, SESHADRI R, RONINSON IB. Localization of the human multiple drug resistance gene, MDR1, to 7q21.1. Hum Genet 1987; 77: 142–144.

[4] CARLSON KM, GRUBER A, LILIEMARK E, LARSSON R, NORDENSKJOLD M. Characterization of drug-resistant cell lines by comparative genomic hybridization. Cancer Genet Cytogenet 1999; 111: 32–36.

[5] CARON H, VAN SLUIS P, BUSCHMAN R, PEREIRA DO TANQUE R, MAES P et al. Allelic loss of the short arm of chromosome 4 in neuroblastoma suggests a novel tumour suppressor gene locus. Hum Genet 1996; 97: 834–837.

[6] CINATL J JR., CINATL J, KOTCHETKOV R, MATOUSEK J, WOODCOCK BG et al. Bovine seminal ribonuclease exerts selectively cytotoxicityx toward neuroblastoma cells both sensitive and resistant to chemotherapeutic drugs. Anti-Cancer Res 2000; 20: 853–859.

[7] EJESKAR K, ABURATANI H, ABRAHAMSSON J, KOGNER P, MARTINSSON T. Loss of heterozygosity of 3p markers in neuroblastoma tumours implicate a tumour-suppressor locus distal to the FHIT gene. Br J Cancer 1998; 77: 1787–1791.

[8] GILBERT F, FEDER M, BALABAN G, BRANGMAN D, LURIE DK et al. Human neuroblastomas and abnormalities of chromosomes 1 and 17. Cancer Res 1984; 44: 5444–5449.

[9] GOLDIE JH, COLDMAN AJ. Drug resistance in cancer. Mechanisms and models. Cambridge: Cambridge University Press, 1998.

[10] HOULDSWORTH J, CHAGANTI RS. Comparative genomic hybridization: an overview. Am J Pathol 1994; 145: 1253–1260.

[11] ISSHIKI K, NAKAO A, ITO M, HAMAGUCHI M, TAKAGI H. P-glycoprotein expression in hepatocellular carcinoma. J Surg Oncol 1993; 52: 21–25.

[12] JULIANO RL, LING V. A surface glycoprotein modulating drug permeability in Chinese hamster ovary cell mutants. Biochim Biophys Acta 1976; 455: 152–162.

[13] KALLIONIEMI A, KALLIONIEMI OP, SUDAR D, RUTOVITZ D, GRAY JW et al. Comparative genomic hybridization for molecular cytogenetic analysis of solid tumors. Science 1992; 258: 818–821.

[14] KOHL NE, KANDA N, SCHRECK RR, BRUNS G, LATT SA et al. Transposition and amplification of oncogene-related sequences in human neuroblastomas. Cell 1983; 35: 359–367.

[15] KOTCHETKOV R, CINATL J, BLAHETA R, VOGEL JU, KARASKOVA J et al. Development of resistance to vincristine and doxo-

rubicin in neuroblastoma alters malignant properties and induces additional karyotype changes: a preclinical model. Int J Cancer 2003; 104: 36–43.

[16] KUČEROVA H, SUMERAUER D, DRAHOKOUPILOVÁ E, PISKOVÁ M, BEDRNÍČEK J et al. Significance of P-glycoprotein expression in childhood malignant tumors. Neoplasma 2001; 48: 472–478.

[17] MARIS JM, MATTHAY KK. Molecular biology of neuroblastoma. J Clin Oncol 1999; 17: 2264–2279.

[18] MARSHALL B, ISIDRO G, MARTINS AG, BOAVIDA MG. Loss of heterozygosity at chromosome 9p21 in primary neuroblastomas: evidence for two deleted regions. Cancer Genet Cytogenet 1997; 96: 134–139.

[19] MELTZER SJ, O'DOHERTY SP, FRANTZ CN, SMOLINSKI K, YIN J et al. Allelic imbalance on chromosome 5q predicts long-term survival in neuroblastoma. Br J Cancer 1996; 74: 1855–1861.

[20] MERTENS F, JOHANSSON B, HOGLUND M, MITELMAN F. Chromosomal imbalance maps of malignant solid tumors: a cytogenetic survey of 3185 neoplasms. Cancer Res 1997; 57: 2765–2780.

[21] NELSON DR, ZELDIN DC, HOFMAN SMG, MALTAIS LJ, WAIN HM et al. Comparison of cytochrome P450 (CYP) genes from the mouse and human genomes, including nomenclature recommendations for genes, pseudogenes, and alternative – splice variants. Pharmacogenetics 2004; 14: 1–18.

[22] NOOTER K, STOTER G. Molecular mechanisms of multidrug resistance in cancer chemotherapy. Pathol Res Pract 1996; 192: 768–780.

[23] POLAND J, SCHADENDORF D, LAGE H, SCHNOLZER M, CELIS JE et al. Study of therapy resistance in cancer cells with functional proteome analysis. Clin Chem Lab Med 2002; 40: 221–234.

[24] REALE MA, REYES-MUGICA M, PIERCEALL WE, RUBINSTEIN MC, HEDRICK L et al. Loss of DCC expression in neuroblastoma is associated with disease dissemination. Clin Cancer Res 1996; 2: 1097–1102.

[25] SCHWAB M. Human neuroblastoma: from basic science to clinical debut of cellular oncogenes. Naturwissenschaften 1999; 86: 71–78.

[26] SELBY CP, SANCAR A. Mechanisms of transcription-repair coupling and mutation frequency decline. Microbiol Rev 1994; 58: 317–329.

[27] STRUSKI S, CORNILLET-LEFEBVRE P, DOCO-FENZY M, DUFER J, ULRICH E et al. Cytogenetic characterization of chromosomal rearrangement in a human vinblastine-resistant CEM cell line: use of comparative genomic hybridization and fluorescence in situ hybridization. Cancer Genet Cytogenet 2002; 132: 51–54.

[28] THOMPSON PM, SEIFRIED BA, KYEMBA SK, JENSEN SJ, GUO C et al. Loss of heterozygosity for chromosome 14q in neuroblastoma. Med Pediatr Oncol 2001; 36: 28–31.

[29] WASENIUS VM, JEKUNEN A, MONNI O, JOENSUU H, AEBI S et al. Comparative genomic hybridization analysis of chromosomal changes occurring during development of acquired resistance to cisplatin in human ovarian carcinoma cells. Genes Chromosomes Cancer 1997; 18: 286–291.

[30] WHITE PS, THOMPSON PM, SEIFRIED BA, SULMAN EP, JENSEN SJ et al. Detailed molecular analysis of 1p36 in neuroblastoma. Med Pediatr Oncol 2001; 36: 37–41.

[31] YASUNO T, MATSUMURA T, SHIKATA T, INAZAWA J, SAKABE T et al. Establishment and characterization of a cisplatin-resistant human neuroblastoma cell line. Anticancer Res 1999; 19: 4049–4057.