RESEARCH ARTICLE

Chromosome Imbalances and Alterations of AURKA and MYCN Genes in Children with Neuroblastoma

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Abstract

Background: Neuroblastoma (NB), like most human cancers, is characterized by genomic instability, manifested at the chromosomal level as allelic gain, loss or rearrangement. Genetics methods, as well as conventional and molecular cytogenetics may provide valuable clues for the identification of target loci and successful search for major genes in neuroblastoma. We aimed to investigate AURKA and MYCN gene rearrangements and the chromosomal aberrations (CAs) to determine the prognosis of neuroblastoma. Methods: We performed cytogenetic analysis by G-banding in 25 cases [11 girls (44%) and 14 boys (66%)] and in 25 controls. Fluorescence *in situ* hybridization (FISH) with AURKA and MYCN gene probes was also used on interphase nuclei to screen for alterations. Results: Some 18.4% of patient cells exhibited CAs., with a significant difference between patient and control groups in the frequencies (P<0.0001). Some 72% of the cells had structural aberrations, and only 28% had numerical chnages in patients. Structural aberrations consisted of deletions, translocations, breaks and fragility in various chromosomes, 84% and 52% of the patients having deletions and translocations, respectively. Among these expressed CAs, there was a higher frequency at 1q21, 1q32, 2q21, 2q31, 2p24, 4q31, 9q11, 9q22, 13q14, 14q11.2, 14q24, and 15q22 in patients. 32% of the patients had chromosome breaks, most frequently in chromosomes 1, 2, 3, 4, 5, 8, 9, 11, 12, 19 and X. The number of cells with breaks and the genomic damage frequencies were higher in patients (p<0.001). Aneuploidies in chromosomes X, 22, 3, 17 and 18 were most frequently observed. Numerical chromosome abnormalities were distinctive in 10.7% of sex chromosomes. Fragile sites were observed in 16% of our patients. Conclusion: Our data confirmed that there is a close correlation between amplification of the two genes, amplification of MYCN possibly contributing significantly to the oncogenic properties of AURKA. The high frequencies of chromosomal aberrations and amplifications of AURKA and MYCN genes indicate prognostic value in children with neuroblastomas and may point to contributing factors in their development.

Keywords: AURKA - MYCN - chromosomal aberrations - neuroblastoma

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Introduction

Neuroblastoma (NB) is the most common extracranial solid tumour in children below the age of 5 years. NB shows an important clinical and genetic heterogeneity (Maris et al., 1999). Genetic methods disclosed genetic abnormalities leading to gain and loss of chromosomal segments in advanced stage of NB. The most frequent genetic alterations of NB are gain of 17q, loss of heterozygosity (LOH) at 11q, MYCN amplification and 1p36 allelic loss. Other alterations described are gains of 4q, 6p, 7q, 11q, and 18q; amplification of MDM2 and MYCL genes; allelic losses at 14q and 10q. The best characterized genetic alterations include amplification of MYCN, gain of chromosome arm 17q and losses of 1p, 3p, and 11q. Based on these genetic aberrations, NB is currently classifed into three major genetic subgroups. Advanced NB often shows deletions and/or translocations involving the short arm of chromosome 1. Allelic loss of chromosome 1p is seen in patients with poor clinical outcome (Brodeur et al., 2003). Allelic loss of 11q can be seen in 15-44% of NB and is associated with poor clinical outcome, suggesting another tumour suppressor gene in this region. Several other chromosomes have been found to show deletions including 2q, 3p, 4p, 9p, 11q and 14q (Brodeur et al., 2003; Spitz et al., 2003).

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AURKA is an oncogene that is amplified in many human tumors, and is key regulators of mitosis. Likewise, the Myc family of transcription factors is also commonly deregulated in cancer, via chromosomal translocation events, gene amplification, and interference with normal protein degradative pathways. AURKA stabilizes the MYCN protein through a direct physical interaction and interferes. MYCN amplification is another feature often noticed in aggressive neuroblastomas and is a strong marker of poor prognosis (Rubie et al., 1997). MYCN gain has recently been observed in about 6-8% of neuroblastomas MYCN showing a poor survival probability is highly amplified in about 20% of neuroblastoma patients (Spitz et al., 2004). In the present study, AURKA and MYCN genes rearrangements and the chromosomal aberrations (CAs) were investigated to determine the prognosis of neuroblastoma.

Materials and Methods

Cytogenetic examination

25 neuroblastoma patients, 11 girls (44 %) and 14 boys (66 %), included to the study. Control group had 25 healthy children, 10 girls (40 %) and 15 boys (60 %), whose no cancer history in their families. The peripheral blood was taken from each patient for karyotype analysis. Each sample examined for cytogenetic anomalies in our genetic laboratory of the Department of Medical Biology and Genetics, Faculty of Medicine, Çukurova University, Adana-Turkey. A 0.3 ml sample of bloods was incubated at 37 °C for 72 h in RPMI-1640. Standard cytogenetic techniques were used for harvesting and slide preparation. The slides were first stained only with Giemsa before the examination in order to avoid missing any gaps. For detailed analysis of the fragile sites, some slides were prepared by GTG banding, and 50 metaphases were scored for each assay. A CA was defined as a fragile site when it was present in 1% of the cells analyzed and in at least 50% of the individuals studied (Fundia et al., 1989). All gaps and breaks were recorded and localized according to the ISCN (1985) (Mitelman et al., 1995). The classification of fragile sites was done according to the nomenclature established in human gene mapping HGM11 (McAlpine et al., 1991). The Fisher's exact test was used to determine the difference in frequency of fragile site observed in patients and control group.

Slide preparation and flourescence in situ hybridization

A paraffin-embedded tissue slides were used paraffin blocks from 9 patients in order to determine the alterations in AURKA and MYCN genes. Poseidon Repeat Free AURKA (on chomosome 20q13/20q11) Dual Color Dual Fusion Amplification probe and LSI MYCN (Vysis) (on chromosome 2p24.1) probes were used. Paraffin sections were cut 4 μm thick using a microtome and a section was mounted on a positively charged slide. Standard techniques were used for harvesting and slide preparation without incubation. The slides were put in *ThermoBrite* Denaturation/Hybridization System and denaturated 5 min at 95°C and hybridized overnight at 37°C. Slides were analysed at flourescent microscopy using red, green and DAPI filters. Interphase cells were analyzed using a BX51 Olympus fluorescence microscope equipped with Cytovision Probe Software (Applied Imaging, Santa Clara, CA). In each patient, 100 interphase cells were evaluated for the signal patterns.

Statistical analysis

Discrete variables and continuous variables were expressed as numerical/percentage and mean±SD, respectively. The comparison of continuous variables between groups was evaluated using non-parametric tests. A p-value <0.05 was considered significant. The SPSS statistical software package program (version 15.0, SPSS, Inc, Chicago, IL, USA) was used to perform all statistical calculations.

Results

25 patients [11 girls (44%) and 14 boys (66%)] aged were between 1 -108 months. Median age was 33.4 months. Mean ages at diagnosis were 10, 55.5 and 33.2 months according to stages 4S, 2b and 4, respectively. 21 (84%) patients revealed predominantly numerical and structural aberrations. CAs was found in 212 (18.4%) cells of 1150 cells analysed. NB patients had a higher incidence of CAs. There was a significant difference between patient and control groups in the frequencies of CAs (numerical and structural abnormalities, and fragile sites) (P<0.0001). Structural abnormality was seen in 212 cells predominantly. 72% and 28% of the all cells had structural and numerical aberrations, respectively. Structural aberrations mostly seen and consisted of deletions, translocations, breaks and fragilities in various chromosomes (Table 1) (Figure 2).

Among these expressed CAs, there was a higher frequency of chromosome at 1q21, 1q32, 2q21, 2q31, 2p24, 4q31, 9q11, 9q22, 13q14, 14q11.2, 14q24, and 15q22 in patients. We observed a significantly greater number of single-cell deletions in patients. 84% of all patients had deletions observed in 63 cells. Deletions were presented as common findings at breakpoints; del(1)(q32-qter)x3, del(15)(q22-qter)x3, del(1)(q21.3-qter)x2, del(2)(p24-pter)x3, del(2)(p23-pter)x2, del(7)(q32-qter)x2, del(9)(q22-qter)x2, del(12)(p11-qter)x2, del(14)

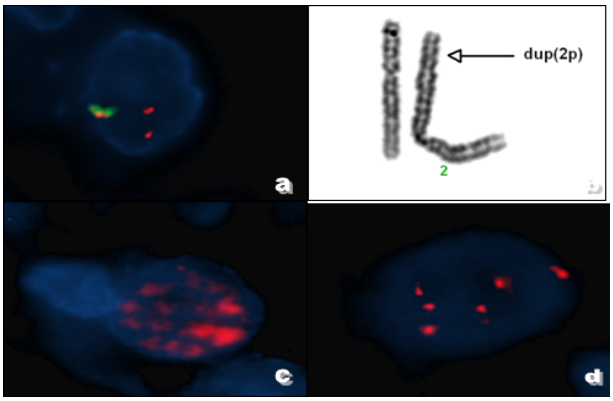

Figure 1. Partial FISH Images Showing Amplification AURKA (a), MYCN (c,d) and Chromosomal amplification MYCN (b)

Table 1. Demographics, Pathological, FISH and Cytogenetic Results in Patients with Neuroblastoma and Cytogenetic Results in Control Group

Patients Group									Control Group	
Patient No	G	Age (m.)	Stage	T.L	R.G	C.A.	MYCN amp.	AURKA amp.	C.G. No	C.A.
P1	M	18	4	S	H	46,XY,del(1)(q21.3-qter);t(7;14)(p22;q24);47,XY,+ace; del(8)(q12-qter); fra(9q22)	-	-	C1	46,XY,gap(1q32);gap(5q32);gap(4q31);gap(10q24)
P2	M	108	4	P	H	No growth	-	-	C2	46,XY,gap(5q31);fra(1q32); gap(4q27)
P3	F	54	4	S	H	46,XX,del(1)(q21.3-qter);del(7)(q32.1-qter);del(13)(q22.1-qter);45,XX,chrb(9q11.1),-15;45,XX,del(9)(p11-pter),-21;del(2)(p24-pter); t(20;22)(p12-pter;q13),del(X)(q21-qter); dup(12)(q24-qter), fra(12q24);dup(2p);del(4)(p12-pter);+1,-14,del(9)(q22-qter); 45,XX,-8;del(3)(p22-pter);45,XX,-10;51,XX,t(21;21)(q22;q22), +5dmin;chtb(5q33); del(14)(q13-qter); t(14;16)(q24;p13)	-	-	C3	46,XX,gap(4q31)
P4	M	108	2b	S	İ.G	46,XY,inv(13)(p13;q14),Yq+;del(4)(q31-qter),del(7)(q32-qter),Yq+; t(8;18)(p11;p11),Yq+; 45,XY,t(3;5)(p21;p11),-8,Yq+;t(10;17)(p14;p13),Yq+;del(10)(q11-qter), Yq+;chrb(4q31),Yq+; dup(19)(p13.2),Yq+;del(8)(q11-qter),Yq+	8%	7%	C4	46,XX,gap(1q32);far(Xq26)
P5	F	22	4	S	H	46,XX,del(1)(q32-qter);del(12)(q11-qter);gap(5q31)	-	-	C5	46,XY,9qh+x5
P6	M	12	4	M	H	43,XY,del(3)(p23-pter),-4,-8,-18;47,XY,+21;chtb(3q21); 8p+,del(14)(q11.2-pter)	-	-	C6	46,XY,gap(1q32)x3;fra(3p25);fra(7q33)
P7	M	42	4	S	I.P	45,XY, -16,del(20)(p12-pter);fra(11q23); 45,XY,-20; 45,XY,-19	32%	25%	C7	46,XX,gap(1q32)x2; gap(3q27)
P8	M	6	4	S	H	46,XY,del(4)(q22-q25);?10q/8q;44,XY,-18,-21;43,XY,-15,-18,-19; 45,XY,-21	36%	25%	C8	46,XX
P9	M	48	4	S	I.P	46,XY,fra(2q21),del(13)(q14-pter);Endoreduplicasion; del(13)(p12-pter),chtb(19q13),fra(2q31);der(21)t(?),der(14)t(14;?)(q11pter;?), der(7)t(7;?)(p11;?),t(1;?)(p16;?); der(7)t(2;7)(q21;p13), chbr(2)(q31),der(4)t(4;?)(q35;?),-19,-22x2;del(8)(q22-qter); 55,XY,der(1)(?),-4,+10x2,+13,+20x2, +21,+22x2,+ace;53,XY,-6,+1,+7,+16x2,+19x2,+21,+22; chtb(1q)(21)(q32),del(6)(p23-pter), fra(7q36),1del(8)(p11-pter), 10p+, +ace	25%	22%	C9	46,XY,gap(1q36); 9qh+x2
P10	F	3	2b	S	L	46,XX,9qh+x50;chtb(3)(p13),9qh+;del(2)(p23-pter),9qh+;chtb(X)(q22),9qh+; chtb(2)(q21),9qh+; del(11)(q13-qter),9qh+	8%	5%	C10	46,XY,fra(12p13)
P11	F	1	4	S	H	46,XX,9qh+x50;chrb(8q22),9qh+;inv(9)(p11;q12),9qh+; 45,XX,9qh+,-17	-	-	C11	46,XY,fra(15q24); fra(7q36)
P12	F	9	4	S	H	48,XX,+ace(1)(q21-qter),+16;del(2)(q12-q21);del(5)(q31-qter); del(15)(q22-qter); 45,X,-8,-9,-X,+21;47,XX,?+(p); der(18)t(13;18)(q11;p11.3); 45,XX,del(1)(p32-pter),-19; del(1)(p22-pter); chrb(8q23); 45,XX,-19	-	-	C12	46,XY
P13	F	10	4S	S	L	46,XX,del(15)(q22-qter);45,XX,rbt(13;22)(p11;p11); 45,XX,del(9)(q22-qter),-21; del(2)(p24-pter);der(4) t(4;7)(p16;q21);der(12)t(12;17)(p13;q12); 45,XX,-8, del(14)(q11.2-q13); 45,XX,-21x2	-	-	C13	46,XX
P14	M	6	4	S	H	46,XY,del(17)(q25-qter);45,XY,der(X)t(8;X)(p23;p22); del(3)(p25-pter); inv(16)(p11.2-pter;q24);chtb(2p11);chtb(11q23)	12%	15%	C14	46,XY,fra(4q27)
P15	M	54	4	S	H	46,XY,gap(9p13); gap(15q15)	18%	12%	C15	46,XY,9qh+x3;chtb(12q13)
P16	F	3	4	S	H	47,XX,+mar; del(1)(q32-qter), t(9;14)(q34;q24.3-qter); chtb(2)(q32.2); del(2)(p24-pter)	-	-	C16	46,XX,gap(7q22)
P17	F	72	4	S	H	46,XX,del(2)(p23-pter);45,XX,der(8)t(8;22)(p23;q11),-22; chtb(4q31)	14%	15%	C17	46,XY,fra(Xq27)
P18	M	30	4	C	H	46,XY,del(9)(q11.1-qter)x2;der(3)t(3;12)(p26;q15-qter); del(12)(p11-qter)x2;del(10)(q24-qter);del(13)(q14.3-p13), del(15)(q25-qter); 45,XY,der(11)t(11;18)(q25;pter-q21), del(18)(q21-qter);del(19)(q13.3-qter); 47,XYY;del(3)(q11-qter); del(1)(q32-qter),del(3)(cen-qter);del(9)(q13-qter); del(7)(q11.1-qter); 47,XY,+ace, del(2)(q31-qter)	-	-	C18	46,XY,9qh+x3
P19	F	48	4	S	H	46,XX	-	-	C19	46,XX,fra(1q42); gap(7q22)
P20	M	48	4	S	H	46,XY	4%	8%	C20	46,XX
P21	M	36	4	S	İ.G	46,XY,15p+x50; der(15)t(15;22)(q11;q11); 47,XYY,15p+; 15p+, dmin(10<);del(15)(q22-qter);-chtb(12)(q14.1),15p+x2; 45,XY,ins(3;8)(q11;q11q24),15p+	-	C21	46,XY	
P22	M	36	4	S	İ.P	No growth	-	-	C22	46,XY,gap(4q27)
P23	F	24	4	P	H	45,XX,-22; 44,XX,-13,-22; 44,XX,-7,-22; 45,XX,-13;44,XX,-13,-16; t(2;22)(p11-pter;p12), t(5;15)(p12-pter;p11) -	-	C23	46,XX,fra(5q31)	
P24	F	12	4	S	H	46,XX,9qh+x50; del(3)(q28-qter),?dup(5)(p12-p14), 9qh+; 45,XX,del(19)(q13-qter),9qh+,-12	-	-	C24	46,XX
P25	M	18	4	S	H	46,XY,9qh+x25;9qh+,t(10;14)(q26;q13);47,XY,+15;del(9)(q32-qter); del(2)(p13-pter),9qh+,+21,-13; 9qh+,del(14)(q11.1-pter)	-	-	C25	46,XX,fra(1q42);fra(1q32);chtb(Xp22); 9qh+

*G: Gender (M:Male, F:Female); T.L.: Tumor Location (S: Suprarenal, P: Paraspinal, C: Cranial, M: Mediasten); R.G: Risk Group (H: High, I.P: İntermediate poor histology, L: Low, I.G: İntermediate good histology); C.A: Chromosomal Aberration; C.G: Control Group; M.: month, amp. Amplification

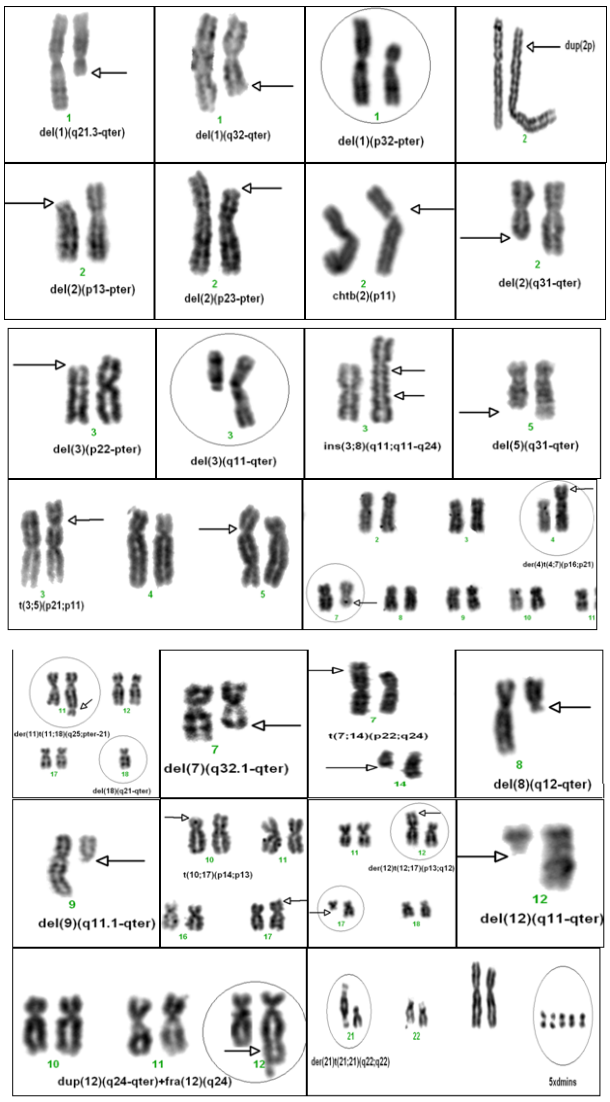

Figure 2. Partial Metaphase Figures Showing Chromosomal Abnormalities

(q11.2-pter)x2, del(19)(q13.3-qter)x2 and others (Table 1). 12% of all patients had duplications and inversions. That of 52% and 32% had translocations and chromosome breaks, respectively. The most frequent breaks were found in the chromosomes 1, 2, 3, 4, 5, 8, 9, 11, 12, 19 and X. 20% of breaks were observed in chromosomes 4, 8 and 9 (Table 1).

Aneuploidies were seen commonly in 26 cells. Particularly 3, 17, 18, 22 and X chromosomes had aneuploidies (monosomies and trisomies). One of the important aneuploidies was numerical sex CAs included 45,X in 3 (10.7%), 47,XXY in 3 (10.7%) and izo(Xq) in 1(3.6%). The distribution of fragile sites according to each chromosome is shown in Table 1. Fragile sites were observed on 16% of our patients. The distribution of fragile sites was in 2, 7, 9, 11 and 12 chromosomes. Chromosome polymorphisms in the form of heterochromatic segments, enlarged long arm, and secondary constrictions were observed at 9qh+, Yqh+ and 15p+ (Table 1).

In the control group, the aberrations were found in 33 (2.6%) cells among 1250 analysed cells. No numerical aberrations were seen. Structural aberrations usually consisted of breaks, fragilities and gaps in various chromosomes (Table 1).

Nine patients with multiple copies of the AURKA and MYCN signal were identified with interphase FISH screening program. The range of FISH signals was between 4% and 36% (Table 2, Figure 1). FISH signals of 6 patients (P7, P8, P9, P14, P15 and P17) were seen significant for both AURKA and MYCN. Whereas 3 patients had 10% AURKA and MYCN signals within the nuclei of tumor cells (P4, P10 and P20) (Table 1).

Discussion

Researchers directed at sites of genetic imbalance will provide insights into the fundamental biology of neuroblastoma initiation and progression. Combined cytogenetic and molecular study might produce additional information for a better understanding of the genetic defects in NB. In the present study, numerical and structural CAs and polymorphic variants were found in 18.4% of cells. 72% of CAs had structural, and only that of 28% had numerical aberrations. There was a significant difference between patients and the control group according to CAs statistically (P<0.0001).

Allelic deletions have been detected at multiple genetic loci in 1p, 2q, 3p, 4p, 9p, 11q, 14q, 16p, and 19q for NB (Brodeur et al., 1977; Guo et al., 1999; Fong et al., 1992; Ejeskar et al., 1998; Weiss et al., 2000; Caron et al., 1996; Mora et al., 2001; Marshall et al., 1997). Gilbert and et al. (1982) reported that the high frequency of deletions and other rearrangements of 1p were held to be the most common genetic aberration of NB tumour cells. 11 aberrations of CAs (particularly deletions) at band p32, q21 and q32 on chromosome 1 were detected in our patients (Table 1). In one study by Sreekantaiah et al. (1988), specific regions on both arms of chromosome 1q21 and 1q32 were overrepresented in changes involving this chromosome in cervical carsinoma. Also it was emphasized that an early cytogenetic event as in neuroblastoma. A copy number polymorphism at 1q21.1 (NBPF23) for sporadic neuroblastoma was reported in other study. These regions are being mapped intensively to identify potential candidate genes for the putative tumour-suppressor gene that has been deleted from this region (Capasso et al., 2010). Therefore it seems likely that a NB suppressor gene is located on 1p, and that this gene is inactivated in at least one third of primary neuroblastomas. It is possible that a tumor suppressor gene(s), located on chromosome 1p and 1q, might be critical to the genesis and/or progression of our patients.

Alterations of the 2p have been of particular interest, since amplification of the MYCN oncogene on 2p24 is associated with an adverse outcome in NB patients (Kohl et al., 1983). We report on the changes of chromosome 2; 6 deletions, 2 translocations, 4 chromotid breaks and 2 fragile sites at band p11.2, p23, p24, q21 and q31 (Table 1) (Figure 2). The results of the present study suggest that the determination of MYCN gene copy number relative to chromosome 2, when evaluating MYCN status at diagnosis, may help to reveal the underlying genetic pattern of these tumors and better understand their clinical behavior. The most frequent changes included losses at

2q31.1, 2q33.3, 2q37.1 were presented by Guled and et al. (2008). The results may help to reveal the underlying genetic pattern of these tumors and better understand their clinical behavior.

On chromosome 4, 3 deletions, 2 translocations, 2 breaks and 2 aneuploidies were presented in our study. The especially 4q31 locus was significantly overrepresented in our patients (Table 1) (Figure 2). Cytogenetic analysis of an extraskeletal Ewing sarcoma/primitive neuroectodermal tumors (PNETs) revealed a novel chromosomal translocation t(4;22)(q31;q12) as the sole anomaly (Sumegi et al., 2011). Recent gene studies of chronic obstructive pulmonary disease (COPD) implicate genetic variants on the chromosomal 4q31 locus. In smokers, genetic variants of the 4q31 locus conferring a protective effect for COPD are also protective in lung cancer (Young et al., 2010). Based on the data presented here we suggest that 4q31 locus should be investigated in NB as well as other cancers.

Chromosomal loss of chromosomes 3, 9, 14 and 15 might confer properties necessary for invasion or metastatic growth in neuroblastoma (Krona et al., 2008). We found the deletions on 9p11, 9q11 and 9q22 were remarkable in our patients. Although several regions of chromosome 9 have been previously reported in NB, fragilities and breaks of 9q22 and 9q13 were also pointed in patients with lung cancer (Karaüzüm et al., 1998). Our findings suggested that losses of chromosome 9p-q played a role in the prognosis of NB. Deletion of the 14q is also a common abnormality in NB. LOH for 14q was first identified in 1989 in six of 12 NBs studied by Suzuki et al (1989). There are only a few reports of cytogenetically visible deletions or rearrangements that involve 14q (Petkovic et al., 1991; Brodeur et al., 1981). A recent study of 372 primary NBs with markers evenly spaced along 14q showed LOH, with a common region of deletion within 14q23-qter (Thompson et al., 2001). Deletions and translocations in bands q11.2, q13 and q24 of chromosome 14 were also often observed in our patients as well. We suggested that losses of 14q11.2, 14q24 and 14q13 regions might play a role in the prognosis of neuroblastoma as well as other previously reported findings. Other alterations described in our patients included deletions and translocations of 15q, with a common region of deletion within 15q22. Although Krona et al. showed chromosomal loss of chromosome 15q, 15q22 was not reported previously in NB. In addition to this result, the loss of 13q14 region was not determined in present studies. Therefore these chromosomal losses can be emphasized in the importance of NB prognosis with further studies.

Aneuploidy is a commonly observed feature in neuroblastic tumours, and has been proposed to drive tumor development by enhancing genomic instability (Ambros et al., 1996). We reported the aneuploidies of chromosomes 3, 17, 18, 22 and X as common findings in 26 cells of patients. Chromosome X aneuploidies were the most commonly detected aberrations in our patients (Table 1). It appears that chromosome X may be preferentially involved and important for detecting cancer development. The monosomy 22 was a common aneuploidy in our patients. Undoubtedly, further studies are necessary to understand the role of X chromosome changes in NB. The chromosome 22 contains a considerable number of uncharacterized disease genes, glioblastoma and other types of astrocytoma, ependymoma, meningioma, schwannomatosis, pheochromocytoma, breast and colon cancer (Dumanski et al., 1996). In cytogenetic studies of colorectal cancers, numerical aberrations of chromosomes 1, 7, 11, 17 and 18 were reported (Nanashima et al., 1997). Trisomy 18 was frequently observed in breast cancers and neurofibrosarcomas (Pandis et al., 1995). Several studies have reported LOH of chromosome 18q in colorectal cancers and inactivation of tumor suppressor genes, and metastasis suppressor genes, such as deleted in colorectal cancer and in pancreatic carcinoma (Fearon et al., 1990; Hahn et al., 1996). In accordance of these studies, our findings indicated that loss and/or gain of chromosome X, 22, 3, 17 and 18 was important in development of NB.

The molecular biology of NB is characterized by somatically acquired genetic events that lead to gene overexpression, gene inactivation, or alterations in gene expression that correlate to various degrees of prognosis, including MYCN and AURKA oncogenes. We found the mean copies of AURKA gene in 19% and that of MYCN in 23% of nine neuroblastoma patients. In six of nine patients, both MYCN and AURKA genes expression were higher than significance level. Therefore there is a close correlation between amplifications of AURKA and MYCN genes in NB. Otto et al. (2009) reported that AURKA had a critical function in stabilizing MYCN protein through a direct physical interaction and interferes in NBs that carried an amplified MYCN gene. AURKA is a key regulatory component of the MYCN pathway, and this is due to elevated levels of AURKA, which inhibit the mitotic degradation of MYCN in such cells. Notably, the relationship of AURKA and MYCN in NB has properties of a positive feedback loop: expression of AURKA is elevated in MYCN amplified NB and induced by activation of MYCN in culture (Berwanger et al., 2002). AURKA is highly expressed relative to normal tissue and amplified in multiple human tumors (Zhou et al., 1998). Amplification of the AURKA gene has been taken as evidence that the kinase activity of AURKA is under selective pressure during tumorigenesis, and, as a consequence, inhibitors of AURKA kinase are being developed as anticancer therapeutics (Carvajal et al., 2006). In addition to neuroblastoma both MYCN and AURKA are expressed at high levels in glioblastoma, astrocytoma, and prostate carcinoma, suggesting that stabilization of MYCN by AURKA may not be restricted to childhood tumors. Finally, both AURKA and MYCN have been implicated in the genesis of NB, arguing that stabilization of MYCN may contribute to AURKA dependent tumorigenesis in several entities.

Study limitations

The major limitations of this study were the small sample size and had no tumor tissue for all patients. Despite this, the aim of this study was primarily to investigate AURKA and MYCN genes rearrangements and the chromosomal aberrations (CAs) to determine the prognosis of neuroblastoma. Thus the tumor tissues were

not expected to provide enough statistical power to detect significant differences in the efficacy parameters for the FISH results.

In conclusion, 1p36, 1q11, 1q42, 2p23, 3p, 5q31, 6q21, 9q and Xp22, and monosomi 22 may be primer genetic lesions predisposed it to tumorogenesis and indicator the contributions to the pathogenesis of NB. However, the loci 1q11, 1q42, 2p23, 5q31, 6p21, and Xp22 expressed in our patients were not reported previously in NB. Further investigation is also necessary to elucidate the role of these chromosomal regions in tumour biology. This study also indicates that there is a close correlation between amplifications of AURKA and MYCN genes in NB. AURKA and MYCN amplifications could have a role in neuroblastoma development and progression and should be a target for molecular cytogenetic detection in NB at diagnosis. Taken together, our data suggest that deregulation of MYCN may contribute significantly to the oncogenic properties of AURKA.

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