# Novel Structural Abnormalities Involving Chromosomes 1, 17 and 2 Identified by Fluorescence *In Situ* Hybridization (FISH) and/or Cytogenetic Karyotyping in Kelly and SH-SY5Y Human Neuroblastoma Cell Lines, Respectively

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**Abstract:** *MYCN* amplification and 1p36 deletion are important poor prognostic factors in neuroblastoma. 1p36 deletion and unbalanced translocations involving chromosomes 1, 17 and 2p were often reported in neuroblastoma cell lines. We aimed to investigate novel chromosomal abnormalities that are likely to affect neuroblastoma progression in Kelly and SH-SY5Y cell lines. Therefore, we analyzed the metaphase chromosomes using fluorescence *in situ* hybridization (FISH) method with probes specific to the chromosome bands 2p24 and 1p36 in SH-SY5Y and Kelly, respectively. Moreover, the rearrangements of chromosomes 1 and 17 from Kelly were re-examined by cytogenetic karyotyping. FISH analysis shows duplication of chromosome 2p24 on the long arm of a partner chromosome resulted from an unbalanced translocation der(?9)t(2;?9)(p24;q?34) in SH-SY5Y, suggesting that duplication of 2p24 locus containing *MYCN* gene may contribute to triggering *MYCN* amplification in neuroblastoma. On the other hand, FISH and karyotype analyses reveal three copies of chromosome 1 that consist of an intact chromosome 1, an extra derivative chromosome 1 with terminal and interstitial deletions (:p32→q25::q41→qter), and another including only interstitial deletion (pter→q25::q41→qter), leading to monosomy of the long arm segment 1q25-q41 in Kelly. These results suggest that chromosome 1q25-q41 may contain one or more tumour suppressor genes important for neuroblastoma progression. Together, FISH analysis shows that an additional 1p36 locus in Kelly is translocated to the short arm of extra chromosome 17, where the *p53* tumour suppressor gene is located. Consequently, these novel structural abnormalities involving chromosomes 1, 17 and 2 could be contributed to the tumourigenicity of neuroblastoma cells.

**Keywords:** Kelly and SH-SY5Y human neuroblastoma cell lines, novel chromosomal abnormalities, *MYCN* amplification, duplication of chromosome 2p24, interstitial 1q deletion, terminal 1p36 deletion, t(1;17) translocation, fluorescence *in situ* hybridization (FISH), Cytogenetic Karyotyping.

## INTRODUCTION

Neuroblastoma derived from primitive cells of the sympathetic nervous system is the most common solid tumour in childhood [1, 2]. *MYCN* amplification and 1p36 deletion are important predictors of poor prognosis in neuroblastoma [3-6]. *MYCN* amplicons are carried via homogeneously staining regions (hsrs) or double minutes (dmins) that are the extrachromosomal structures in *MYCN*-amplified neuroblastoma cell lines [7, 8]. The short arm rearrangements of chromosome 2 formed by unbalanced translocations with various partner chromosomes resulting in consistent gain of chromosome segment 2p22-pter including *MYCN* gene were detected in many neuroblastoma cell lines [9].

In addition, 1p36 deletion and unbalanced t(1;17) translocations involving the short arm of chromosome 1 are often observed in neuroblastoma cell lines [10, 11]. Moreover, it was reported the gain of chromosome 17q23.1-qter resulting from unbalanced translocations with numerous chromosomal partners in more than 90% of untreated high-grade neuroblastomas [12]. Gain of 17q is associated with 1p and/or 11q deletions as well as advanced stage in primary neuroblastoma tumours [13]. The neuroblastoma tumours with 17q gain lacking *MYCN*-amplification or 11q deletion have worse prognosis than those without 17q gain [14]. On the other hand, 11q deletion is a poor prognostic factor independent from *MYCN* amplification in high-risk neuroblastoma tumours [14].

The loss of heterozygosity at 19q13.3 occurs primarily in a defined subgroup of high-risk neuroblastoma patients with propensity for local recurrence [15]. Trisomy of chromosomes 1, 17 and their long arms is also observed in human neuroblastomas [16]. Chromosome 1q21-q25 gain is strongly associated with progressive stage 4 neuroblastoma resistant to aggressive treatment [17].

To investigate novel chromosomal abnormalities, we analyzed the metaphase chromosomes using fluorescence *in situ* hybridization (FISH) method in Kelly and SH-SY5Y human neuroblastoma cell lines. In addition, the rearrangements of chromosomes 1 and 17 from Kelly cells were re-examined by cytogenetic karyotyping. In conclusion, FISH analysis showed an

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unbalanced translocation der(?9)t(2;?9)(p24;q?34) in SH-SY5Y. On the other hand, Kelly cells harbour an interstitial deletion on two of three chromosomes 1 leading to monosomy of the long arm segment 1q25-q41, suggesting that chromosome 1q25-q41 is likely to contain one or more tumour suppressor genes important for neuroblastoma progression. In addition, Kelly carries a translocation der(17)t(1;17)(p36;p13). These novel structural abnormalities involving chromosomes 1, 17 and 2 may play a key role in the tumourigenicity of neuroblastoma cells.

## MATERIALS AND METHODS

### Ethics Approval

All the experiments were carried out in established human neuroblastoma cell lines. This study was approved by the Local Ethics Committee of Clinical and Laboratory Researches of the Faculty of Medicine, Dokuz Eylul University (04.10.2005/223, Protocol No: 188).

### Cell Culture

Kelly (ACC 355) and SH-SY5Y (ACC 209) cell lines were purchased from the 'German Collection of Microorganisms and Cell Cultures' (DSMZ, Braunschweig, Germany). Kelly cells were cultured in RPMI 1640 (Biochrom AG, Ankara, Turkey) supplemented with 10% foetal bovine serum (FBS) (Biochrom AG, Ankara, Turkey), 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. SH-SY5Y cells were grown in Dulbecco's modified Eagle's medium (DMEM) (Biochrom AG, Ankara, Turkey) supplemented with 20% FBS, 2 mM L-glutamine, 100 units/ml penicillin and 100 µg/ml streptomycin. Cells were incubated at 37 °C in a humidified atmosphere containing 5% $CO_2$.

### Fluorescence *In Situ* Hybridization (FISH)

FISH method was performed as previously described [6]. *MYCN* gene (2p24)/Chromosome 2 Alpha-Satellite (Qbiogene, Ankara, Turkey; cat. no., PONC0224), 1p36/Chr 1 satellite enumeration (SE) (Kreatech/Poseidon, Ankara, Turkey; cat. no., KB-10705) and 1p36/1q25 (Abbott Molecular/Vysis, Istanbul, Turkey; cat. no., 32-231004) probes were used in FISH experiments.

Neuroblastoma cells were seeded into 25 $cm^2$ tissue culture flasks and grown in culture medium until cells reach near-confluency in a humidified atmosphere containing 5% $CO_2$ at 37 °C. The cells were detached with trypsin-EDTA (Biochrom AG, Ankara, Turkey) solution and later transferred into sterile polystyrene tubes containing 5 ml culture medium. After 80 µl colcemid per tube was added, the cells were incubated at 37 ºC in a water bath for 30 minutes. After centrifugation, 8 ml hypotonic solution (0.075 M) (Biochrom AG, Ankara, Turkey) was added to each cell pellet, and tubes were incubated at 37 ºC in a water bath for 30 minutes. The cell pellets were fixed five times with 5 ml Carnoy's fixative (3:1 Methanol: Glacial Acetic acid; Merck) after centrifugation. After the homogenized cells were dropped onto slides, the slides were incubated in a coplin jar containing 2X SSC/0.5% NP-40 at 37 ºC in a water bath for 30 minutes.

For denaturing double-stranded DNA, the slides were incubated at 70 ˚C for two minutes (five minutes for Vysis 1p36 probe) in a coplin jar containing 70% formamide/2X SSC after they were dehydrated in 70%, 85% and 96% ethanol series, respectively. Probes were denatured at 96 ˚C in a water bath for five minutes (five and ten minutes at 75 ˚C for Vysis and Poseidon 1p36 probes, respectively) in a 2 ml eppendorf tube. After the probes were added into hybridization areas on the slides, the cover slips were sealed to the slides with rubber cement, followed by overnight incubation at 37 ˚C in a hybridization box. Slides were washed in 0.5X SSC/0.1% SDS, 1X PBD including NP40 and Tween 20, and 70% ethanol solutions, respectively. Cell nuclei were counterstained with 4',6-diamidino-2-phenylindole II (DAPI II, Abbott Molecular/Vysis, Istanbul, Turkey; cat. no., 30-804841) suspended in an antifade solution.

### Dual-Color FISH Analysis

FISH slides were analyzed at 100x magnification on a Nikon Eclipse E600 epifluorescence microscope (Nikon corp., Tokyo, Japan) equipped with DAPI, FITC, rhodamine and triple band-pass filter sets. FISH images from interphase/metaphase areas were captured using high-sensitivity monochrome charge-coupled device camera integrated with a Macintosh computer and processed with MacProbe imaging software (PCI Scientific Systems).

The *MYCN* gene (2p24)/Chromosome 2 Alpha-Satellite (Qbiogene) FISH probe is a dual-color probe. The *MYCN* gene (2p24) locus-specific DNA probe is direct-labelled with Rhodamine (red signal), while the chromosome 2 alpha satellite DNA probe (internal control) is direct-labelled with Fluorescein (green

signal). In FISH analyses, two red and two green signals for *MYCN* gene and internal control per diploid genome were considered normal. Three, four and five red signals versus two green signals per diploid genome show low copy number of *MYCN* gene. Six, seven, eight, nine and ten red signals versus two green signals per diploid genome reveal intermediate copy number of *MYCN* gene, while red signals more than 10 versus two green signals per diploid genome indicate high copy number of *MYCN* gene.

1p36/Chr 1 SE (Poseidon) FISH probe is a dual-color probe. 1p36 probe is direct-labeled with Platinum *Bright* 550 (red signal), while the chromosome 1 SE probe (internal control) is direct-labeled with Platinum *Bright* 495 (green signal). 1p36/1q25 (Vysis) FISH probe is also dual-color probe for 1p36 locus. 1p36 probe is labeled with Spectrum Orange, while 1q25 probe (internal control) is labeled with Spectrum Green. 1p36 alterations were analyzed according to the guidelines of European Neuroblastoma Quality Group (ENQUA) [18]. In FISH analyses, two orange/red and two green signals for 1p36 and internal control per diploid genome were considered normal. Only one orange/red signal versus at least two green signals per diploid genome was considered 1p36 deletion. Two orange/red signals versus three green signals per diploid genome and so forth (represents at least one more signal number of control than that of 1p36 probe) were considered 1p36 imbalance. Three orange/red and three green signals per diploid genome were considered 1p36-3/3 balanced alteration. Four orange/red signals versus three green signals per diploid genome were considered 1p36-4/3 over-balanced alteration. Three orange/red signals versus two green signals per diploid genome were considered 1p36-3/2 over-balanced alteration.

### Cytogenetic Karyotyping

Fixed cells dropped onto slides were prepared exactly as described in FISH method. After the slides were baked overnight at 65 ºC followed by three hours at 80 ºC, the chromosome banding was performed using trypsin-Giemsa technique [19]. The slides were analyzed using a Nikon Eclipse E600 light microscope (Nikon corp., Tokyo, Japan). Karyotyping was carried out with MacKtype software. Chromosomal aberrations were named according to the International System for Human Cytogenetic Nomenclature (ISCN 1995) [20].

## RESULTS

To investigate the chromosomal abnormalities involving 1p and 2p in Kelly and SH-SY5Y neuroblastoma cell lines, respectively, we performed molecular cytogenetic analysis of the metaphase spreads using FISH method with probes specific to the chromosome bands 1p36 and 2p24 including *MYCN* gene.

### An Unbalanced Translocation der(?9)t(2;?9)(p24; q?34) Causing Duplication of 2p24 in SH-SY5Y Cells

In SH-SY5Y cell line, four metaphases and six interphase areas hybridized with *MYCN* gene

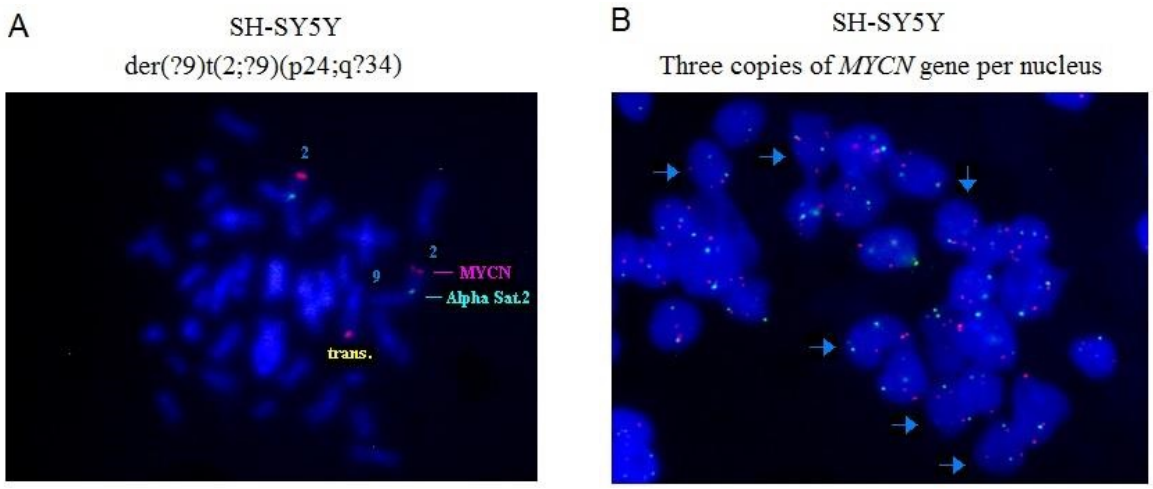


**Figure 1:** Duplication of chromosome 2p24 locus including *MYCN* gene in SH-SY5Y cells.

(**A**) An unbalanced translocation of 2p24 locus including *MYCN* gene onto probably chromosome 9q34 was detected using FISH method. (**B**) Three copies of *MYCN* gene versus two copies of internal control in interphase nuclei are indicated by arrows. FISH probe: *MYCN* gene (2p24)/Chromosome 2 Alpha-Satellite (red/green, Qbiogene, cat. no., PONC0224). Abbreviations: Alpha Sat. 2, alpha satellite 2; FISH, fluorescence *in situ* hybridization; trans., translocation.

(2p24)/Chromosome 2 Alpha-Satellite (red/green, Qbiogene) probe were analyzed. FISH analyses show that SH-SY5Y cells harbour an unbalanced translocation der(?9)t(2;?9)(p24;q?34) detected in four metaphases (Figure **1A**), causing the duplication of chromosome 2p24 locus including *MYCN* gene probably at band q34 of the long arm of chromosome 9. Three copies of *MYCN* gene that were mostly detected in interphase nuclei from SH-SY5Y cells may be occurred through the duplication of 2p24 locus (Figure **1B**).

**Mainline Clone with 1p36-3/3 Balanced Alteration from Kelly Cells Harbours Novel Structural Abnormalities such as an Interstitial Deletion del(1)(q25q41) and a Translocation der(17) t(1;17)(p36;p13)**

Present FISH analyses confirm that Kelly cells harbour 2p24 hsr containing *MYCN* amplification on the q arm of both chromosomes 17 (Figure **2A**), which is among previously identified chromosomal abnormalities (Figure **2A**). On the other hand, Kelly cell clones with different signal ratios for 1p36/Internal control carry novel structural aberrations involving chromosomes 1 and 17 detected by FISH method, with the exception of 1p36 deletion (Table **1**).

To identify the breakpoint location of novel chromosomal abnormalities in Kelly, cytogenetic karyotype analysis from four metaphases was also done. As compared with a cytogenetic karyotype (Figure **2B**), FISH analysis reveals that the mainline clone of Kelly cells harbouring del(1p), del(1q) and a translocation der(17)t(1;17)(p36;p13) detected in 35 metaphases has a signal ratio of 3/3 of 1p36/Internal control probe set hybridized to specific loci on three chromosomes 1 and one chromosome 17 (classified based on shape and size) (Figure **2B**). In Kelly, 1p36-3/3 balanced alteration from 500 interphase/metaphase cells analyzed by FISH method has the highest percentage (49.8%), whereas 1p36 deletion was observed at very low level (0.1%) in only interphase cells (our unpublished data).

FISH and cytogenetic analyses reveal that the mainline clone 3/3 contains two chromosomes 1 with conserved 1p36 locus, but one of them bears deletion of the interstitial segment 1q25-q41 (Figure **3A**, **B**), at the same time, this clone harbours an extra derivative chromosome 1 with deletion of the segments 1p32-pter and 1q25-q41 (Figure **3C**), leading to monosomy of the long arm segment 1q25-q41 in Kelly. Therefore, chromosome 1q25-q41 is likely to contain one or more tumour suppressor genes important for neuroblastoma progression.

In addition, it was observed an extra chromosome 17 (Figure **3D**), besides two chromosomes 17 that carry 2p24 hsr containing *MYCN* amplification (Figure **3E**). Moreover, FISH analysis showed that an additional 1p36 locus is translocated to the short arm of extra chromosome 17 (Figure **3D**) including an insertion ins(17;?)(q12~q21;?) identified by karyotype analysis (Figures **2B** and **3D**).

**Sideline Clones with 1p36-4/3 and 1p36-3/2 Over-Balanced Alterations from Kelly also Harbour del(1q) and der(17)t(1;17)(p36;p13)**

Present FISH analyses reveal that the sideline 1 clone with a signal ratio of 4/3 of 1p36/Internal control from Kelly cells includes two extra chromosomes 17 fused with 1p36 locus besides chromosome 1 abnormalities like those of mainline clone 3/3, which was detected in five metaphases (Figure **4A**). In addition, the sideline 2 clone with a signal ratio of 3/2 of 1p36/Internal control harbours the structural abnormalities like those of mainline clone 3/3 except extra chromosome 1 detected in three metaphases

**Table 1: Characteristic Features of Kelly Cell Clones with Different Signal Ratios for 1p36/Internal Control[a]**

| 1p36/1SE or 1q25 Cell clone | 3/3 mainline | 4/3 sideline 1 | 3/2 sideline 2 | 2/2 stemline |
|---|---|---|---|---|
| Metaphase number | 35 (77.78%) | 5 (11.11%) | 3 (6.67%) | 2 (4.44%) |
| Chromosome 1 | x3 | x3 | x2 | x2 |
| Extra derivative chr. 1 | + | + | - | - |
| der(17)t(1;17) | x1 | x2 | x1 | x1 |
| 1p36 deletion | + | + | - | + |
| 1q deletion | x2 | x2 | x1 | ? |

[a]Signal ratio of 1p36/Internal control and chromosomal abnormalities in metaphases were determined using FISH method. The plus (+) and minus (-) signs indicate presence and absence, respectively. Abbreviations: Chr., chromosome; FISH, fluorescence *in situ* hybridization; SE, satellite enumeration.

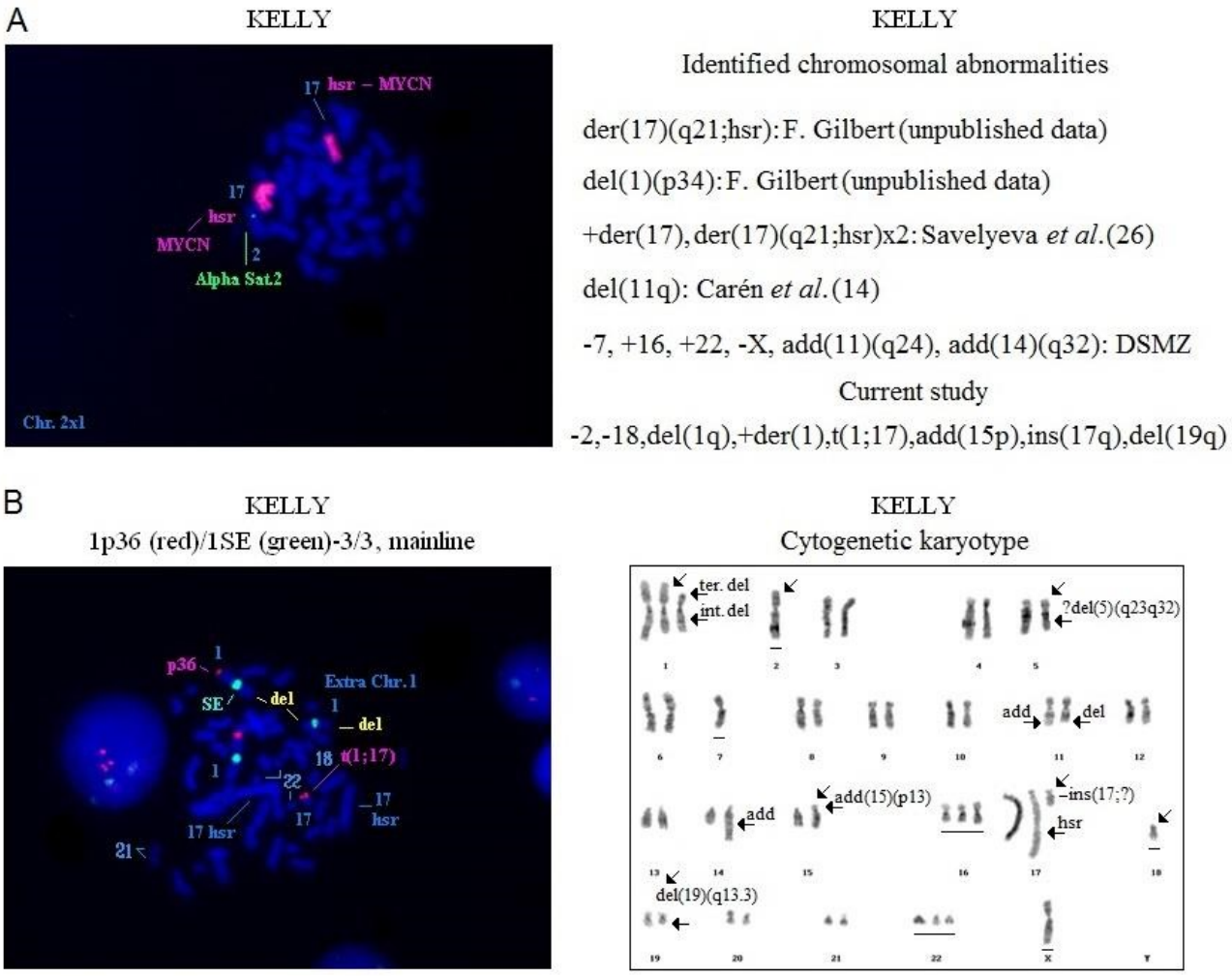


**Figure 2:** Chromosome 1 abnormalities of the mainline clone in *MYCN*-amplified Kelly cells.

(**A**) The 2p24 hsr containing *MYCN* amplification from Kelly cells is located on the q arm of both chromosomes 17. Kelly also harbours many previously identified chromosomal abnormalities along with newly detected ones here. FISH probe: *MYCN* gene (2p24)/ Chromosome 2 Alpha-Satellite (red/green, Qbiogene, cat. no., PONC0224). (**B**) Mainline clone 3/3 from Kelly cell line carries three copies of chromosome 1 that consist of an intact chromosome 1, an extra derivative chromosome 1 with del(1p) and del(1q) and another including only same del(1q). This clone also contains the translocation der(17)t(1;17)(p36;p13). In addition, a cytogenetic karyotype from Kelly cells confirms the structural abnormalities of chromosome 1 detected by FISH. Top arrows (karyotype) indicate novel chromosomal aberrations of Kelly cells identified in our study. FISH probe: 1p36/1SE (red/green, Kreatech/Poseidon, cat. no., KB-10705). Abbreviations: Alpha Sat. 2, alpha satellite 2; Chr., chromosome; FISH, fluorescence *in situ* hybridization; hsr, homogeneously staining region; int., interstitial; SE, satellite enumeration; ter., terminal.

(Figure **4B**). On the other hand, stemline clone with a signal ratio of 2/2 of 1p36/Internal control contains a 1p36 deletion and a translocation der(17)t(1;17)(p36; p13) detected in two metaphases (Figure **4C**).

## DISCUSSION

This study presents multiple structural and numerical chromosomal abnormalities newly identified by metaphase FISH and/or cytogenetic karyotyping in Kelly and SH-SY5Y cell lines (Table **2**). As a result of FISH analysis, we report here an unbalanced translocation der(?9)t(2;?9)(p24;q?34) in SH-SY5Y cells (Figure **1A**). By cytogenetic karyotyping, an addition at band 9q34 from SH-SY5Y cell line was demonstrated by Khan *et al.* [21] and DSMZ. In addition, Do *et al.* [22] showed a chromosomal gain in the segment between bands 2p16.3 and 2p25.3 containing *MYCN* gene locus, which is identified by analyzing array-CGH data with CGH-Miner program in SH-SY5Y cells. In some neuroblastoma cases, partial duplication of chromosome 2p including *MYCN* gene at bands 13q34, 17q25 and 18q23 resulted from an unbalanced translocation was reported [23-25]. Present findings suggest that duplication of *MYCN* gene may contribute to triggering *MYCN* amplification in neuroblastoma.

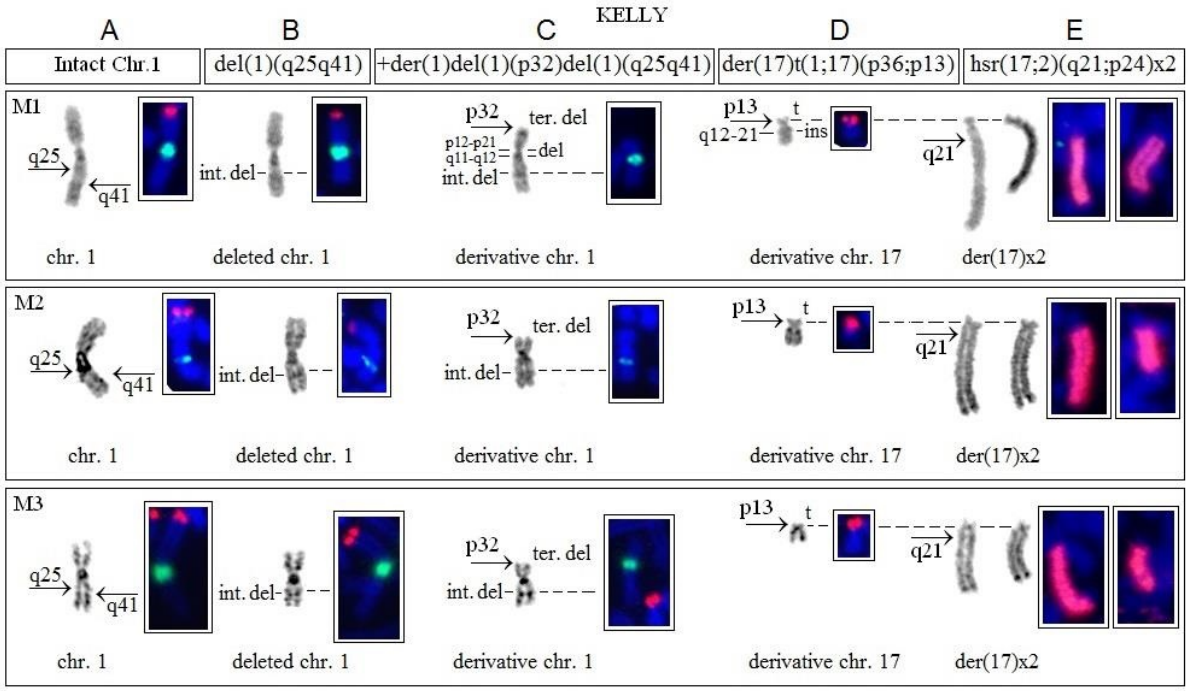


**Figure 3:** Chromosome 1 rearrangements and t(1;17) translocation in Kelly.

In Kelly, chromosomes 1 and 17 from three metaphase spreads (M1-M3) prepared by cytogenetic method are presented along with those prepared by FISH method. First three columns show (**A**) an intact chromosome 1, (**B**) a chromosome 1 deleted between bands 1q25 and 1q41 (pter→q25::q41→qter) and (**C**) an extra derivative chromosome 1 with terminal and interstitial deletions (:p32→q25::q41→qter). Derivative chromosome 1 also bears interstitial deletions del(1)(p12p21) and del(1)(q11q12). (**D**) A derivative chromosome 17 resulted from a translocation between chromosomes 1 and 17 (1pter→1p36::17p13→17qter) detected by FISH method is demonstrated (see also Figure **2B**). Besides, this derivative chromosome 17 includes an insertion ins(17;?)(q12~21;?) identified by cytogenetic karyotyping. (**E**) Two derivative chromosomes 17 with 2p24 hsr containing *MYCN* amplification (17pter→17q21::hsr::2p24::17q21→17qter) are shown. FISH probes: (**A-D**) M1, M3; 1p36/1SE (red/green, Kreatech/Poseidon, cat. no., KB-10705), M2; 1p36/1q25 (orange/green, Abbott Molecular/Vysis, cat. no., 32-231004) and (**E**) M1-M3; *MYCN* gene (2p24)/Chromosome 2 Alpha-Satellite (red/green, Qbiogene, cat. no., PONC0224). Arrows indicate chromosome breakpoints. Abbreviations: Chr., chromosome; FISH, fluorescence *in situ* hybridization; hsr, homogeneously staining region; int., interstitial; M, metaphase; SE, satellite enumeration; ter., terminal.

Aygun [26] recently reviewed the proposed mechanisms for the formation of *MYCN* amplification. Neuroblastoma cell lines including Kelly harbour a head-to-tail tandem array of amplicon units containing *MYCN* gene [27], suggesting that *MYCN* amplification can occur via a mechanism other than those involving breakage-fusion-bridge (BFB) cycles that produce inverted duplication [28]. Multiple replication-based mechanisms for the formation of *MYCN* or general gene amplification have been proposed [26, 27, 29-32]. Aygun [26] also showed a significant association between long inverted repeats (LIRs) and both 5' and 3' boundaries of the amplicon units containing *MYCN* gene in 14 neuroblastoma cell lines and 42 other primary solid tumours. In addition, there was found a microhomology of mean 5.18 bp (range: 2-14 bp) between both 150 bp DNA sequences encompassing 5' and 3' boundaries of same study group. Aygun [33] had also previously demonstrated a significant association between LIRs and the breakpoint regions of gross gene deletions in human cancers and inherited diseases. Taken together, our findings support that a replication-based mechanism involving the LIRs using microhomology could be involved in formation of the *MYCN* amplification, as presented in reference [26].

On the other side, our results demonstrate that Kelly cells carry a translocation der(17)t(1;17)(p36;p13) causing a rearrangement on the short arm of extra chromosome 17, where the *p53* gene is located (Figures **2B** and **3D**). Unbalanced 1;17 translocations resulting in integration of a 17q material into the short arm of derivative chromosome 1 were often reported in neuroblastoma cell lines [34]. Savelyeva *et al.* [34] also showed that Kelly cell line harbours an extra derivative chromosome 17 with an additional material of unknown origin besides other two chromosomes 17 containing the hsr (see also Figure **2A**).

*p53* tumour suppressor gene is not expressed under normal conditions in Kelly (our unpublished

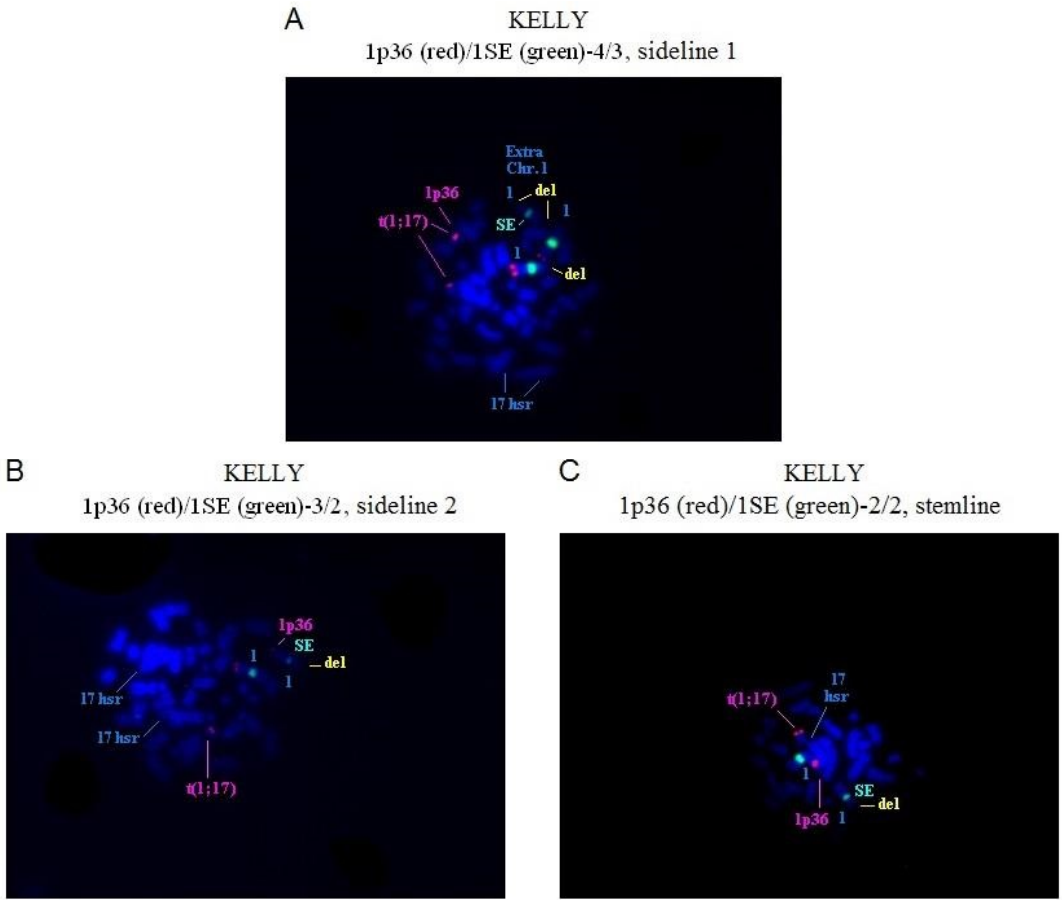


**Figure 4:** Structural abnormalities involving chromosomes 1 and 17 from the sidelines and stemline in Kelly.

(**A**) A sideline 1 metaphase with a signal ratio of 4/3 of 1p36/Internal control from Kelly cells is presented. An intact chromosome 1, a deleted one at 1q, an extra derivative chromosome 1 with 1p and 1q deletions, and also two copies of the translocation der(17)t(1;17)(p36;p13) are indicated. (**B**) A sideline 2 metaphase with a signal ratio of 3/2 of 1p36/Internal control from Kelly cells is presented. An intact chromosome 1, a deleted chromosome 1 at 1q and a translocation der(17)t(1;17)(p36;p13) are indicated. (**C**) A stemline metaphase with a signal ratio of 2/2 of 1p36/Internal control from Kelly cells is presented. A translocation der(17)t(1;17)(p36;p13) and a 1p36 deletion in only one of two chromosomes 1 are indicated. FISH probe: 1p36/1SE (red/green, Kreatech/Poseidon, cat. no., KB-10705). Abbreviations: Chr., chromosome; FISH, fluorescence *in situ* hybridization; hsr, homogeneously staining region; SE, satellite enumeration.

**Table 2: Novel Structural and Numerical Chromosomal Abnormalities Identified in Kelly and SH-SY5Y Cell Lines**

| Chromosomes | Region | Kelly | Zygosity | Method |
|---|---|---|---|---|
| 1 | 1p | del(1)(p32) | 1 of 3 Chr. 1 | FISH+CYT |
| 1 | 1p | del(1)(p12p21) | 1 of 3 Chr. 1 | FISH+CYT |
| 1 | 1q | del(1)(q11q12) | 1 of 3 Chr. 1 | FISH+CYT |
| 1 | 1q | del(1)(q25q41) | 2 of 3 Chr. 1 | FISH+CYT |
| 1;17 | 1p;17p | der(17)t(1;17)(p36;p13) | 1 of 3 Chr. 17 | FISH |
| 2 | whole | -2[a] | hemizygous | FISH+CYT |
| 5 | 5q | ?del(5)(q23q32) | hemizygous | CYT |
| 15 | 15p | add(15)(p13) | heterozygous | CYT |
| 17 | 17q | ins(17;?)(q12~21;?) | 1 of 3 Chr. 17 | CYT |
| 18 | whole | -18 | hemizygous | FISH+CYT |
| 19 | 19q | del(19)(q13.3) | hemizygous | CYT |
| Chromosomes | Region | SH-SY5Y | Zygosity | Method |
| 2;9 | 2p;9q | der(?9)t(2;?9)(p24;q?34) | heterozygous | FISH |

[a]This finding was used as unpublished data in reference (26). Abbreviations: Chr., chromosome; CYT, cytogenetic; FISH, fluorescence *in situ* hybridization.

data), suggesting that the translocation t(1;17)(p36;p13) may impair transcriptional expression of *p53* gene located at 17p13 [35]. However, it remains to be determined whether *p53* gene is transcriptionally and/or epigenetically repressed in other chromosomes 17 containing 2p24 hsr. A constitutional balanced translocation t(1;17)(p36.2;q11.2) disrupts an isoform of *ACCN1*, a potential glioma tumour suppressor gene, overlapped with chromosome 17q breakpoint in a neuroblastoma patient [36].

FISH and karyotype analyses also reveal three copies of chromosome 1 that consist of an extra derivative chromosome 1 with terminal del(1p) and interstitial del(1q), another including only same del(1q) and an intact one, which are observed in mainline and sideline 1 clones, leading to monosomy of the long arm segment 1q25-q41 in Kelly (Figures **2B**, **3A-C** and **4A**). These findings suggest that chromosome 1q25-q41 may contain one or more tumour suppressor genes important for neuroblastoma progression. Taken together, our results show that mainline clone 3/3 acquired a proliferative advantage via translocation der(17)t(1;17)(p36;p13) and extra chromosome 1 with terminal and interstitial deletions in Kelly.

The expression level of differentially expressed genes from the long arm segment q21.2-q42 of chromosome 1 was mostly higher in spontaneously regressing stage 4s than in stage 4 neuroblastoma [37]. In this regard, monosomy of the long arm segment q25-q41 of chromosome 1 might be associated with advanced stage neuroblastoma. A new recurrent distal chromosome 1q deletion del(1)(q42.2qter) was mostly found in high risk 11q-deleted tumours without *MYCN* amplification [38].

A 195 kb gene cluster encoding interleukin-10 (IL-10) cytokine family members IL-10, IL-19, IL-20 and mda-7 (IL-24) maps to human chromosome 1q32 [39, 40], moreover, IL-10 is significantly lower expressed in mostly *MYCN*-amplified high risk neuroblastoma patients [41], and in neuroblastoma cell lines [42, 43]. In addition, pro-inflammatory cytokine IL-1β could not induce the expression of IL-10 in high risk SK-N-SH neuroblastoma cell line [41]. These papers suggest that a deletion at 1q32 may frequently occur in high risk neuroblastoma. In addition, in SH-SY5Y cells, IL-24 promotes differentiation, growth inhibition and apoptosis, whereas it blocks proliferation, furthermore, adenovirus-mediated IL-24 significantly inhibits tumour growth *in vivo* in a xenograft neuroblastoma tumour in athymic nude mice [44, 45]. *IL-24* functions as a tumour suppressor gene *in vivo* in a breast cancer developed in immune-competent transgenic mice [46].

In this study, we found novel structural abnormalities involving chromosomes 1, 17 and 2 using FISH method in Kelly and SH-SY5Y neuroblastoma cell lines, respectively. In addition, we identified the breakpoint location of chromosome 1 rearrangements by cytogenetic karyotyping in Kelly. Our analyses demonstrated the structural chromosomal abnormalities involving the translocation der(17)t(1;17)(p36;p13) and deletion del(1)(q25q41) in Kelly, and duplication of 2p24 in SH-SY5Y. These novel structural chromosomal abnormalities could be contributed to the tumourigenicity of neuroblastoma cells. Taken together, our findings provide new insights into clonal evolution of neuroblastoma cells. Future studies are needed to investigate these chromosomal aberrations and their effect on survival in neuroblastoma patients.

## CONFLICTS OF INTEREST

The authors declare no conflict of interest.

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## REFERENCES

[1] Gurney JG, Ross JA, Wall DA, Bleyer WA, Severson RK, Robison LL. Infant cancer in the U.S.: histology-specific incidence and trends, 1973 to 1992. J Pediatr Hematol Oncol 1997; 19: 428-32. https://doi.org/10.1097/00043426-199709000-00004

[2] Brodeur GM. Neuroblastoma: biological insights into a clinical enigma. Nat Rev Cancer 2003; 3: 203-16. https://doi.org/10.1038/nrc1014

[3] Brodeur GM, Seeger RC, Schwab M, Varmus HE, Bishop JM. Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. Science 1984; 224: 1121-4. https://doi.org/10.1126/science.6719137

[4] Seeger RC, Brodeur GM, Sather H, Dalton A, Siegel SE, Wong KY, *et al*. Association of multiple copies of the N-myc oncogene with rapid progression of neuroblastomas. N Engl J Med 1985; 313: 1111-6. https://doi.org/10.1056/NEJM198510313131802

[5] Spitz R, Hero B, Westermann F, Ernestus K, Schwab M, Berthold F. Fluorescence in situ hybridization analyses of chromosome band 1p36 in neuroblastoma detect two classes of alterations. Genes Chromosomes Cancer 2002; 34: 299-305. https://doi.org/10.1002/gcc.10070

[6] Altungoz O, Aygun N, Tumer S, Ozer E, Olgun N, Sakizli M. Correlation of modified Shimada classification with MYCN

and 1p36 status detected by fluorescence in situ hybridization in neuroblastoma. Cancer Genet Cytogenet 2007; 172: 113-9. https://doi.org/10.1016/j.cancergencyto.2006.10.005

[7] Solovei I, Kienle D, Little G, Eils R, Savelyeva L, Schwab M, *et al*. Topology of double minutes (dmins) and homogeneously staining regions (HSRs) in nuclei of human neuroblastoma cell lines. Genes Chromosomes Cancer 2000; 29: 297-308. https://doi.org/10.1002/1098-2264(2000)9999:9999<::AID-GCC1046>3.0.CO;2-H

[8] Storlazzi CT, Lonoce A, Guastadisegni MC, Trombetta D, D'Addabbo P, Daniele G, *et al*. Gene amplification as double minutes or homogeneously staining regions in solid tumors: origin and structure. Genome Res 2010; 20: 1198-206. https://doi.org/10.1101/gr.106252.110

[9] Van Roy N, Van Limbergen H, Vandesompele J, Van Gele M, Poppe B, Laureys G, *et al*. Chromosome 2 short arm translocations revealed by M-FISH analysis of neuroblastoma cell lines. Med Pediatr Oncol 2000; 35: 538-40. https://doi.org/10.1002/1096-911X(20001201)35:6<538::AID-MPO8>3.0.CO;2-C

[10] White PS, Thompson PM, Gotoh T, Okawa ER, Igarashi J, Kok M, *et al*. Definition and characterization of a region of 1p36.3 consistently deleted in neuroblastoma. Oncogene 2005; 24: 2684-94. https://doi.org/10.1038/sj.onc.1208306

[11] Van Roy N, Laureys G, Cheng NC, Willem P, Opdenakker G, Versteeg R, *et al*. 1;17 translocations and other chromosome 17 rearrangements in human primary neuroblastoma tumors and cell lines. Genes Chromosomes Cancer 1994; 10: 103-14. https://doi.org/10.1002/gcc.2870100205

[12] Meddeb M, Danglot G, Chudoba I, Vénuat AM, Bénard J, Avet-Loiseau H, *et al*. Additional copies of a 25 Mb chromosomal region originating from 17q23.1-17qter are present in 90% of high-grade neuroblastomas. Genes Chromosomes Cancer 1996; 17: 156-65. https://doi.org/10.1002/(SICI)1098-2264(199611)17:3<156::AID-GCC3>3.0.CO;2-3

[13] Plantaz D, Mohapatra G, Matthay KK, Pellarin M, Seeger RC, Feuerstein BG. Gain of chromosome 17 is the most frequent abnormality detected in neuroblastoma by comparative genomic hybridization. Am J Pathol 1997; 150: 81-9.

[14] Carén H, Kryh H, Nethander M, Sjöberg RM, Träger C, Nilsson S, *et al*. High-risk neuroblastoma tumors with 11q-deletion display a poor prognostic, chromosome instability phenotype with later onset. Proc Natl Acad Sci U S A 2010; 107: 4323-8. https://doi.org/10.1073/pnas.0910684107

[15] Mora J, Cheung NK, Chen L, Qin J, Gerald W. Loss of heterozygosity at 19q13.3 is associated with locally aggressive neuroblastoma. Clin Cancer Res 2001; 7: 1358-61.

[16] Gilbert F, Feder M, Balaban G, Brangman D, Lurie DK, Podolsky R, *et al*. Human neuroblastomas and abnormalities of chromosomes 1 and 17. Cancer Res 1984; 44: 5444-9.

[17] Hirai M, Yoshida S, Kashiwagi H, Kawamura T, Ishikawa T, Kaneko M, *et al*. 1q23 gain is associated with progressive neuroblastoma resistant to aggressive treatment. Genes Chromosomes Cancer 1999; 25: 261-9. https://doi.org/10.1002/(SICI)1098-2264(199907)25:3<261::AID-GCC8>3.0.CO;2-#

[18] Ambros IM, Benard J, Boavida M, Bown N, Caron H, Combaret V, *et al*. Quality assessment of genetic markers used for therapy stratification. J Clin Oncol 2003; 21: 2077-84. https://doi.org/10.1200/JCO.2003.03.025

[19] Seabright M. A rapid banding technique for human chromosomes. Lancet 1971; 2: 971-2. https://doi.org/10.1016/S0140-6736(71)90287-X

[20] Mitelman F. ISCN (1995): An international system for human cytogenetic nomenclature. Mitelman F (ed). Basel: S. Karger Press; 1995.

[21] Khan FH, Pandian V, Ramraj S, Natarajan M, Aravindan S, Herman TS, *et al*. Acquired genetic alterations in tumor cells dictate the development of high-risk neuroblastoma and clinical outcomes. BMC Cancer 2015; 15: 514. https://doi.org/10.1186/s12885-015-1463-y

[22] Do JH, Kim IS, Park TK, Choi DK. Genome-wide examination of chromosomal aberrations in neuroblastoma SH-SY5Y cells by array-based comparative genomic hybridization. Mol Cells 2007; 24: 105-12.

[23] Patel JS, Pearson J, Willatt L, Andrews T, Beach R, Green A. Germline duplication of chromosome 2p and neuroblastoma. J Med Genet 1997; 34: 949-51. https://doi.org/10.1136/jmg.34.11.949

[24] Yuksel A, Seven M, Karaman B, Yilmaz S, Deviren A, Hacihanefioglu S, *et al*. Neuroblastoma in a dysmorphic girl with a partial duplication of 2p caused by an unbalanced translocation. Clin Dysmorphol 2002; 11: 39-42. https://doi.org/10.1097/00019605-200201000-00008

[25] Lipska BS, Koczkowska M, Wierzba J, Ploszynska A, Iliszko M, Izycka-Swieszewska E, *et al*. On the significance of germline cytogenetic rearrangements at MYCN locus in neuroblastoma. Mol Cytogenet 2013; 6: 43. https://doi.org/10.1186/1755-8166-6-43

[26] Aygun N. Acquired chromosomal abnormalities and their potential formation mechanisms in solid tumours. In: Larramendy ML, Soloneski S, editors. Chromosomal abnormalities - a hallmark manifestation of genomic instability. InTech 2017; p. 27-70. https://doi.org/10.5772/67733

[27] Amler LC, Schwab M. Amplified N-myc in human neuroblastoma cells is often arranged as clustered tandem repeats of differently recombined DNA. Mol Cell Biol 1989; 9: 4903-13. https://doi.org/10.1128/MCB.9.11.4903

[28] Lo AW, Sabatier L, Fouladi B, Pottier G, Ricoul M, Murnane JP. DNA amplification by breakage/fusion/bridge cycles initiated by spontaneous telomere loss in a human cancer cell line. Neoplasia 2002; 4: 531-8. https://doi.org/10.1038/sj.neo.7900267

[29] Schwab M. Human neuroblastoma: from basic science to clinical debut of cellular oncogenes. Naturwissenschaften 1999; 86: 71-8. https://doi.org/10.1007/s001140050574

[30] Watanabe T, Tanabe H, Horiuchi T. Gene amplification system based on double rolling-circle replication as a model for oncogene-type amplification. Nucleic Acids Res 2011; 39: e106. https://doi.org/10.1093/nar/gkr442

[31] Blumrich A, Zapatka M, Brueckner LM, Zheglo D, Schwab M, Savelyeva L. The FRA2C common fragile site maps to the borders of MYCN amplicons in neuroblastoma and is associated with gross chromosomal rearrangements in different cancers. Hum Mol Genet 2011; 20: 1488-501. https://doi.org/10.1093/hmg/ddr027

[32] Slack A, Thornton PC, Magner DB, Rosenberg SM, Hastings PJ. On the mechanism of gene amplification induced under stress in Escherichia coli. PLoS Genet 2006; 2: e48. https://doi.org/10.1371/journal.pgen.0020048

[33] Aygun N. Correlations between long inverted repeat (LIR) features, deletion size and distance from breakpoint in human gross gene deletions. Sci Rep 2015; 5: 8300. https://doi.org/10.1038/srep08300

[34] Savelyeva L, Corvi R, Schwab M. Translocation involving 1p and 17q is a recurrent genetic alteration of human neuroblastoma cells. Am J Hum Genet 1994; 55: 334-40.

[35] McBride OW, Merry D, Givol D. The gene for human p53 cellular tumor antigen is located on chromosome 17 short arm (17p13). Proc Natl Acad Sci USA 1986; 83: 130-4. https://doi.org/10.1073/pnas.83.1.130

[36] Vandepoele K, Andries V, Van Roy N, Staes K, Vandesompele J, Laureys G, *et al*. A constitutional translocation t(1;17)(p36.2;q11.2) in a neuroblastoma patient disrupts the human NBPF1 and ACCN1 genes. PLoS One 2008; 3: e2207. https://doi.org/10.1371/journal.pone.0002207

[37] Lavarino C, Cheung NK, Garcia I, Domenech G, de Torres C, Alaminos M, *et al*. Specific gene expression profiles and chromosomal abnormalities are associated with infant disseminated neuroblastoma. BMC Cancer 2009; 9: 44. https://doi.org/10.1186/1471-2407-9-44

[38] Fieuw A, Kumps C, Schramm A, Pattyn F, Menten B, Antonacci F, *et al*. Identification of a novel recurrent 1q42.2-1qter deletion in high risk MYCN single copy 11q deleted neuroblastomas. Int J Cancer 2012; 130: 2599-606. https://doi.org/10.1002/ijc.26317

[39] Blumberg H, Conklin D, Xu WF, Grossmann A, Brender T, Carollo S, *et al*. Interleukin 20: discovery, receptor identification, and role in epidermal function. Cell 2001; 104: 9-19. https://doi.org/10.1016/S0092-8674(01)00187-8

[40] Huang EY, Madireddi MT, Gopalkrishnan RV, Leszczyniecka M, Su Z, Lebedeva IV, *et al*. Genomic structure, chromosomal localization and expression profile of a novel melanoma differentiation associated (*mda*-7) gene with cancer specific growth suppressing and apoptosis inducing properties. Oncogene 2001; 20: 7051-63. https://doi.org/10.1038/sj.onc.1204897

[41] Gowda M, Godder K, Kmieciak M, Worschech A, Ascierto ML, Wang E, *et al*. Distinct signatures of the immune responses in low risk versus high risk neuroblastoma. J Transl Med 2011; 9: 170. https://doi.org/10.1186/1479-5876-9-170

[42] Xu Y, Sun J, Sheard MA, Tran HC, Wan Z, Liu WY, *et al*. Lenalidomide overcomes suppression of human natural killer cell anti-tumor functions by neuroblastoma microenvironment-associated IL-6 and TGFβ1. Cancer Immunol Immunother 2013; 62: 1637-48. https://doi.org/10.1007/s00262-013-1466-y

[43] Asgharzadeh S, Salo JA, Ji L, Oberthuer A, Fischer M, Berthold F, *et al*. Clinical significance of tumor-associated inflammatory cells in metastatic neuroblastoma. J Clin Oncol 2012; 30: 3525-32. https://doi.org/10.1200/JCO.2011.40.9169

[44] Li Y, Zhang H, Zhu X, Feng D, Gong J, Han T. Interleukin-24 induces neuroblastoma SH-SY5Y cell differentiation, growth inhibition, and apoptosis by promoting ROS production. J Interferon Cytokine Res 2013; 33: 709-14. https://doi.org/10.1089/jir.2013.0004

[45] Zhuo B, Wang R, Yin Y, Zhang H, Ma T, Liu F, *et al*. Adenovirus arming human IL-24 inhibits neuroblastoma cell proliferation *in vitro* and xenograft tumor growth *in vivo*. Tumor Biol 2013; 34: 2419-26. https://doi.org/10.1007/s13277-013-0792-1

[46] Menezes ME, Shen XN, Das SK, Emdad L, Guo C, Yuan F, *et al*. MDA-7/IL-24 functions as a tumor suppressor gene *in vivo* in transgenic mouse models of breast cancer. Oncotarget 2015; 6: 36928-42. https://doi.org/10.18632/oncotarget.6047

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