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MYCN gene amplification in patients with neuroblastic tumors

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Abstract
Although neuroblastic tumors are the most prevalent solid tumors, little is known about the genetic basis underlying their progression. The prognostic role for the MYCN gene in neuroblastic tumors is irrefutable. The aim of this study is to identify the frequency of MYCN gene amplification and its relationship with clinico-pathological and prognostic factors in 40 patients with neuroblastic tumors by using real-time quantitative PCR. There was significant association between the age of older than 18 months and the high number of metastasis. 83.3% of metastatic neuroblastic tumors in patients aged more than 18 months were in stage 4, while it was about 12.5% in patients aged less than 18 months. We found an amplification of MYCN in 19 out of 40 patients. Also, we found MYCN gene amplification in 64% of neuroblastoma (NB) and 8% of gangelioneuroblastoma (GNB) cases. There was a significant association between the histological type of samples with MYCN gene amplification. Neuroblastic tumors have a varied range of MYCN gene amplification depend on histopathology types. No significant associations have been found between MYCN gene amplification and tumor evaluation, CNS involvement, metastasis, stage of disease and patients outcome.

Key words: Gangelioneuroblastoma, MYC, Neuroblastoma.

Introduction

Neuroblastic tumors arise from primitive cells of the sympathetic system and are the most prevalent solid tumors, occurring in childhood period. However, little is known about the genetic basis of these types of tumors (1). Clinical behaviour of neuroblastic tumors has been shown to be diverse among patients, ranging from rapid progression despite intensive therapy in the vast majority of them, to spontaneous differentiation and regression in some others. This has led to intense study of the biological and prognostic features of these kinds of tumors.

Neuroblastoma (NB) accounts for about 15% of cancer mortality among children, and in the USA is responsible for 6-10% of all childhood malignancies (2, 3). In Iran, NB patients shown to have more advanced disease, characterized by poor biologic markers and the lower overall survival compared to developed countries (4). The lower survival rate can arise from late diagnosis, inability to distinct high risk group during the years of study and utilization of single treatment procedure for all enrolled patients (4). Therefore, it would be fundamental to determine parameters for early diagnosis and personalized treatment to improve patients' survival rate. Clinical parameters such as age, stage, histology and biological markers (such as MYCN amplification, 1p deletion) were introduced into clinical use to help to establish the prognosis and treatment (5-7). MYCN is a member of transcription factor that controls expression of many target genes and consequently regulate major cellular processes including proliferation, cell growth, protein synthesis, metabolism, apoptosis and differentiation. It plays a wide variety of roles in cancers including altering metabolic programs, supporting angiogenesis, promoting self-renewal and driving proliferation, while inhibiting differentiation. Amplified MYC is one of the most frequent genetic mutations that occur in several types of cancers (8-11).

MYCN expression is pivotal for central nervous system (CNS) development and neurogenesis through rapid expansion of neural progenitor cells (5, 12, 13) and impediment of neuronal differentiation. Gene dosage increment mechanisms are important molecular events in tumorigenesis that lead to upregulation of gene expression. Gene amplification is one of the important molecular events in which increase in the copy number cause excessive production of a given protein (14). MYCN ability in tumorigenesis is presumably the result of an increased gene dose while no mutations have not shown to be responsible instead and all existing copies were transcriptionally active (15, 16).

MYCN amplification is mostly characterized by increase of the MYCN gene copy number for more than four-fold relative to the number of chromosomes 2 (17). The vast majority of NB patients have shown to develop metastatic tumors with poor prognosis, and are not suitably treated with the existing therapeutic protocols. Importantly, MYCN gene amplification have been determined to be in charge for a group of aggressive tumors with poor prognosis distinguished by rapid progression, regardless of age and clinical stage (18, 19).

The prognostic role for the MYCN oncogene in neuroblastic tumors is undisputed (5). Thus, we present here a retrospective study of MYCN amplification in patients with neuroblastic tumors. The aims of this stu-

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dy are, firstly, to identify the frequency of MYCN gene amplification in child patients with neuroblastic tumors referring to Children Hospital of Tabriz and, secondly to determine the relationship between the clinicopathological, prognostic factors and MYCN amplification.

Materials and methods

Patients and DNA extraction

We determined the MYCN amplification of 40 patients with neuroblastic tumors under 14 years old. All the parents of participants have given an informed written consent, and the study protocol was approved by the Ethics Committee of Tabriz University of Medical Sciences (TUMS), which was in compliance with the Helsinki declaration. Tissue samples were collected in patients referring to Children Hospital of TUMS that is a major child patient referral center for the north west of Iran. Inclusion criteria were age younger than 14 years with neuroblastic tumors and the exclusion criteria were age older than 14 years, any other underlying disease and the lack of completion of treatment.

All patients were diagnosed with neuroblastic tumors by oncologist. The pathologist examined all these cases histologically, confirmed the diagnosis of NB or GNB. Patients demographic and clinicopathological data were collected with fulfilling checklists. All patients were untreated before the operation. Specimens from each tumor were taken at the time of the initial operation and then immediately frozen in liquid nitrogen and stored at –71°C. Histological analysis was made to confirm the presence of tumor cells in all specimens. DNA samples from the blood of healthy subjects were used as controls. DNA isolation was performed according to the protocol supplied with the DNeasy tissue kit (Qiagen, Hilden, Germany). The amount of purified DNA was then quantified on a spectrophotometer (ND-1000 Spectrophotometer; NanoDrops, Wilmington, DE).

Real-time quantitative PCR

RQ-PCR was performed with the ABI stepone plus System (Applied Biosystems, Foster City, CA). qPCR was carried out in a 25-mL reaction volume composed of: RealityTM universal PCR mix (Cat. #RR039A, TAKARA) with specific primers (20), probe and 2 μL of a 100–500 ng of template DNA. The sequences of the original primers and TaqMan probes labeled with FAMs and TAMRAs (Molecular Probes, Carlsbad, CA) were as follows. The primers for b-actin gene amplification were 5`-TCACCCACACTGTGCCCATC-TACGA-3` (forward primer, positions 2141–2165) and 5`-CAGCGGAACCGCTCATTGCCAATGG-3` (reverse primer, positions 2411–2435). The sequence of the TaqMan fluorogenic probe for the b-actin gene was 5`-ATGCCCTCCCCCATGCCATCCTGCGT-3` (positions 2171–2196). For the MYCN oncogene, the primers and probe were 5`-CCCCTGGGTCTGCCCCGTTT-3` (forward primer, positions 1456–1475),5`-GCCGAAG-TAGAAGTCATCTT-3` (reverse primer, positions 1720–1739), and 5`-CCCACCCTCTCCGGTGTGTC-TGTCGGTT-3` (fluorogenic probe, positions 1477–1501). The final optimized concentration of each primer and TaqMan probe was 100 nM. Thermal cycling conditions included an initial denaturation step at 95°C for 10 min, followed by 45 cycles of 15 sec at 95°C and 30 sec at 60°C. One run contained two sets of measurements: for target and control genes (for accuracy rating increasing and for reaction conditions unification). Five points in five replicates of plasmid DNA serial dilutions, a no template control, unknown and control (normal) samples were present in each set. The threshold cycles (Ct) were determined manually. Reference evaluations for each DNA sample were performed according to the endogenous control b-actin gene. The absolute target copy number was calculated using a standard calibration curve generated from PCR reactions with serially diluted plasmids (pGEM-T vector; Amersham, Netherlands) containing the respective sequence verified inserts. All results were expressed as arbitrary units; representing the ratio of mean of MYCN copy number/ mean of copy numbers of b-actin target (normalized copy number, NCN). All the experiment lots were carried out in the minimal time period. Amplification of MYCN represents more than four-fold increase in the MYCN gene copy number (17, 21).

Statistical analyses

Descriptive statistics and chi-square tests were performed by using the statistical package from the Social Science (SPSS) software (version 20) using $p<0.05$ for calculation of the statistical significance.

Results

The mean age of patients at diagnosis was 2.86 years (range, 0.5–8); twenty (50%) of them were males and twenty (50%) were females. Out of 40 patients, 24 patients had developed metastasis. Primary location of tumors at diagnosis was different among patients; 75% in Adrenal, 10% in Thoracic paravertebral, 7.5% in Lumbar paravertebral, 5% in Kidney and 2.5% in Sacrum. Thus, Adrenal gland was the most common site for primary tumors (In 30 out of 40 patients). At the time of diagnosis, 7.5% of patients were in stage 1, 22.5% in stage 2, 12.5% in stage 3 and 57.5% in stage 4. Overall, 22 patients out of 40 (55%) with neuroblastic tumors were cured and 18 (45%) patients were dead. CNS was involved in 20% of patients with neuroblastic tumors, and all of them were dead. Metastasis occurrence of all patients was analyzed according to the age (younger than 18 months or older than 18 months) of patients. There was significant association between the age of older than 18 months and the high number of metastasis. Also, it was shown that patients in stage 4 compared to patients in different other stages had the most frequently metastasized ($P<0.05$). 83.3% of metastatic neuroblastic tumors in patients aged more than 18 months were in stage 4 and it was about 12.5% in patients aged less than 18 months. We found an amplification of MYCN in 19 out of 40 patients (47.5%). Clinicopathological factors were analyzed in relation to MYCN gene amplification (Table 1). As shown in Table 1, 8 out of 19 patients with MYCN gene amplification were dead while, 10 out of 21 patients with non-amplified MYCN gene were dead. There was a significant relation between the histopathological type of samples with MYCN gene amplification ($P=0.001$). Out of 40 patients, 28 ones (70%) were NB and 12 (30%) were GNB. MYCN gene amplification

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Table1. Clinicopathological factors in relation to MYCN gene amplification

	MYCN+	MYCN-	P value
Gender			0.49
Male	9 (22.5%)	11 (27.5%)	
Female	10 (25%)	10 (25%)	
Age (years old)			0.37
3>=	11 (27.5%)	15 (37.5%)	
3<	8 (20%)	6 (15%)	
Histopathology			0.001
Neuroblastoma	18 (45%)	10 (25%)	
Gangelioneuroblastoma	1 (2.5%)	11 (27.5%)	
Stage			0.6
1	1 (2.5%)	2 (5%)	
2	3 (7.5%)	6 (15%)	
3	2 (5%)	3 (7.5%)	
4	13 (32.5%)	10 (25%)	
Metastasis			0.3
Absent	6 (15%)	10 (25%)	
Present	13 (32.5%)	11 (27.5%)	
CNS involvement			0.87
Absent	15 (37.5%)	17 (42.5%)	
Present	4 (10%)	4 (10%)	
Outcome			0.72
Cured	11 (27.5%)	11 (27.5%)	
Expired	8 (20%)	10 (25%)	

was observed in 18 out of 28 NB (64%) and in 1 out of 12 GNB patients (8%). Clinicopathological characteristics of 28 NB patients are shown in Table 2.

Discussion

Several prognostic markers, such as the patients' age and the presence of metastasis at the time of diagnosis, have been identified in neuroblastic tumors; (22). Weinstein et al. reported that the age at diagnosis can be considered as the only independent clinical prognostic factor, as infants before first year of age have significantly better survival rates compared to older children with the same stages of disease (23). Similarly, the present study identified that infants less than 1 year of age have significantly better and cured outcomes. We identified a significant association between patients' outcome with CNS involvement and the occurrence of metastasis. Of the various biological risk factors, the status of the MYCN gene has been shown to be the most predictive of the clinical outcome in NB patients (24). We investigated the status of MYCN gene amplification in patients with neuroblastic tumor by RQ-PCR. RQ-PCR method is uniquely capable to determine gene copy number within a small percentage of observed cancer cells and can be used as an accurate and quicken detection way for MYCN status, like for gene amplification even in a low-level state (13, 25-27). Melagh et al. showed that RQ-PCR is an easy and reliable method to use and determine copy numbers of MYCN gene in compare to southern blot and FISH method (28). In a study conducted in Iran by Poopak et al. 47% MYCN amplification was showed in NB patients (mean age of patients were 4.1 years) (29). Additionally, in a recent study in Japan, 47% MYCN amplification was found in NB patients (30). Consistent with our study, these recent Asian studies demonstrate high rate of MYCN amplification in NB patients. As for MYCN gene amplification in NB and GNB cases, our findings are in line with the finding of Wang et al. in which they found 1 out of 32 GNB cases and 13.5% NB cases with MYCN amplification (31). Many lines of studies on NB patients reported that MYCN gene amplification appears approximately in 9-30% of primary tumors (5, 32-37). For instance, Valentijn et al. reported that 20% of NB patients have amplification of MYCN, and it is associated to poor prognosis (32). Recently, Tabyaoui et al. found MYCN gene amplification in 27.8% of patients with neuroblastic tumors (38).

Compared with the results of previous studies, in this study the number of patients that showed MYCN amplification is remarkably high. It is likely due to some factors; First, it may be due to racial issues, as in other studies, conducted in Iran, the numbers of NB patients with MYCN amplification were shown to be more than other countries (29, 39). Second, it could be due to differences in the methods of evaluation for MYCN gene amplification. And finally, it could be due to effect of other clinicopathogical characteristics, for example age at the time of diagnosis. In a recent study, it was reported that MYCN gene amplification status ranged between 9-28% depending on children with different age group (34). We found that the stage 4 is the most prevalent extend of NB at diagnosis, which is in line with a similar study in Iran (4). GM Brodeur et al. examined MYCN gene amplification in NB patients and the

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Table 2. Clinicopathological characteristics of 28 NB patients.

Case	Age at diagnosis (years old)	Primary tumor localization	Stage	MYCN amplification	Metastasis	Outcome
1	8.0	Ad*	IV	+	+	Expired
2	3.0	Ad	III	-	-	cure
3	4.0	Ad	II	+	-	cure
4	1.5	Ad	IV	+	+	cure
5	6.0	Ad	IV	+	+	expired
6	3.0	Ad	IV	+	+	expired
7	2.0	Ad	IV	+	+	cure
8	3.5	Ad	III	+	-	cure
9	2.0	Ad	II	+	-	cure
10	3.5	Ad	IV	+	+	cure
11	2.0	Ad	IV	+	+	cure
12	1.0	Ad	II	+	-	cure
13	2.0	Ad	IV	+	+	expired
14	4.0	Ad	IV	-	+	expired
15	6.0	Ad	IV	+	+	expired
16	1.0	Ad	III	+	-	cure
17	2.0	Ad	IV	-	+	expired
18	3.0	Ad	IV	-	+	expired
19	0.5	Ad	I	-	-	cure
20	1.0	Ad	IV	-	+	cure
21	2.0	Ad	IV	+	+	cure
22	1.5	Ad	IV	+	+	expired
23	4.0	Ad	III	-	+	expired
24	6.0	Ad	IV	+	+	expired
25	4.5	L.Pψ	IV	-	+	expired
26	2.0	L.P	IV	-	+	expired
27	3.0	T.P†	II	-	-	cure
28	0.5	Kidney	I	+	-	cure

*Adrenal, ψ Lumbar paravertebral, † Thoracic paravertebral pos.mediastan, + Positive, - Negative

results did not showed MYCN gene amplification in any of 15 NB patients with stage 1 and 2 of NB. Thus, they concluded that MYCN gene amplification is associated with high stage of disease (27). Moreover, their results showed that 24 out of 48 patients with stage 3 and 4 have MYCN gene amplification, while in our study 15 out of 28 patients with stage 3 and 4 showed MYCN gene amplification. MYCN gene amplification is roughly the most frequent in patients with high stage disease; however, it was not statistically significant. Inandikhoglu et al. indicated that evaluating MYCN status at diagnosis may help the better understanding of clinical behavior of the disease (18). Our results revealed that MYCN does not appear to have an adverse effect on tumor evolution, CNS involvement, metastasis, stage of disease and patient outcome. MYCN gene amplification is a well-established factor for an unfavourable prognosis in many studies. Due to these controvertible results, there is crucial need for complementary studies with larger sample size in different ethnic groups.

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References

1. Maris, J.M., Recent advances in neuroblastoma. *New. Engl. J. Med*. 2010, **362** (23): 2202-2211. doi: 10.1056/NEJMra0804577

2. Hiyama, E., Neuroblastoma screening in Japan: population-based cohort study and future aspects of screening. *Annal. Academy. Med.*. 2008,**37**: 88.

3. ACW, L., Neuroblastoma: the challenge remains. *Singapore medical journal*. 2012, **53** (1): 1-2.

4. Mehdiabadi, G.B., Arab, E. and Arjmandi, K., Neuroblastoma in Iran: An Experience of 32 Years at a Referral Childrens Hospital. *Asian. Pac. J. Cancer. P*. 2013, **14** (4): 2739-2742. doi: 10.7314/APJCP.2013.14.5.2739

5. Spitz, R., Hero, B., Skowron, M., Ernestus, K. and Berthold, F., MYCN status in neuroblastoma: characteristics of tumours showing amplification, gain, and non-amplification. *Eur. J. Cancer*. 2004, **40** (18): 2753-2759. doi: 10.1016/j.ejca.2004.05.002

6. Attiyeh, E.F., London, W.B., Mossé, Y.P., Wang, Q., Winter, C., Khazi, D., McGrady P.W., Seeger, R.C., Look, T., Shimada, H., Brodeur, G.M., Cohn, S.L., Matthay, K.K. and Maris, J.M., Chromosome 1p and 11q deletions and outcome in neuroblastoma. *New. Engl. J. Med.*. 2005, **353** (21): 2243-2253. doi: 10.1056/NEJMoa052399

7. Riley, R.D., Heney, D., Jones, D.R., Sutton, A.J., Lambert, P.C., Abrams, K.R., Young, B., Wailoo, A.j. and Burchill, S.A., A system-

Copyright © 2014. All rights reserved.

atic review of molecular and biological tumor markers in neuroblastoma. *Clin. cancer. Res*. 2004, **10** (1): 4-12. doi: 10.1158/1078-0432.CCR-1051-2

8. Schwab, M., Alitalo, K., Klempnauer, K.H., Varmus, H.E., Bishop, J.M., Gilbert, F., Brodeur, G., Goldstein, M. and Trent, J., Amplified DNA with limited homology to myc cellular oncogene is shared by human neuroblastoma cell lines and a neuroblastoma tumour. *Nature*. 1983, **305** (6): 245. doi: 10.1038/305245a0

9. Westermark, U.K., Wilhelm, M., Frenzel, A. and Henriksson, M.A., The MYCN oncogene and differentiation in neuroblastoma. *In: Seminars in cancer biology*. 2011, 256-266. doi: 10.1016/j.semcancer.2011.08.001

10. Bell, E., Chen, L., Liu, T., Marshall, G.M., Lunec, J. and Tweddle, D.A., MYCN oncoprotein targets and their therapeutic potential. *Cancer lett*. 2010, **293** (2):144-157. doi: 10.1016/j.canlet.2010.01.015

11. Beltran, H., The N-myc Oncogene: Maximizing its Targets, Regulation, and Therapeutic Potential. *Mol. Cancer. Res*. 2014, **12** (6):1-8. doi: 10.1158/1541-7786.MCR-13-0536

12. Knoepfler, P.S., Cheng, P.F. and Eisenman, R.N., N-myc is essential during neurogenesis for the rapid expansion of progenitor cell populations and the inhibition of neuronal differentiation. *Gene. Dev.*. 2002, **16** (20): 2699-2712. doi: 10.1101/gad.1021202

13. Malakho, S., Korshunov, A., Stroganova, A. and Poltaraus, A., Fast detection of MYCN copy number alterations in brain neuronal tumors by real-time PCR. *J. Clin. Lab. Anal*. 2008, **22** (2): 123-130. doi: 10.1002/jcla.20232.

14. Schwab, M., Oncogene amplification in solid tumors. *In: Seminars in cancer biology*. Elsevier. 1999, 319-325. doi: 10.1006/scbi.1999.0126

15. Hogarty, M.D. and Brodeur, G.M., Wild-sequence of MYCN in neuroblastoma cell lines. *Int. J. Cancer*. 1999, **80** (4):630-631. doi: 10.1002/(SICI)1097-0215(19990209)80:4<630::AID-IJC24>3.0.CO;2-7

16. Lutz, W. and Schwab, M., In vivo regulation of single copy and amplified N-myc in human neuroblastoma cells. *Oncogene*. 1997, **15** (3): 303-315.

17. Ambros, P.F. and Ambros, I.M., Pathology and biology guidelines for resectable and unresectable neuroblastic tumors and bone marrow examination guidelines. *Med. pediatr. oncol*. 2001, **37** (6): 492-504. doi: 10.1002/mpo.1242

18. İnandıklıoğlu, N., Yılmaz, S., Demirhan, O., Erdoğan, Ş. and Tanyeli, A., Chromosome Imbalances and Alterations of AURKA and MYCN Genes in Children with Neuroblastoma. *Asian. Pac. J. Cancer. P*. 2012, **13** (11): 5391-5397. doi: 10.7314/APJCP.2012.13.11.5391

19. Brodeur, G.M., Knowing Your ABCCs. Novel Functions of ABCC Transporters. *J. Natl. Cancer. I.*. 2011, **103** (16): 1207-1208. doi: 10.1093/jnci/djr277

20. Raggi, C.C., Bagnoni, M.L., Tonini, G.P., Maggi, M., Vona, G., Pinzani, P., Mazzocco, K., DeBernaardi, B., Pazzagli, M. and Orlando, C., Real-time quantitative PCR for the measurement of MYCN amplification in human neuroblastoma with the TaqMan detection system. *Clin. Chem*. 1999, **45** (11): 1918-1924.

21. Valent, A., LeRoux, G., Barrois, M., Terrier-Lacombe, M.J., Valteau-Couanet, D., Léon, B., Spengler, B., Lenoir, G., Benard, G. and Bernheim, A., MYCN gene overrepresentation detected in primary neuroblastoma tumour cells without amplification.*J. Pthol*. 2002, **198** (4): 495-501. doi: 10.1002/path.1244

22. Sartelet, H., Grossi, L., Pasquier, D., Combaret, V., Bouvier, R., Ranchere, D., Plantaz, D., Munzer, M., Philip, T., Birembaut, P., Zahm, J., Bergeron, C., Gaillard, D. and Pasquier, B., Detection of N-myc amplification by FISH in immature areas of fixed neuroblastomas: more efficient than Southern blot/PCR. *J. pathol*. 2002, **198** (1): 83-91.

23. Weinstein, J.L., Katzenstein, H.M. and Cohn, S.L., Advances in the diagnosis and treatment of neuroblastoma. *Oncologist*. 2003, **8** (3): 278-292. doi: 10.1634/theoncologist.8-3-278

24. Rubie, H., Hartmann, O., Michon, J., Frappaz, D., Coze, C., Chastagner, P., Baranzelli, M.C., Plantaz, D., Avet-Loiseau, H., Benard, J., Delattre, O., Favrot, M., Peyroulet, M.C., Thyss, A., Perel, Y., Bergeron, C., Courbon-Collet, B., Vannier, J.P., Lemerle, J. and Sommelet, D., N-Myc gene amplification is a major prognostic factor in localized neuroblastoma: results of the French NBL 90 study. Neuroblastoma Study Group of the Société Francaise d'Oncologie Pédiatrique. *J. Clin. Oncol*. 1997, **15** (3): 1171-1182.

25. Park, J.R., Eggert, A., Caron, H., Neuroblastoma: biology, prognosis, and treatment. *Pediatr. Clin. N. Am.*. 2008, **55** (1): 97-120. doi: 10.1016/j.pcl.2007.10.014

26. Tanaka, S., Tajiri, T., Noguchi, S-i., Shono, K., Ihara, K., Hara, T. Suita, S.,. Clinical significance of a highly sensitive analysis for gene dosage and the expression level of MYCN in neuroblastoma. *J. pediatr. surg*. 2004, **39** (1): 63-68. doi: 10.1016/j.jpedsurg.2003.09.015

27. Brodeur, G.M., Seeger, R.C., Schwab, M., Varmus, H.E .and Bishop, J.M., Amplification of N-myc in untreated human neuroblastomas correlates with advanced disease stage. *Science*. 1984; **224** (4653): 1121-1124. doi: 10.1126/science.6719137

28. Melegh, Z., Bálint, I., Tóth, E., Csernak, E., Szentirmay, M. and Nagy, K., Detection of n-myc gene amplification in neuroblastoma by comparative, in situ, and real-time polymerase chain reaction. *Fetal. Pediatr. Pathol*. 2003, **22** (3): 213-222.

29. Poopak, B., Arzanian, M.T., Vosough, P., Gharib, A., Hamidieh, A.A. and Ehsani, M., Parsania, M., Yousefian, A., Jahangirpour, M.A. and Farahani, K., Evaluation of MYCN amplification in Iranian patients with neuroblastoma by conventional and real time PCR. *Med. Sci. J. Islamic. Azad. Uni.Tehran. Med. Branch*. 2011, **21** (3): 188-195.

30. Kojima, M., Hiyama, E., Fukuba, I., Yamaoka, E., Ueda, Y., Onitake, Y., Kurihara, S. and Sueda, T., Detection of MYCN amplification using blood plasma: noninvasive therapy evaluation and prediction of prognosis in neuroblastoma. *Pediatr. Surg. Int*. 2013, **29** (11): 1139-1145.

31. Wang, M., Zhou, C., Cai, R., Li, Y. and Gong, L., Copy number gain of MYCN gene is a recurrent genetic aberration and favorable prognostic factor in Chinese pediatric Neuroblastoma patients. *Diagn. Pathol*. 2013, **8** (5): 1-6. doi: 10.1186/1746-1596-8-5

32. Valentijn, L.J., Koster, J., Haneveld, F., Aissa, R.A., van Sluis, P., Broekmans, M.E., Molenaar, J.J., Nes, J.V. and Versteeg, R., Functional MYCN signature predicts outcome of neuroblastoma irrespective of MYCN amplification. *P. Natl. Acad. Sciences*. 2012, **109** (47):19190-19195. doi: 10.1073/pnas.1208215109

33. Bourdeaut, F., Grison, C., Maurage, C-A., Laquerriere, A., Vasiljevic, A., Delisle, M-B., Michalak, S., Figarella-Branger, D., Doz, F., Richer, W., Pierron, G., Miguel, C., Delattre, O. and Couturier, J., MYC and MYCN amplification can be reliably assessed by aCGH in medulloblastoma. *Cancer. Genet*. 2013, **206** (4): 124-129. doi: 10.1016/j.cancergen.2013.02.003

34. Mossé, Y.P., Deyell, R.J., Berthold, F., Nagakawara, A., Ambros, P.F., Monclair, T., Cohn, S.L., Pearson, A.D., London, W.B. and Matthay, K.K., Neuroblastoma in older children, adolescents and young adults: A report from the International Neuroblastoma Risk Group project. *Pediatr. blood. cancer*. 2014, **61** (4): 627-635. doi: 10.1002/pbc.24777

35. Huang, M. and Weiss, W.A., Neuroblastoma and MYCN. *Cold. Spring. Harbor. Perspectives. Med*. 2013, **3** (10): a014415. doi: 10.1101/cshperspect.a014415

36. Brodeur, G., Molecular basis for heterogeneity in human neuroblastomas. *Eur. J. Cancer*. 1995, **31** (4): 505-510. doi: 10.1016/0959-

Copyright © 2014. All rights reserved.

8049(95)00040-P
37. Look, A.T., Hayes, F.A., Shuster, J.J., Douglass, E.C., Castleberry, R.P., Bowman, L.C., Smith, E.I., Brodeur, G.M., Clinical relevance of tumor cell ploidy and N-myc gene amplification in childhood neuroblastoma: a Pediatric Oncology Group study. *J. Clin. Oncol.*. 1991, **9** (4): 581-591.
38. Tabyaoui, I., Tahiri-Jouti, N., Serhier, Z., El Maani, K., Cherkaoui, S., Al Zemmouri, M., Mohamed, M. and Soumaya, A., High incidence of MYCN amplification in a Moroccan series of neuroblastic tumors: Comparison to current biological data. *Diagn. Mol. Pathol.*. 2013, **22** (2): 112-118. doi: 10.1097/PDM.0b013e318277448e
39. Pedram, M., Heidari, M., Keikhaei, B., Malamiri, R.A., Poopak, B., Fekri, K., Impact of N-myc Amplification on Median Survival in Children With Neuroblastoma. *J. Comprehensive. Pediatr.*. 2012, **3** (1): 29-33. doi: 10.5812/jcp.7273

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