DETERMINING GENOMIC PROFILE AND APPLICATION IN TREATMENT OF NON-AMPLIFIED *MYCN* NEUROBLASTOMA PATIENT

Vu Dinh Quang[1]; Nguyen Thi Hong Van[2]; Phung Tuyet Lan[1]
Nguyen Xuan Huy[1]; Ngo Diem Ngoc[1]; Bui Ngoc Lan[1]; Pham Duy Hien[1]
Le Dinh Cong[1]; Le Thi Kim Ngoc[1]; Hoang Ngoc Thach[1]
Hoang Quoc Chinh[3]; Nguyen Thanh Liem[3]; Le Thanh Hai[1]

SUMMARY

Background: Neuroblastoma is the most common extracranial solid cancer of childhood and is characterized by a remarkable biological heterogeneity, cause multiple genetic changes. The genetic profiles are the powerful tools for the clinician in risk stratification and treatment tailoring in neuroblastoma patients. This will increase the chance of treatment's success and minimize the dose of chemotherapy for these patients. Subjects: 6 neuroblastoma patients under 18 months, non-amplified MYCN were diagnosed and treated in National Children's Hospital. Method: The CGH technique is performed on the Agilent's system with the 400k chip at Vinmec International Hospital. Results: 4 patients were found the numerical chromosomal abnormalities (both stage L2), the others were the segmental chromosomal abnormalities (1 stage L2 and 1 stage M). Based on this results, 4/5 patients could be stopped the chemotherapy, 1 patient had to continue the treatment. The stage M patient had the 50% of chance of success in high-dose chemotherapy and stem cell transplantation. Conclusion: The genomic profile by CGH is established successfully in Vietnam. The integration of this technique allows more precise prognostication and refined treatment assignment which contribute to improve survival with decreased toxicity.

** Keywords: Neuroblastoma; Genomic hybridization.*

INTRODUCTION

Neuroblastoma (NBL), an embryonic tumour of the sympathetic nervous system, often affects children age 5 or younger [1]. It's the most common solid tumor in first year of life, with the prevalence approximately 1/7,000 live births. The median age at diagnosis is around 18 months [2].

Some specific genetic alterations in NBL had been discovered from 1980s, including the amplification of *MYCN* gene, gain 17q, loss 1p, loss 11p...

These genetic markers had provided more prognostic information, and contributed significantly in risk stratification and treatment tailoring in NBL patients. For example,

1. National Children's Hospital
2. VNU University of Science
3. International Vinmec Hospital
Corresponding author: Vu Dinh Quang (vudinhquang@nhp.org.vn)
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the (near) triploid tumour has the good prognosis; or the amplified *MYCN* often occurs in high risk, worse prognotic patients [3]. Those aberations have divided into 2 separate groups: the numerical chromosomal abnormalities (NCA) and the segmental chromosomal abnormalities (SCA). The NCA tumour has found in infants, low stage, spontaneous regression and better prognosis case. Otherwise, the SCA profile, including the amplification of *MYCN* gene, alterations at 1p, 3p, 4p, 11q, 17q, exposure the worst prognostic for NBL patient [4].

The genetic alterations could be detected by classic karyotype or fluorescent in-situ hybridization (FISH) technique. While the karyotype shows time-consuming and low effective because of the requirement of metaphases from tumour cells, the limitations of FISH technique are expensive and low throughput. The apperance of array comparative genomic hybridization (aCGH), which has the posibility of whole chromosomes analysis, enabled the determination of genetic profile on NBL patients swiftly and high reliably. This profile have been used to classify NBL into risk groups based on the specific characteristics, corresponds with the diffenrent treatment plans and outcomes [4, 5].

The aCGH had been established in Untied States of America in 1992. Up to now, this technique had been optimized and became popular in genetic field. The first and most important component of aCGH technique is the DNA chip (or array), a region on the glass slide contains from thousands to millions distinct oligonucleotides (probes). Normally, the resolution using for NBL varies from 60,000 (60k) to 180,000 (180k) oligonucleotides per chip. The second component is the mix of 2 fluorescent DNA: target DNA dyed with Cy5 (blue) and control DNA dyed with Cy3 (dark pink), which have been put on the array to hybrid with the oligonucleotides. The ratio of fluorescent intensity displays the gain and loss at each probe position [6, 7].

At National Children's Hospital, there are 50 - 60 new diagnosed cases anually which investigate *MYCN* gene status by FISH technique for risk assessment. The low risk NBL (*MYCN* not-amplified) need the type of chromosomal alterations to choose the appropriate treatment protocol. Based on the collaboration between the National Children's Hospital, Vinmec International Hospital and Vinmec Research Institute of Stem Cell and Gene Technology, the study has been established for: *Either determining genomic profile on some NBL or tailoring the treatment in order to increase the chance of treatment's success and minimize the dose of chemotherapy for these patients.*

SUBJECTS AND METHODS

1. Subjects.

6 NBL patients in National Children's Hospital, under 18 months, without *MYCN* amplified have been selected from January to April 2017, including five L2 stage cases and one M stage case.

2. Methods.

** Samples:*

Fresh tumour samples (not fix in formol) before chemotherapy is collected after the biopsy and store in -80°C until the test.

** aCGH technique:*

The aCGH technique have been performed in Vinmec Research Institute of Stem Cell and Gene Technology on the Agilent system. DNA chip used was the SurePrint G3 Human CGH Microarray Kit, 2 x 400k (Agilent) with the resolution of 400,000 oligonucleotides covered 23 chromosomes.

The DNA was extracted by the kit of Qiagen Company and measured the concentration on the Nanodrop 2,000 (Thermo). Target DNA dyed with Cy5 and control DNA dyed with Cy3 were mixed and put on the slide, hybrid at 67°C in 40 hours. The result has analyzed by CytoGenomics software (Agilent) with the helps from Curie Institute (Paris, France).

RESULTS AND DISCUSSION

1. Determination of genetic profiles.

The clear results enabled for analysis of genetic profiles accurately, in which 4 NCA cases and 2 SCA cases.

Table 1: List of NBL cases and the results.

Order	Labcode	Age at diagnosis	Stage	Genetic profile
1	NBL001	11 months	L2	NCA (-3, -4, +7, -10, -11, -13, -14, -16, +17, -19, -21)
2	NBL002	13 months	L2	NCA (-4, -5, +7, +8, -10, -14, -16, +17, +18, -19, -21)
3	NBL003	2 months	L2	NCA (-4, +7, -9, -10, -11, -14, -17, -21)
4	NBL004	15 months	L2	SCA ($1p^-$, -4,$9q^-$, $11q^-$, $17p^-$, $17q^+$, -19)
5	NBL005	12 months	L2	NCA (-4, -5, +7, +8, -10, -14, -16, +17, +18, -19, -21)
6	NBL006	12 months	M	SCA ($1p^-$, $2q^+$, $3p^-$, $12q^+$, +13, $17q^+$, $19p^-$)

(-: Loss; +: Gain; p: Short arm; q: Long arm)

Some genetic profiles on NBL were below.

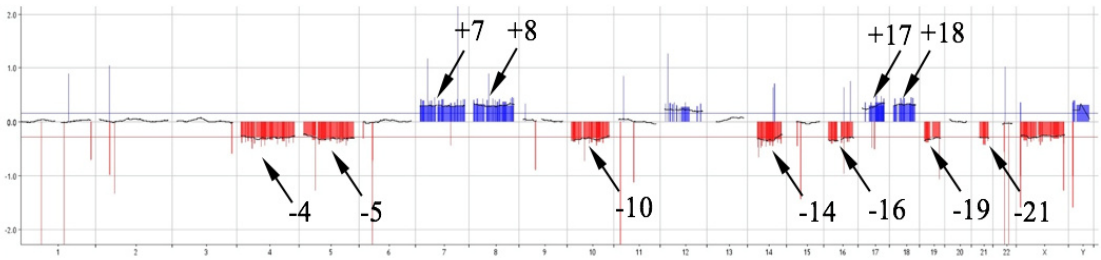

Figure 1: The results of NBL005 patient (NCA type).

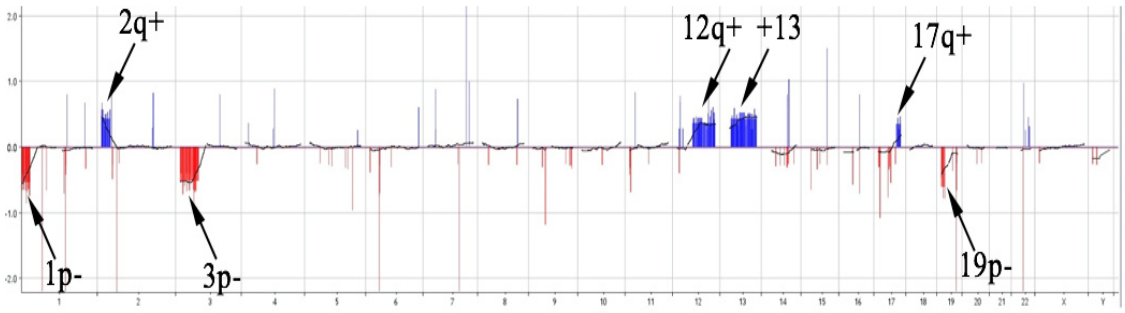

Figure 2. The results of NBL006 patient (SCA type).

So, the genetic profiles of NBL had been well determined by aCGH, and beneficial in risk stratification and treatment plan.

***2.* Clinical significance in treatment tailoring.**

The NBL patients in National Children's Hospital had been treated following the protocol of the International Society of Paediatric Oncology (SIOPEN). In five L2 stage NBL, 3 cases were unresectable and following-up after 2 courses of Carbo-VP16, 1 unresectable case after 3 courses of chemotherapy (2 courses of Carbo-VP16 and 1 course of CADO) and 1 new case. The decision of next chemotherapy courses depended on the genetic profile. If the genetic profile is NCA, the patient could be stopped chemotherapy and just follow-up. On the contrary, in case of SCA, the patient would be continued more 2 courses of chemotherapy.

Otherwise, the M stage patient had undergone the intensive chemotherapy based on the high risk treatment protocol, and now are having the palliative chemotherapy. The result of aCGH could change the future treatment plan, either draw up the chemotherapy (NCA type) or keep on the high dose chemotherapy, stem cell transplantation, surgery and radiotherapy with the successful rate of about 50% (SCA type).

The genetic profiles have assisted the clinical in tailoring the treatment in order to maximize the outcomes, specially in three L2 NBL: NBL002, NBL004 and NBL005. The NBL002 have abandoned the 4th course of chemotherapy (CADO) because of NCA type. The NBL004, a following-up case, by the SCA profile must be treated with 2 additional courses of chemotherapy and surgery for decreasing the risk of relapse. About the NBL005, this is a new NBL boy and the NCA profile help him avoid the chemotherapy while the size of tumor reduced by 40% in one month. Obviously, the determination of genetic profile by aCGH is the reliable tool, play an important role in risk stratification and treament tailoring.

CONCLUSION

The application of comparative hybridization technique in definition of the genomic profile has showed the clear

benefit on low-risk NBL patient, avoiding overtreatment or undertreatment for young patients. This is a grand step in developping the personalized medicine, resulting in high therapeutic effect as well as minimizing the complications of treatment for Vietnamese NBL patients.

REFERENCES

1. Brodeur G.M. Neuroblastoma: biological insights into a clinical enigma. Nature Reviews Cancer. 2003, 3 (3), pp.203-216.

2. London W.B et al. Evidence for an age cutoff greater than 365 days for NBL risk group stratification in the children's oncology group. Journal of Clinical Oncology. 2005, 23 (27), pp.6459-6465.

3. Maris J.M. Recent advances in NBL. The New England Journal of Medicine. 2010, 362 (23), pp.2202-2211.

4. Thorner P.S. The molecular genetic profile of neuroblastoma. Diagnostic Histopathology. 2014, 20 (2), pp.76-83.

5. Janoueix-Lerosey I et al. Overall genomic pattern is a predictor of outcome in NBL. Journal of Clinical Oncology. 2009, 27 (7), pp.1026-1033.

6. Pinkel D, D.G. Albertson. Array comparative genomic hybridization and its applications in cancer. Nature Genetics. 2005, 37 Suppl, pp.S11-17.

7. Garnis C et al. High-resolution array CGH increases heterogeneity tolerance in the analysis of clinical samples. Genomics. 2005, 85 (6), pp.790-793.