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Coamplification of the L-*myc* and N-*myc* Oncogenes in a Neuroblastoma Cell Line

Takeshi Jinbo,[1] Yukio Iwamura,[2] Michio Kaneko[1,3] and Shigenori Sawaguchi[1]

[1]*Department of Pediatric Surgery, Institute of Clinical Medicine and* [2]*Department of Microbiology, Institute of Basic Medical Science, University of Tsukuba, 1-1-1, Tennoudai, Tsukuba-shi, Ibaraki 305*

The L-*myc*, N-*myc* and c-*myc* genes are members of the *myc* oncogene family. In particular, L-*myc* is novel, and amplification of L-*myc* is still unknown except in small cell lung carcinoma. We examined L-*myc* amplification in 30 human neuroblastomas using Southern blot hybridization, and found that the L-*myc* gene was amplified approximately 5-fold in GOTO, a human neuroblastoma cell line. The N-*myc* gene was also amplified approximately 60-fold and furthermore, over-expression of L-*myc* and N-*myc* genes was observed in this cell line. In this report, we describe the coamplification of the *myc* gene family in the GOTO neuroblastoma cell line.

Key words: Coamplification — L-*myc* — N-*myc* — Neuroblastoma

Cellular oncogenes are thought to play an essential role in cell growth, differentiation and embryogenesis.[1] In some cancers, activated oncogenes are observed as a result of mutation, DNA rearrangement or gene amplification.[1,2] The L-*myc*, N-*myc* and c-*myc* cellular oncogenes are members of the *myc* oncogene family and the amplification of these genes has been detected in many malignant tumors. However, no coamplification of *myc* oncogenes has ever been found in human tumors. Amplification of c-*myc* gene has been reported in various malignant tumors[3] and that of N-*myc* gene is believed to be specific to neurogenic tumors such as neuroblastoma,[4,5] retinoblastoma[6] and small cell lung carcinoma (SCLC).[7] However, L-*myc* amplification is still unknown except in SCLC.[8] It has been reported that *Eco*RI restriction endonuclease digests of human genomic DNA contain two L-*myc*-related fragments (10.0 kb and 6.6 kb). The formation of these two *Eco*RI fragments is due to *Eco*RI restriction site polymorphism in two alleles for L-*myc*. Further, amplification of the L-*myc* gene has frequently been observed in human SCLC cell lines.[8] Recently, a correlation between L-*myc* restriction fragment length polymorphism (RFLP) and malignancy of human lung cancers has been reported,[9] but no correlation between L-*myc* RFLP and malignancy of colorectal cancers was found.[10] However, no report concerning L-*myc* gene in neuroblastoma has appeared. In this work, we studied L-*myc* amplification and RFLP patterns in human neuroblastomas.

Cells examined were obtained from 30 neuroblastomas (20 primary tumors, 2 nude mouse xenografts and 8 cell lines including GOTO) and were kept frozen at −80°C until DNA extraction. GOTO, a human neuroblastoma cell line, established by Sekiguchi *et al.*[11] was provided by the Japanese Cancer Research Resources Bank, Tokyo.

High-molecular-weight DNAs were isolated from cells as described previously.[12] Briefly, 10 μg of DNA sample was digested with *Eco*RI restriction endonuclease, electrophoresed through 0.8% agarose gel and then Southern blot hybridized. Total cellular RNA was extracted by the method reported previously.[13] Then 20 μg of total RNA was denatured, electrophoresed on a 1% agarose-formaldehyde gel,[14] and northern blot hybridized. L-*myc*,[8] N-*myc*[5] and c-*myc* genes[15] used as probes were provided by the Japanese Cancer Research Resources Bank. Each fragment was nick-translated with [α-^{32}P]-dCTP to give a specific activity of 5×10^7 cpm/μg.

It is known from previous reports that the *Eco*RI digestion of the genomic DNAs gives 3 different DNA patterns, namely, L-L type (patients homozygous for 10.0-kb L-*myc* fragment), S-S type (patients homozygous for 6.6-kb L-*myc* fragment), and L-S type (patients heterozygous for L-*myc*).[8] In the neuroblastomas examined, the RFLP patterns of L-*myc* gene were as follows: 4 cases were of L-L type, 11 cases were of S-S type and 15 cases including GOTO were of L-S type.

L-*myc* amplification was seen in the GOTO neuroblastoma cell line (Fig. 1A). The amplification of L-*myc* gene in this cell line was approximately 5-fold. This degree of L-*myc* amplification was determined by measuring the ratio of the L and S bands in GOTO cell line by densitometric scanning. The N-*myc* gene has been amplified approximately 60-fold in this cell line, and this was also determined by densitometric scanning. No amplification of c-*myc* gene was detected (Fig. 1B). In addition, over-expression of the L-*myc* and N-*myc* genes was detected in this cell line (Fig. 2). This is the first report of amplification and/or over-expression of the

[3] To whom correspondence should be addressed.

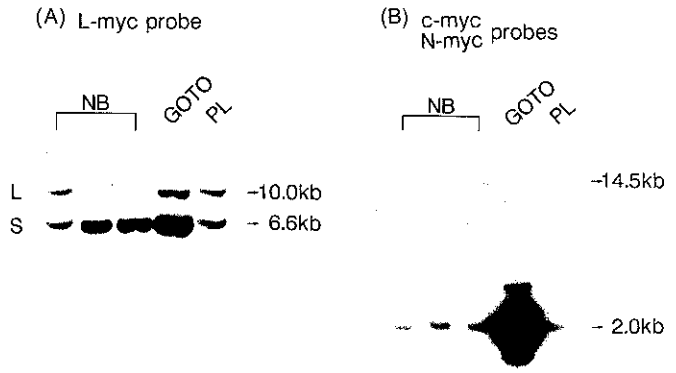

Fig. 1. Coamplification of both the L-*myc* and N-*myc* genes in neuroblastomas. Genomic DNAs were digested with *Eco*RI and hybridized with L-*myc* (A), c-*myc* and N-*myc* (B) probes as described previously. In (A), the L-*myc* probe used is the 1.8-kb *Sma*I-*Eco*RI genomic L-*myc* fragment.[8] In (B), the c-*myc* and N-*myc* probes used are the 1.0-kb *Pst*I-*Pst*I[13] and 2.0-kb *Eco*RI-*Eco*RI fragments,[5] respectively. The 14.5-kb position represents the *Eco*RI genomic c-*myc* fragment and the 2.0-kb position represents the *Eco*RI genomic N-*myc* fragment. NB, surgically removed human neuroblastomas; GOTO, a human neuroblastoma cell line; PL, placental DNA (as a control).

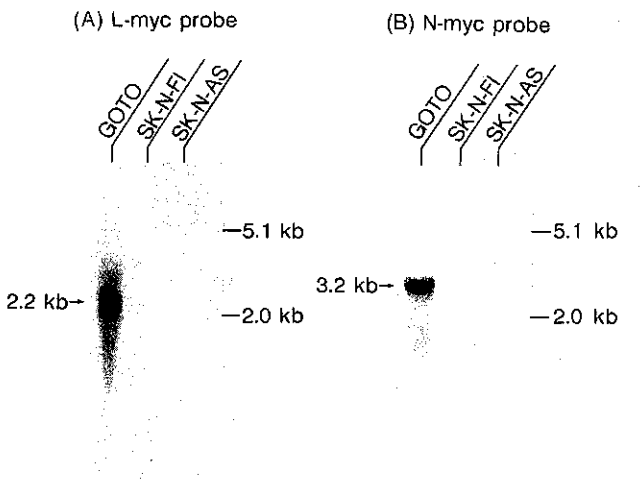

Fig. 2. Over-expression of both the L-*myc* and N-*myc* genes in neuroblastoma cell lines. Total RNA (20 μg) was hybridized with L-*myc* (A) and N-*myc* probes (B) as described previously. In (A), the L-*myc* probe used is the 1.8-kb *Sma*I-*Eco*RI fragment[8] and the 2.2-kb position represents human N-*myc* RNA. The 5.1-kb and 2.0-kb positions indicate 28S and 18S human ribosomal RNAs, respectively. GOTO, SK-N-FI and SK-N-AS are all human neuroblastoma cell lines. SK-N-FI and SK-N-AS were provided by Memorial Sloan-Kettering Cancer Center, New York.

L-*myc* and N-*myc* genes simultaneously in a human malignant cell line.

Coamplification of the *hst-1* and *int-2* genes has been reported previously in human cancers.[16] These genes are closely linked and have been mapped to chromosome 11q13. We have demonstrated the coamplification of genes on different chromosomes, because L-*myc* gene has been mapped to chromosome 1p32[8] and N-*myc* gene has been mapped to chromosome 2p23-24.[5, 17]

The L-*myc* gene has been cloned from SCLC DNA with homology to a small region of both the c-*myc* and N-*myc* genes as a third *myc*-related gene.[8] Amplification of the L-*myc* gene has not been observed in tumors except for lung cancers (mainly SCLC) so far. It is said that those with amplified L-*myc* sequences may show malignant transformed phenotype and also be resistant to chemotherapy and/or radiation. In SCLC, amplification and over-expression have been seen not only with L-*myc* but also with c-*myc* and N-*myc* genes.[8] However, it was not known whether two or more *myc* genes could be simultaneously activated in the same tumor. In this report, we have shown that L-*myc* was amplified in a neuroblastoma cell line. Furthermore, in contrast to the case of SCLC, coamplification of genes of the *myc* gene family (L-*myc* and N-*myc*) was observed in the neuroblastoma cell line. These findings raise the possibility that co-activation of two or more *myc* genes is related to malignant transformation in neuroblastoma.

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