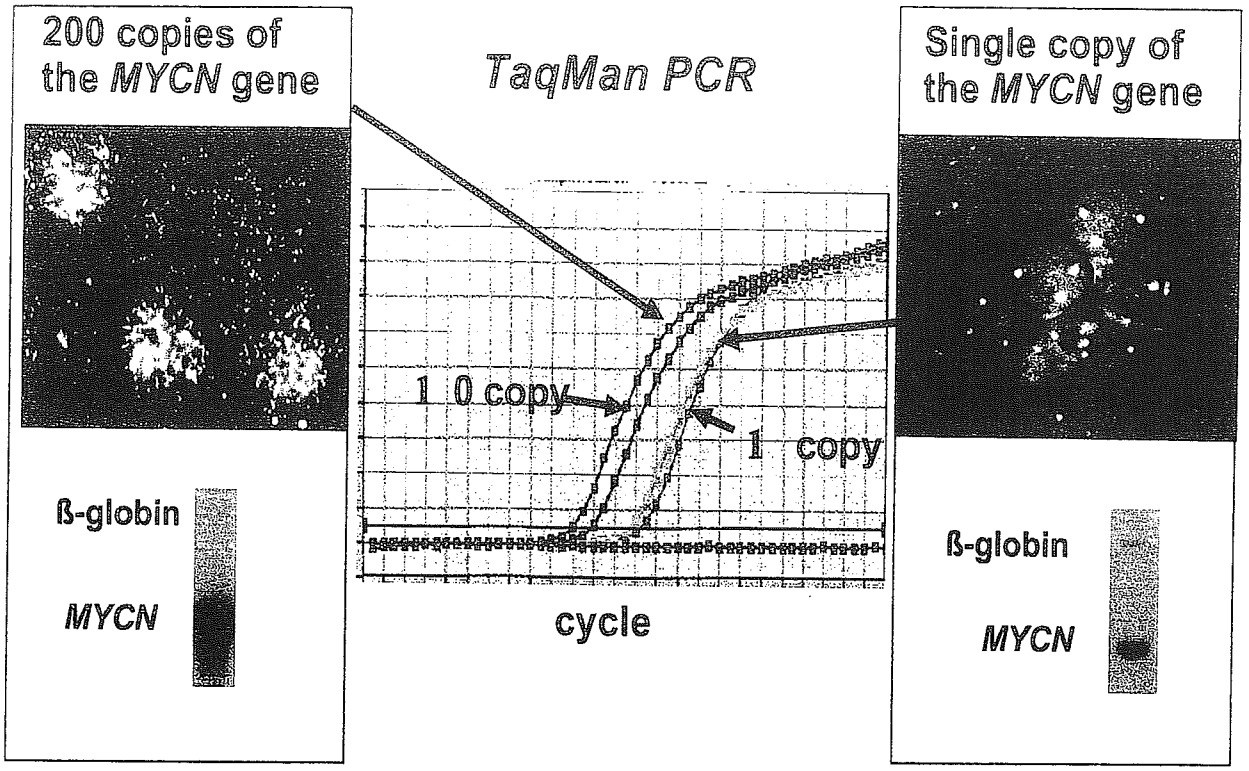

Fig. (1). Detection of *MYCN* amplification in neuroblastoma. *MYCN* copy number is usually determined by Southern blot, quantitative real time PCR (TaqMan®), or fluorescence *in situ* hybridization.

Several reports demonstrated that chromosome 1p deletion occurs in approximately 35% of all neuroblastomas [38, 39] and a smallest region of overlapping deletion (SRO) was mapped to 1p34-p36 [40], suggesting that tumor suppressor genes for the development of neuroblastoma might be located in this region. Recently, the SRO was refined to a size of approximately 1 Mb within 1p36.3, which was defined by the region of LOH in a primary tumor that extends distally from *DIS214*. Patients whose tumors had large 1p deletions showed poorer outcome than patients with short or interstitial deletions [41, 42], suggesting the existence of more than one deleted 1p loci in neuroblastoma. The tumors with large 1p deletions were associated with adverse prognostic factors, such as diploidy or tetraploidy and amplified *MYCN*, while the tumors with small interstitial deletions of 1p associated with triploidy in favorable tumors. The regions of 1p deletions of *MYCN*-amplified tumors are very large including a region from 1p32 to telomere [24]. In contrast, in single-copy *MYCN* cases, 1p deletions were described to be consistently smaller, and a commonly deleted region maps to 1p36.1. Thus, the second tumor suppressor gene, which is correlated with progressive neuroblastoma, was suggested to be localized at proximal (1p32) or distal (1p36.3) to the deletion border of the smallest 1p deletion found in single-copy *MYCN* tumors [43, 44]. The SRO of the single-copy *MYCN* tumors is included in the larger SRO of *MYCN*-amplified tumors, implying that more than two suppressor loci in 1p must be simultaneously deleted in *MYCN*-amplified tumors.

Preferential deletion of the maternal allele in single copy *MYCN* tumors [45] would imply that the distal locus of tumor suppressor gene (TSG) at 1p36.3 may be subject to genomic imprinting. Recent study indicated that the origin of the affected allele is frequently parental of random distribution in neuroblastoma with a large 1p deletion and in amplified *MYCN* gene [46]. Now, the reports for preferential loss of the allele have given conflicting results [43, 45, 47, 48].

Thus, the biological effect of 1p deletions has remained unclear. Several candidate TSGs have been proposed, but none has been shown to contain tumor-specific mutations, indicating that alternate mechanisms of TSG inactivation, such as epigenetic mechanisms of gene inactivation or haploinsufficiency, also have to be considered. 1p alterations are frequently detected together with other genetic alterations, such as amplified *MYCN*, 17q gain, diploidy/triploidy, and each combination appears to have a divergent impact on tumor growth characteristics. In the recent study, LOH at 1p36 was a significant independent predictor of decreased event-free survival, but had no significant effect on overall survival in multivariate analysis [49]. In contrast, amplified *MYCN* was a more powerful prognostic factor for decreased overall survival. This implies that 1p36 allelic status may be useful for predicting which neuroblastoma patients with otherwise favorable clinical and biological features are more likely to have disease progression. Closer surveillance is needed after surgery for disease progression because these patients can be salvaged with additional therapy.

Cytogenetic studies also revealed additional copies of 17q in cell lines and primary tumors [50]. Several studies of neuroblastoma cells and tumors have indicated a high frequency of unbalanced translocations involving chromosome

17q [30, 51, 52]. Clinically, gain of 17q is more common at an advanced stage, in tumors from children aged over 1 year, and in tumors showing 1p loss, *MYCN* amplification and diploidy/ tetraploidy. On the other hand, triploidy with whole chromosome 17 gain is associated more often with neuroblastomas showing favorable clinical features [26]. The report from six European centers with more than 300 cases identified that 17q gain was the most powerful prognostic factor of survival in multivariate analysis with other clinical and tumor genetic parameters, including 1p deletion and *MYCN* amplification. In stepwise multivariate analysis, significant independent predictors of lethal outcome were 1p deletion ($P = 0.02$), stage 4 disease ($P = 0.004$), and 17q gain ($P < 0.001$) [26]. These studies suggested that unbalanced gain of distal 17q is the independent prognostic factor for predicting high risk for tumor progression and that the region of chromosome 17q gain includes a gene critical for tumor progression. FISH analyses indicated that the breakpoints were clustered in the proximal half of 17q from *D17Z1* to *MPO* (17q23.1), with a shortest region of gain extending from *MPO* to 17qter [53, 54]. Several candidate genes have been proposed to be responsible for the 17q-gain effect on tumor growth characteristics. Survivin, an anti-apoptosis protein, which was recently mapped to 17q25, is one candidate as its expression correlates strongly with adverse clinical data [55]. *NM23-H1* located at 17q21-22 is another candidate because overexpression of nm23-H1 has been demonstrated in aggressive neuroblastoma tumors [56, 57].

Cytogenetic analyses have also reported 11q deletion in about 15% of neuroblastomas [58]. Constitutional rearrangements of 11q have been reported in some neuroblastoma patients, including a deletion of 11q23-qter, [59-61]. These constitutional changes may predispose paitents to the development of neuroblastoma. On the other hand, LOH studies revealed 11q loss in 5-32% of the tumors [62-64]. Loss of the whole chromosome 11 was observed in 19%, while unbalanced 11q LOH was observed in 22% of primary neuroblastomas [65]. Loss of the whole chromosome 11 was mainly detected in low stage tumors, whereas unbalanced deletion of 11q was predominantly detected in high stage tumors without amplified *MYCN* [65, 66]. Loss of 11q was significantly correlated with adverse clinical parameters, including age over 1 year, stage 4 tumors and unfavorable histology [67]. The SRO in 11q23.3 is between markers *D11S1340* and *D11S1299* in the tumors with 11q LOH [66]. Unbalanced 11q deletion is considered as a frequent event in the *MYCN* non-amplified tumors and COG study recently revealed that 11q deletion is an independent prognostic indicator for predicting high risk for tumor progression, especially in *MYCN* non-amplified tumors [35, 68].

Recently, genome-wide linkage analysis revealed that a candidate locus for the gene has been localized to chromosome band 16p12 [69, 70] in familial neuroblastomas [71], whereas other loci, such as chromosome 1p, have been excluded [49, 72]. Although a familial neuroblastoma predisposition gene would function as a tumor suppressor, it is possible that dominant mutations may predispose to neuroblastoma.

Representational difference analysis (RDA) revealed that the most frequently deleted region, apart from 1p, was chromosomal region 3p [73]. In this study, a SRO encompassing chromosome bands 3p25.3-p14.3 with 46 cM was defined [74]. Recently, a novel 3p21.3 candidate tumor suppressor, a *RAS* association domain family protein (*RASSF1A*), which was identified within the SRO [75], has been shown to be epigenetically silenced by promoter methylation in primary neuroblastomas and other cancer types[76]. Silencing of *RASSF1A* may contribute to aberrations of *RAS* signal pathways observed in neuroblastomas [77]. In addition, a significant correlation between *RASSF1A* and *CASP8* methylation in neuroblastoma was demonstrated [76], suggesting that some neuroblastomas may have a CpG island methylator phenotype.

Recently, comparative genome hybridization (CGH) has been used for genome-wide screening of gains and losses in neuroblastoma [35-37, 78]. With respect to ploidy changes in neuroblastoma, CGH analyses have confirmed the findings of flow cytometry and classical cytogenetic analysis: triploid tumors with favorable outcomes are characterized by numerical chromosome imbalances with only few structural abnormalities, including the typical pattern of gains for chromosomes 1, 2, 6, 7, 8, 12, 13, 17, 18, and 22, and losses for chromosomes 3, 4, 9, 11, 14, and X [37]. Partial loss of the chromosomes is usually detected in advanced stage tumors. Similarly, gain of the whole chromosome 17 is predominantly observed in low stage tumors, whereas partial gain of 17q is usually detected in advanced stage tumors [37]. A number of CGH studies have also confirmed the unbalanced 11q deletion in approximately 20% of primary neuroblastomas, and loss of whole chromosome 11 was a frequent finding in near-triploid low stage neuroblastomas [35, 78, 79]. Unbalanced 11q deletion was detected in more than 50% of stage 4 neuroblastomas without *MYCN* amplification [35]. This genetic subgroup is also characterized by a positive correlation with deletion events, such as losses of 3p, 4p, and 14q, and an inverse correlation with 1p deletion, and furthermore 17q gain was consistently present [35, 37]. More recently, array-based CGH, a powerful tool for survey whole chromosomal changes in the tumor cells, revealed that major alternations in neuroblastoma cells are loss of 1p, 11q, and gain of 17q [80]. Array-based CGH and SNPs array have been developed for gemone-wide screening at approximately 1 Mb and 100 Kb mapping resolution, respectively. These methods will clarify the genetic differences between these two groups in future.

Allelic Loss and Tumor Suppressor Genes (TSGs)

In addition to amplified *MYCN* gene, 1p deletion, 17q gain, 11q deletion, and ploidy changes, further genetic alterations exist in neuroblastomas (Table (**1**)). In general, deletions are more common than defined chromosomal gains in neuroblastoma. Molecular genetic characterization of deleted regions in neuroblastoma largely depends upon the two-hit hypothesis [81], predicting that LOH events are the second step in the inactivation of both alleles of a TSG. LOH analyses in a large number of cases were used to define a shortest region of overlapping region (SRO) containing one or more putative TSGs. In the chromosome 1p, the numerous researches have been performed and the SRO in 1p has been narrowed into 1p36 region [24, 41, 42, 49, 63, 72]. The number of candidate genes that have emerged from LOH analyses is small, but none has been identified as the classi-

cal TSG in neuroblastoma. There may be several explanations as follows: (1) Chromosomal instability, which especially occurs in advanced stage neuroblastomas, frequently causes marker chromosomes and unidentified products of unbalanced translocations. This chromosomal instability may increase the rate of LOH, and it seems possible that not all of these LOH events are critical for tumor progression. (2) LOH could result in haploinsufficiency and the reduced activity of a particular gene at a given locus, alone or in combination with other genes, could contribute to tumorigenesis. (3) Epigenetic mechanisms such as silencing, could play an important role for tumorigenesis, then mutation is undetectable in the loci of LOH.

Table 1. Genetic and Molecular Abnormalities in Neuroblastoma

Abnormalities	Associated genetic/ molecular abnormalities	Prognosis
Triploid/ pentaploid	Unknown	Good
Diploid/ tetraploid	*MYCN* amplification	Poor
LOH 1p	*MYCN* amplification	Poor*
LOH 3p	LOH 11q, 14q, *MYCN* normal	Intermediate
LOH 4p	Unknown	Unknown
LOH 9p	Unknown	Unknown
LOH 11q	LOH 3p, 14q, *MYCN* normal	Intermediate
LOH 14q	LOH 3p, 11q, *MYCN* normal	Intermediate
17q gain	t (1;17) or t (11; 17)	Poor
	NM23-H1 overexpression	
	Survivine overexpression	
	MYCN amplification	
MYCN amplification	LOH 1p, 17q gain	Poor
CCND1 amplification	*CCND1* overexpression	Unknown

LOH, Loss of heterozygosity

*1p 36 small deletion is not correlated with poor prognosis

The *TP53* gene is one of the most famous TSGs in human neoplasia. Several studies have examined TP53 gene mutation but it was rare in neuroblastoma [82]. However, there is still controversy about this involvement of TP53 in neuroblastoma. Some reports showed cytoplasmic sequestration in undifferentiated neuroblastoma [83], whereas p53 mutations develop frequently in relapsed neuroblastoma, correlating with multi-drug resistance [84, 85]. The *CDKN2A* (INK4A/p16) gene is sometimes deleted or mutated in adult cancers, while inactivation of this gene has not been reported in primary neuroblastoma [86].

Apoptosis related genes have been emerged as one of the strongest candidate TSGs in neuroblastoma. Caspase 8, a cysteine protease involved in death-receptor induced apoptosis, is activated in programmed cell death. Alterations of the caspase 8 gene, *CASP8*, have been described in neuroblastomas [87-89]. Activated caspase 8 will lead to activation of pro-caspase 3 and initiation of the final pathway to apoptosis. *CASP8* is located at human chromosome band 2q33, a region associated with LOH in neuroblastomas and several other tumor types [39, 87, 90]. The overall loss of *CASP8* expression in neuroblastomas is estimated at 25–35%, predominantly in high-risk tumors, and seems strongly associated with amplified *MYCN* [87, 88]. The lack of *CASP8* expression was associated with hypermethylation of its promoter region in neuroblastoma tumor samples. *CASP8* acts as a tumor suppressor gene, and inactivation will result in cell survival because it exists in the central position of the extrinsic apoptotic route. Tumor cells with loss of *CASP8* do not respond to TNF-receptor mediated triggers like TNF receptor apoptosis inducing ligand (TRAIL) or Fas ligand (FasL) [87, 91, 92]. Therefore, the down-regulation of *CASP8* may be the first evidence of alterations in apoptosis in neuroblastomas. Flice inhibitory protein (FLIP), a caspase 8-related protein, is an important regulator of caspase 8. The *FLIP* gene is a structural homologue of *CASP8* and co-localizes at chromosomal band 2q33. In neuroblastoma cell lines, a strong association was found between silencing of *CASP8* and *FLIP*. The majority of these cell lines were hypermethylated for *Casp8* and *FLIP* [93].

Expression of Neurotrophin Receptors

Neuroblastoma originates from sympathetic neurons, which are derived from the neural crest. The migration of neural crest cells during embryonic development is controlled by several molecules including the bone morphogenetic proteins, MASH1 (mammalian Achaete-Scute homolog 1), Ret, MYCN, and Trk family tyrosine kinase receptors. Trk receptors encoding the high-affinity receptor tyrosine kinases for neurotrophins are important to regulate growth, differentiation and apoptosis of neuroblastoma. High expression of TrkA, a high affinity receptor of NGF, which is expressed in cells at the late stage of embryonic development of sympathetic neurons, was observed in neuroblastomas with favorable prognosis which often showed spontaneous regression or maturation [94-97]. A trophic theory is that normally developing sympathetic neurons survive and differentiate by the target-derived supplement of neurotrophins. The stromal cells such as schwannian cells and fibroblasts, may supply a limited amount of NGF which partly regulates differentiation and programmed cell death of the neuroblast as shown in normal sympathetic neurons [98]. Thus, in neuroblastomas with good prognosis, tumor cells expressing TrkA receptor may be dependent on a limited amount of NGF supplied from the stromal cells and the tumor cells, when they could obtain enough amount of NGF, become to maturate or to regress spontaneously [98, 99]. On the other hand, the levels of TrkA expression are extremely low in aggressive tumors with *MYCN* amplification and/or 1p loss [94, 99]. In contrast, TrkB is preferentially expressed in aggressive neuroblastomas and its preferred ligands, BDNF and NT-4/5, are also expressed together with an autocrine/ paracrine manner [100, 101]. Thus, aggressive neuroblastomas shut off TrkA signals by down-regulating its expression or disturbing the downstream signaling cascades, whereas BDNF or NT-4/TrkB autocrine system may stimulate to grow much efficiently. TrkC is expressed rather in favorable neuroblastomas at variable levels [102], but its preferred ligand, *NTF3*, is usually undetectable by RT-PCR in primary tumors [98]. Thus, expression levels of Trks show one of heterogeneous characteristics in neuroblastoma.

Telomere and Telomerase Biology

Normal cells have a limited life span, only dividing 20 to 80 times before undergoing growth arrest (senescence) and

eventually dying. Telomere, specialized DNA-protein structure at the ends of eukaryotic chromosomes, consists of a large number of tandem repeats of short guanin-rich sequence which is highly conserved throughout evolution [103, 104]. The gradual erosion with each cell division of chromosomal telomeres plays an integral role in cell senescence and activation of a mechanism for maintaining telomeres is a key to cell immortality [105]. Telomerase is a unique reverse transcriptase capable of maintaining telomere length that is expressed in germ-line cells and immortal cells, not in most somatic cells, due to the repression of telomerase during development. Expression of telomerase activity and stabilization of telomeres are frequently found in malignant cells, which is consistent with the hypothesis that telomere maintenance is essential for attainment of immortality in tumor cells (Fig. (2)) [106, 107]. Using a highly sensitive, polymerase chain reaction-based assay for measuring telomerase activity [108, 109], which is known as the TRAP (telomeric repeat amplification protocol) assay, several studies have reported telomerase activity in neuroblastoma tissue (Table (2)) [79, 110-113]. Telomerase activity was not detectable in adrenal gland or in ganglioneuromas, but was detectable in almost all untreated neuroblastoma specimens except for stage 4S tumors [110, 111]. Moreover, high expression of telomerase activity has been shown to correlate with advanced stages of disease and with tumor biological features that predict an adverse prognosis [110-112, 114].

TRF (terminal restriction fragment) length, an indicator of telomere length, of normal adrenal glands in neuroblastoma patients ranged between 8 and 15 kb [114, 115], which are similar in size to other normal somatic cells.

Neuroblastomas with high telomerase activity have various telomere lengths, but these are presumably stabilized and maintained at a constant length, and in some cases are elongated far beyond that detected in normal cells (Fig. (3)). On

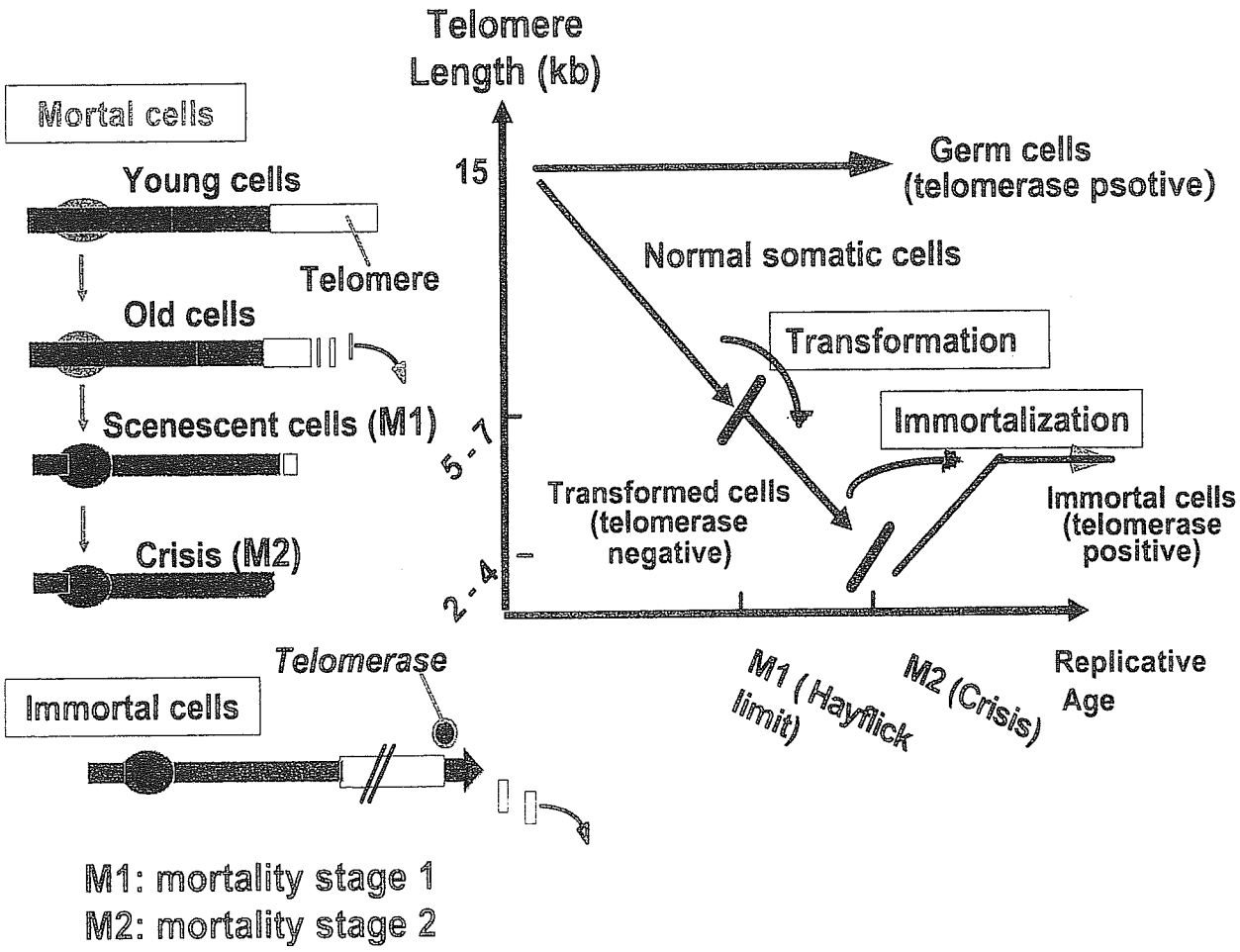

Fig. (2). The telomere hypothesis. These diagrams show the changes that occur in telomere length (vertical axis) in cells undergoing many cycles of division (horizontal axis). Human germ cells express high levels of telomerase and thus are able to keep their telomere lengths approximately 15 kilobases (kb). Germ cells differentiate into somatic cells, which do not express telomerase or do so at very low levels. Each time somatic cells divide, their telomeres shorten. When telomere attrition becomes critical (~4 kb), somatic cells senesce and can no longer divide. Telomere shortening in normal somatic cells leads to senescence which is likely triggered by a DNA damage response due to critical telomere loss or uncapping on one or a few chromosome ends (M1: mortality stage 1). If cells lack this checkpoint, or suffer a transforming growth control mutation, they can continue to divide, losing telomeric DNA until the crisis phase characterized by major telomere dysfunction, genetic instability (M2: mortality stage 2). Immortal cancer cells overcome M1 and M2 by maintaining their telomeres by the expression of telomerase. Immortal cancer cells with telomerase activity typically have short but maintained telomeres after more than 300 times replication.

Table 2. Reports of Telomerase Study in Neuroblastoma

Study	Summary of Data	Ref.
Kim *et al.* (n = 5)	Telomerase positive 5/5 (100%)	[108]
Hiyama *et al.* (n = 105)	Detectable telomerase 101/105 (96%) Stage I,II 51 (low 48, high 3) Stage III,IV 45 (low 25, high 19) Stage IVS 9 (low 5, high 1)	[110, 111]
Reynolds *et al.* (n = 150)	High hTR expression 60 (40%). Stage I = 7%, II = 18%, III = 38%; IV = 80%, IVS = 30%	[112, 134]
Brinkschmidt *et al.* (n = 14)	Telomerase positive Stage IVS 4/14 Telomerase positive Regressed Stage IVS 1/10	[79]
Poremba *et al.* (n = 16)	Telomerase positive 12/16 (75%)	[113]
Dockhorn-Dworniczak *et al.* (n = 77)	TRAP positive 22/77 (29%) TRAP positivity correlated with poor survival in stage IVS	[135]
Choi *et al.* (n = 106)	Telomerase positive 43/106 (41%), hTR positive 43/106 (41%). High telomerase activity or hTR correlated with poor prognosis	[136]
Isobe *et al.* (n = 16)	High hTERT expression correlated with unfavorable tumor	[137]
Krams *et al.* (n = 124)	Full-length hTERT (30/124) correlated with poor prognosis	[121]
Streutker *et al.* (n = 38)	Detectable telomerase (19/38, 50%) correlated with poor prognosis. Dead cases: undetectable 1/19, low 0/4, high 6/15	[138]
Nozaki *et al.* (n = 65)	High telomerase activity and low NTRK1 expression correlated with poor prognosis. The 5-year event-free survival rates:low 86.5%	[139]
Maitra *et al.* (n = 32)	High hTR expression 11 (40%) Advanced stages: 9/12, early stages: 2/8 stage 4S: 0/4, ganglioneuroblastoma/ ganglioneuroma: 0/8	[140]

the other hand, none of those tumors with low or undetectable telomerase activity have elongated telomeres and those with shortened telomere lengths may be the result of repeated replication without sufficient telomerase activity. Alternative telomere lengthening (ALT), which is an alternative mechanism to elongate telomere in some immortal culture cells, has not been reported in primary neuroblastoma. Indeed, most stage 4S tumors examined showed shortened telomeres relative to normal tissue, suggesting that telomere shortening with low or absent telomerase activity may be a factor in promoting the spontaneous regression of the tumors seen in some patients.

Most patients whose tumors showed low or undetectable telomerase activity were younger than 1 year old at diagnosis, while high telomerase activity was also associated with advanced stages. More than one half of patients with high telomerase activity died of disease [111]. Amplification of the *MYCN* gene and 1p loss were usually detectable in the tumors with high telomerase activity, but rarely in the tumors with low or undetectable activity [111]. While most tumors with undetectable or low telomerase activity showed high expression of *NTRK1* and *HRAS*, more than one half of those tumors with high telomerase activity showed no expression of these genes. Thus, high telomerase activity correlates with molecular biological features of neuroblastoma that predict aggressive clinical behavior while low telomerase activity is associated with tumor biological features known to be associated with less-aggressive tumors (Table **3**).

In human germ cells, telomerase is expressed to maintain telomere length, while in embryonal somatic tissues telomerase is likely repressed gradually before birth as cells differentiate [116]. Neuroblastoma is likely derived from primitive cells that originate in the embryonal neural crest, either from multipotent stem cells or from more differentiated neuroblasts. In fetal adrenal glands, neuroblasts increase in number and size until 14-20 weeks of gestational age and then regress [117, 118]. Adrenal gland tissues of fetuses at 16 and 18 weeks of gestational age exhibited low telomerase activity. As shown in Fig. (**4**), neuroblastomas could acquire telomerase activity from a failure to repress telomerase activity during development (i.e. retention of telomerase activity in pre-neoplastic neuroblasts). Alternatively, reactivation of telomerase could occur in the tumor cells, perhaps as a consequence of other genetic alterations. High telomerase activity seen in many aggressive neuroblastomas is likely to be due to reactivation of telomerase by the tumor during progression. The fact that most of these aggressive tumors are found in children older than one year is also compatible with

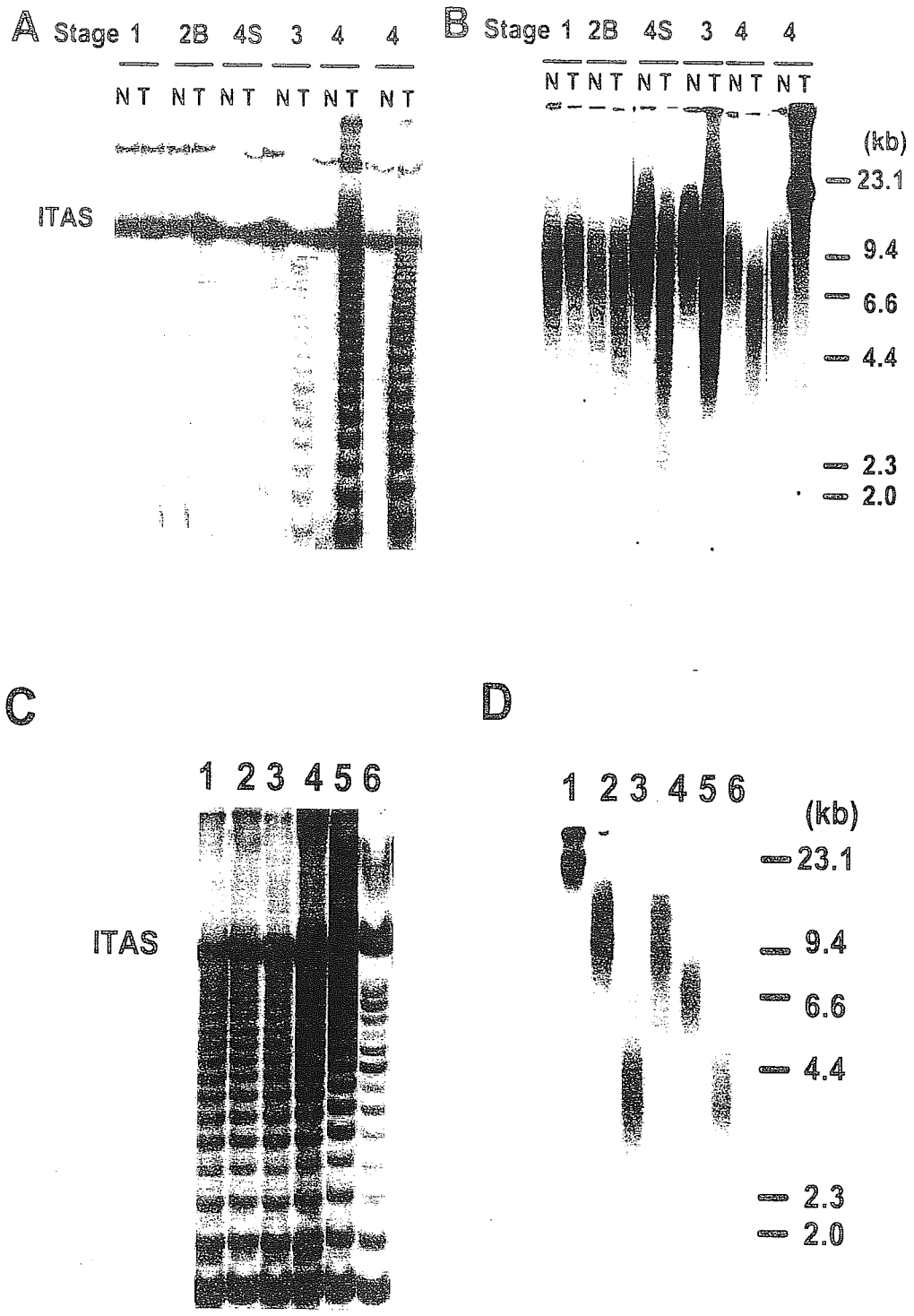

Fig. (3). Telomerase activity and telomere length in neuroblastoma specimens (A, B) and in neuroblastoma cell lines (C, D) N, normal adrenal gland; T, neuroblastoma tissue. (A) Telomerase activity was measured by TRAP assay in 6 representative neuroblastoma samples with each normal adrenal gland tissue. Stages were classified according to International Neuroblastoma Staging System (INSS). No noncancerous adrenal gland tissues showed telomerase activity. In neuroblastoma tissues, cases with stage 1, 2B, 4S tumors showed no detectable activity, while stage 3 case showed low activity and 2 stage 4 cases showed high activity. (B) Telomere length was measured by Southern blot analysis in 6 representative neuroblastoma samples. In cases of stage 1, 2B and 3, telomere length in noncancerous tissues was undistinguishable from that of each neuroblastoma sample. In stage 4S case, telomere length was shorter than that of noncancerous tissue. In this case, the tumor did not have telomerase activity, suggesting that telomere shortening may occur with regression of this tumor. However, in two stage 4 cases, tumor samples showed shorter and longer telomere, respectively. Since telomerase was activated in these tumors, telomere lengths were stabilized in these lengths. (C) Telomerase activity was measured by TRAP assay in 6 representative neuroblastoma cell lines. All showed high levels of telomerase activity. (D) Telomere length was measured by Southern blot analysis in 6 representative neuroblastoma cell lines. Since telomerase was activated in these cell lines, telomere lengths were stabilized in various lengths.

Table 3. Correlation Between Telomerase Activity and Other Prognosis-Associated Factors in Neuroblastoma Cases [110, 111]

Telomerase activity*		undetectable (n = 9)	low (n = 107)	high (n = 35)
Stage	I, II (n = 72)	2	66	6
	III, IV (n = 63)	1	34	28
	IV-S (n = 14)	6	7	1
MYCN amplification		0	0	24
1p LOH¶ / informative cases		0/6	4/66	18/26
NTRK1 expression		4	77	8

According to serial dilution analysis of telomerase assay, telomerase positive samples were classified into two groups: high telomerase activity (e.g., a TRAP signal retained using 100 times diluted extracts containing 0.06 μg of protein) and low telomerase activity (e.g. a TRAP signal was detectable using the extract containing 6 μg of protein but not using 100-times diluted extracts containing 0.06 μg of protein).

¶1p LOH: loss of heterozygosity in short arm of chromosome 1

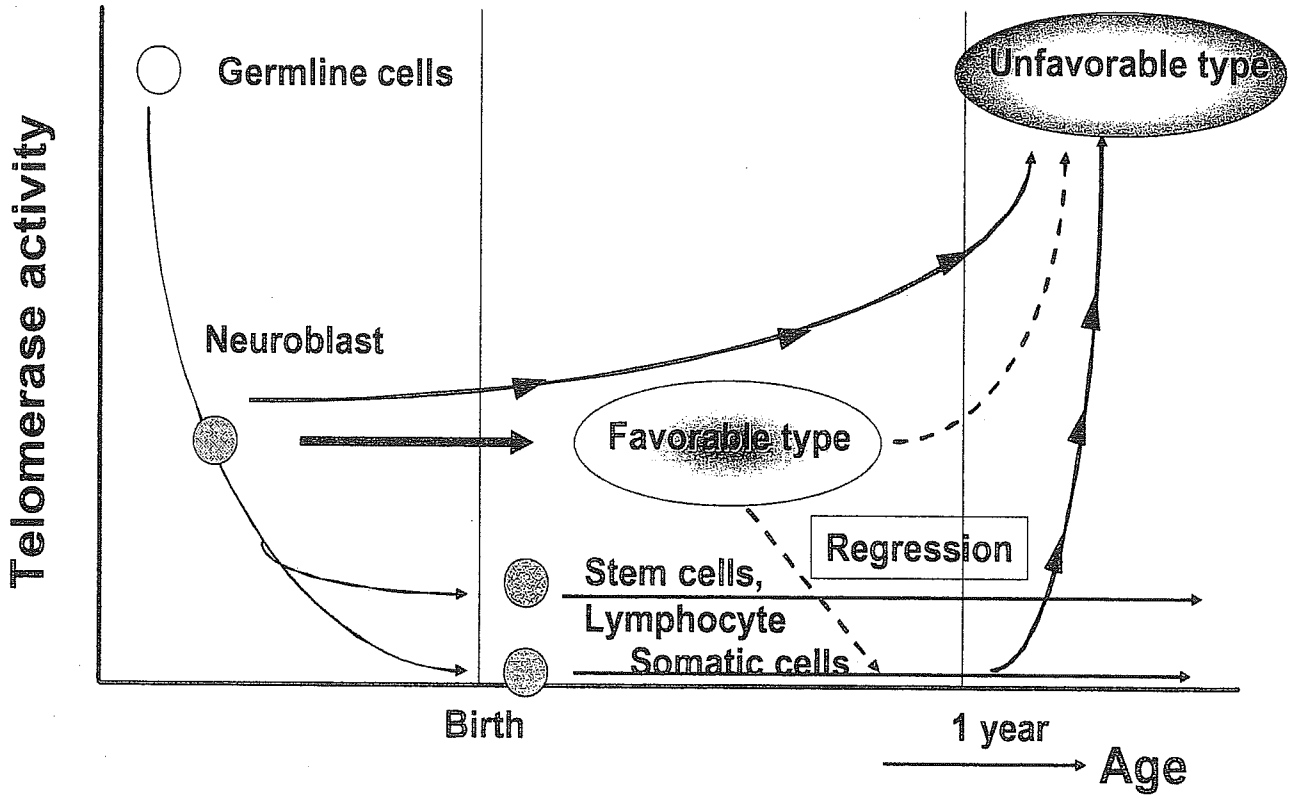

Fig. (4). Hypothesis of telomerase activity and neuroblastoma development. Normal neuroblasts, which develop from germline cells, have low telomerase activity at fetal development. Telomerase activity is repressed in normal neuroblasts and other somatic cells except for stem cells and lymphocytes before birth. Favorable neuroblastoma is developed from the neuroblasts retaining the expression of fetal levels of telomerase activity. Most of these tumors occur in young infants and some of them regress concomitant with a loss of telomerase activity. Telomere lengths of these tumors are indistinguishable or shorten from those of normal tissues. On the other hand, unfavorable tumors occur with telomerase reactivation. In these tumors, other genetic aberrations such as *MYCN* amplification promote cell division and shortening of telomere. Critical shortening of telomere causes chromosomal instability which contributes to the accumulation of additional genetic alterations and telomerase reactivation. This reactivation stabilize telomeres at various length in tumor cells capable of indefinite proliferation. These tumors occur in elder children and frequently show poor prognosis. Thus, telomerase expression may be required as a critical step in the multigenetic process of tumorigenesis, and two different pathways may exist for the development of neuroblastoma.

the hypothesis that such tumor cells likely have emerged after accumulating several genetic changes during the course of multiple cell divisions. Favorable neuroblastomas appear to share many features of neuroblasts. Most of these favorable tumors occur in infants (< one year old) and appear to have few genetic aberrations as described previous sections. In some of these cases, of which stage 4S patients may be the best example, a lack of telomerase activity may be related to

regression of the tumors. In such tumors the levels of telomerase activity are likely insufficient to maintain telomere length, so that as the tumors continue to proliferate, the telomeres shorten, eventually resulting in senescence or cell death. Thus, a failure to maintain telomeres may be one mechanism by which some neuroblastomas undergo spontaneous regression.

Human telomerase activity is associated with the expression of two major components: human telomerase RNA (hTR) [104] and human telomerase reverse transcriptase (hTERT) [119]. Recent studies have targeted the expression of these two components as surrogates of telomerase activity and discussed the feasibility of their quantitative evaluation. Since hTR is expressed at low level even in cells without telomerase activity [120], detection of *hTERT* mRNA expression is believed to be a more reliable marker for existing cancer cells in neuroblastoma [121-123]. However, the existence of splicing variants of *hTERT* mRNA that do not produce telomerase activity [124] is also problematic in detection of *hTERT* mRNA as a surrogate of telomerase activity.

In situ hybridization (ISH) of telomerase components (hTR and *hTERT* mRNA) and hTERT immunohistochemistry (IHC) are applicable to fixed cells [125-127]. However, hTR is detectable at low level in cells without telomerase activity, and it does not always represent telomerase activation. On the other hand, hTERT is the catalytic component of human telomerase and its expression levels appear to parallel with those of telomerase activity. Thus, ISH of *hTERT* mRNA and hTERT IHC are preferable to evaluate telomerase activation [125, 126, 128]. The expression of hTERT appears to be heterogeneously distributed in adult cancer tissues and the distribution shows regional variability in some cases. By contrast, in neuroblastoma, the level of hTERT expression in each cell differed between the unfavorable tumors with high telomerase activity and the favorable ones with low telomerase activity. The levels of hTERT expression may reflect the differentiating process of these tumor cells [125]. The detail analysis of hTERT, hTR, and telomerase associated proteins should contribute to clarify the biology of neuroblastoma in future.

Telomerase activity might also provide a therapeutic target against malignant cells, including neuroblastoma. Agents that inhibit telomerase activity which are under development include reverse transcriptase inhibitors [129], and antisense strategies (oligonucleotide, peptide nucleic acids (PNA) [130]) or ribozymes [131] against hTR. Since telomerase activity is repressed in most human somatic tissues, toxicity of agents targeting for telomerase will be minimal. The antitelomerase agent GRN163, which is a 13-mer oligonucleotide complementary to the template region of the human telomerase RNA subunit hTR, inhibits growth of glioblastoma *in vivo* [132]. This finding supports further development of this compound as a potential anticancer agent for neuroblastoma. However, such a therapeutic strategy may be limited by the length of therapy required if telomere shortening is required to trigger tumor cell death, and by alternative, non-telomerase mechanisms for telomere maintenance [133]. The successful application of such therapy will require a thorough understanding of the biology of telomeres and telomerase in both normal and neuroblastoma cells.

PERSPECTIVES

Neuroblastoma, despite many advances in the understanding of its biological heterogeneity and developmental molecular pathways, has remained serious in young children. Basic research and clinical efforts will lead to an understanding of the molecular pathways governing both progression and spontaneous regression of neuroblastoma. Neuroblatoma mass-screening project revealed that more than half of infant neuroblastomas regress or mature. These events should provide the platform from which new diagnostic tools can be developed and from which new types of therapies for individual patient can be attempted. Recently, genome-wide genetic aberrations and gene expression profiles are able to be identified by microarray analysis. More precise definition of the molecular changes in neuroblastomas may allow for more specific therapies with subsequent improvements in overall rates and quality of cure.

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報　告

神経芽腫

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1　はじめに

神経芽細胞腫は，神経提由来の腫瘍で，小児固形悪性腫瘍の中では多い腫瘍の一つである．また，この腫瘍は，小児がんの中でも腫瘍特性が最も多様性を示すがんとして知られ，その治療は，リスク分類に従って選択されるのが一般的である．診断は病理で神経芽腫と確定するか，尿中のvanillylmandelic acid (VMA)，homovanillic acid (HVA) の上昇と骨髄で神経芽腫を示唆する細胞塊を検出することによってなされる．治療方針決定には，正確な病理診断と生物学的特性によるリスク分類が必要で，手術的摘出または生検での腫瘍の解析が必須となる．リスク分類は，診断時年齢，組織分類（嶋田分類，INPC 分類：International Neuroblastoma Pathology Classification）[1-3)]，病期（INSS分類:International Neuroblastoma Staging System），*MYCN* 遺伝子増幅，腫瘍細胞の染色体数（Ploidy），等の分子生物学的因子を組合わせて判定する．通常では，高リスク群，中等度リスク群，低リスク群に分類して，治療方針を決定する．こうした状況で，手術不能の進行がんは，生検後にリスク判定を行い，それに応じた化学療法を行い，そののちに摘出術を行う．

2　組織学的分類

神経芽腫群腫瘍の組織分類は，従来の日本小児外科学会悪性腫瘍分類では，良性の神経節腫と，悪性は神経節細胞の混在の有無による神経節芽腫と神経芽腫に分類し，前者は高分化型，混在型，低分化型に，後者は花冠細繊維型と円形細胞型に亜分類していた．嶋田らは，年齢によって分化傾向を示すことに着目し，年齢因子を加味した病理所見からのリスク分類として嶋田分類を提唱した．この考え方がベースとなり，今は，International Neuroblastoma Pathology Classification (INPC) System が提唱されており，これに基づいて分類する（表1）[1-4)]．

3　病期分類とリスク分類

病期は，従来，Evans の分類[5)]に従い，I，II，III，IV 期と IV-S 期に分類されていたが，本邦の日本小児外科学会悪性腫瘍分類[6)]では病期 IV 期を IV-A 期と IV-B 期（IV-S 期と同じ転移部位であるが，原発巣が進行しているもの）に分けていた．最近は，手術による切除範囲を加味した INSS 分類[7, 8)]で分類されることが多い．

リスク分類は，表3に示した様に，診断時年齢，病期（INSS 分類：International Neuroblastoma Staging System），組織分類（嶋田分類，INPC 分類：International Neuroblastoma Pathology Classification）[1-3)]，*MYCN* 遺伝子増幅，腫瘍細胞の染色体数（Ploidy），等の因子を組合わせて判定する[9)]．通常では，高リスク群，中等度リスク群，低リスク群に分類するのが一般的であるが，表4に示した一番染色体短腕（1p）欠失，17番染色体長腕増加，Trk A 発現などのマーカーで3群（タイプ1から3）に分けられている腫瘍特性からみたサブセット分類を参考に決定する[10-12)]．

4　治療法の概要

低リスク症例の治療は，ほとんどの症例は外科

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的摘出のみであるが，一部の症例に 6-12 週の化学療法を付加する．脊髄の圧迫症状，病期 4S で肝腫大のために呼吸障害がある症例などである．薬剤毒性を起こさないように各薬剤の投与総量を最小限にする．中等度リスク群では，外科切除後に 12-72 週の化学療法を行うのが原則である．腹部の神経芽細胞腫で腎臓を巻き込んだ症例に対して，化学療法前に腎臓合併切除は行うべきでない[14]．（エビデンスのレベル：III，勧告のグレード：B）．高リスク群では，大量の cisplatin や ifosfamide などを用いた大量多剤併用化学療法を行い，その後に外科切除を行い，さらにその後に全身照射，造血幹細胞移植などの治療法を組合わせた化学療法を行う．放射線療法はこうした化学療法前，施行中，終了後に組合わせる．終了後，分化誘導療法を行うことがある[15]．一部では，大量化学療法，放射線療法を施行し，最後に局所療法として手術を行うことが試みられているが，治

表 1　International Neuroblastoma Pathology Classification (Shimada System)[1, 2]．

以下の４グループとそれぞれの亜分類に分ける

1. Neuroblastoma (Schwannian, stroma-poor)
   - a. Undifferentiated
   - b. Poorly differentiated
   - c. Differentiating
2. Ganglioneuroblastoma, Intermixed (Schwannian stroma-rich, GNB stroma-rich, mature Schwannian cell > 50%)
3. Ganglioneuroma (stromal dominant)
   - a. Maturing (scattered neuroblasts, not in nests)
   - b. Mature ganglio-neuromatous tumour with a few randomly dispersed immature neuroblasts. No distinct nests of neuroblasts are found.
4. Ganglioneuroblastoma, Nodular

Unfavorable histology group は以下のように定義する:

1. All undifferentiated neuroblastomas
2. All nodular ganglioneuroblastomas
3. All neuroblastomas in patients older than 5 yrs of age
4. Poorly differentiated / differentiated neuroblastomas with high MKI in patients less than 1.5 yrs
5. Poorly differentiated neuroblastomas with low MKI or differentiated neuroblastomas with high/ intermediate MKI's in patients 1.5-5 yrs

表 2　INSS 病期分類[7, 8]

Stage 1	原発部位に限局した腫瘍：肉眼的完全切除で，組織学的腫瘍残存は問わない．ただし，腫瘍に接して切除されたリンパ節は転移があってもよい．
Stage 2A	限局性の腫瘍で肉眼的にも不完全切除；組織学的に腫瘍に接していない同側のリンパ節転移を認めない．
Stage 2B	限局性の腫瘍で完全または不完全切除．同即の腫瘍に接していないリンパ節転移あり．組織学的に対側のリンパ節転移を認めない．
Stage 3	切除不能の片側性腫瘍が正中線を越えたもの（局所リンパ節転移はありまたはなし）．または片側性腫瘍で対側の局所リンパ節転移があるもの．
Stage 4	原発腫瘍の進展範囲に問わず，遠隔リンパ節，骨，骨髄，肝，皮膚，さらに／または他の臓器に進展するもの（stage 4S を除く）
Stage 4S	限局性の原発腫瘍（stage 1, 2A, 2B）で，転移部位が皮膚，肝，骨髄にとどまるもの．ただし，年齢は 1 歳未満． 骨髄転移は浸潤腫瘍細胞が有核細胞の 10％未満．

療成績は明らかでない．（エビデンスのレベル：IV，勧告のグレード：D）

放射線療法は，化学療法に反応せず生命危機あるいは臓器障害をきたす症状がある時，中等度リスク群で化学療法と手術にて治療が不十分の時，および高リスク群に施行する．高リスク群では，全摘後の局所照射が行われる場合もある．

脊髄圧迫症状で緊急の対処を要する場合，化学療法，放射線療法，椎弓切除術がある．椎弓切除術は側彎という晩期障害をきたす．脊髄圧迫症状が出現して72時間以上を経ると非可逆性の神経障害を残すので，緊急椎弓切除は72時間以内症例に限定される．低リスク群あるいは中等度リスク群では，晩期障害が少ない化学療法が選択され

表3　神経芽細胞腫のリスク分類[9]

INSS病期分類	診断時年齢*	MYCN増幅	INPC病理分類	DNA ploidy	リスク分類
1	—	—	—	—	低
2A/2B	1才未満	—	—	—	低
	1才以上	なし	—	—	低
		あり	favorable	—	
		あり	unfavorable	—	高
3	1才未満	なし	—	—	中等度
		あり	—	—	高
	1才以上	なし	favorable	—	中等度
			unfavorable	—	高
		あり	—	—	高
4	1才未満	なし	—	—	中等度
		あり	—	—	高
	1才以上	問わない	—	—	高
4S	1才未満	なし	favorable	hyperdiploid	低
			—	diploid	中等度
			unfavorable	—	中等度
		あり	—	—	高

＊診断時年齢においても，1才半で分けるべきであるとの報告[10]もある．
—：結果を問わない項目

表4　神経芽細胞腫の生物学的因子によるサブセット分類[11-13]

生物学的因子	タイプ1（低悪性度）	タイプ2（悪性度中等度）	タイプ3（高悪性度）
MYCN 増幅	なし	なし	あり
核型	hyperdipolid	near-diploid	near-diploid
	near-triploid	near-tetraploid	near-tetraploid
1p 欠失	稀	少数	あり
17q 増加	稀	あり	あり
Trk A 発現	高発現	低発現／なし	低発現／なし
Ha-ras 発現	高発現	低発現／なし	低発現／なし
テロメラーゼ	低発現／なし	低発現	高発現
年齢	通常1才未満	通常1才以上	1　5才
INSS 病期分類	1，2，4S	3，4	3，4
無病3年生存	90%以上	30-50%	20%以下

ることがある.

マスクリーニングで発見された乳児例などでは,手術による合併症を避ける目的で,外科的な処置による確定的な組織診断なく経過観察される場合がある[16,17]. 米国では,周産期のEvansの病期分類Iでこのような試みがなされている.

(エビデンスのレベル:IV, 勧告のグレード:D)

5 化学療法ガイドライン

A) 低リスク神経芽腫

外科的に全摘できた低リスク症例に対する化学療法:

低リスク群の治癒(長期生存)率は90%以上であることから[18-22],外科的摘出術のみにて経過観察する[18,20-22]. (エビデンスのレベル:I, 勧告のグレード:A)

外科的に全摘できた低リスク症例で,化学療法施行による有意な成績向上が得られた報告はない.以下の症例が対象となる.

1) INSS 2の低リスク腫瘍で50%以上の腫瘍が残存した症例
2) INSS 2の低リスク腫瘍で,術後なお生命危機や臓器障害をきたすおそれがある症例. 臓器障害には呼吸障害,腎障害,脊髄圧迫症状,消化管閉塞,尿路閉塞や凝固障害などがある[23,24]. (エビデンスのレベル:III, 勧告のグレード:B)

化学療法の期間は6-24週間, cisplatin, cyclophosphamide, doxorubicin, etoposide, vincristineを副作用予防のため比較的低濃度で使用する. vincristineとcyclophosphamideを一週毎に投与して,3クール後の効果を判定し,必要な場合さらに3クールを追加する. Carboplatinまたはcyclophosphamideまたはcyclophosphamideとdoxorubicin1回とetoposideを3回/3日間, carboplatin, cyclophosphamide, doxorubicinを各1回のレジメンを3週ごと6-24週行う.

化学療法が奏功しない症例には放射線療法を併用する場合もある[23]. これらの薬剤を低リスク腫瘍に使用した群と使用しない群での長期生存率の有意差は得られていない[20]. (エビデンスのレベル:IV, 勧告のグレード:D)

病期4Sの症例の治療は,臨床症状によって異なる. このタイプの腫瘍は臨床的に安定していれば治療は不要である. 生後2-3ヶ月未満児に多い巨大な肝腫大による心肺や大血管の圧迫などの合併症は治療対象となる[25-27]. 80例の病期4S症例の長期生存率は,保存的治療例が100%であったのに対し,低用量の化学療法を受けた症例は81%であった. 原発巣の切除は治癒(長期生存)率の改善につながらない. (エビデンスのレベル:II・勧告のグレード:B)[22].

B) 中等度リスク群の化学療法

中等度リスク群の化学療法の対象は

1) 1歳未満の*MYCN*遺伝子増幅がないINSS3・4期の症例
2) 1歳以上で*MYCN*遺伝子増幅がなくINSS3期でINPC(嶋田)分類favorable typeの症例,
3) 1歳未満のINSS4S期で*MYCN*遺伝子増幅がなく, INPC(嶋田)分類unfavorable typeかdiploid核型など予後不良因子を持つ症例である.

薬剤の組み合わせとコースは, cyclophosphamide, vincristine, pirarubicin, cisplatin(持続静注)を組み合わせた5日間のレジメンとcyclophosphamideとDTICからなる5日間のレジメンを組みあわせて4週毎に52-76週まで行う.

INSS3症例の化学療法は欧米ではcyclophosphamide, etoposide, doxorubicin, cisplatinを組み合わせたレジメンを3-4週ごとに行い,18週で手術を行った後,化学療法,放射線療法施行し,維持療法としてcyclophosphamide; doxorubicin, cisplatin + etoposideを交互に4回繰り返し,最後に残存腫瘍の手術を行うプロトコールと,先の4剤を4週ごとに行い,12週で骨髄を採取し,17週で手術,化学療法と放射線療法後に自家骨髄移植を行うプロトコールが用いられている. 移植後は維持療法を行うが,13シスレチノイン酸による分化誘導療法を組み合わせることもある[18].

また，最近は，carboplatin，cyclophosphamide，doxorubicin，etoposideを組み合わせてたレジメンも試みられている．予後不良因子を有する症例では，各薬剤の投与量と投与回数を増量させる．治療期間については，favorable typeでは12週，unfavorable typeでは24週行う．1才未満でMYCN遺伝子増幅がないINSS 4期の症例は中等度リスク群であるが，favorable histologyでtriploidであれば，化学療法は12週，それ以外は24週とする．favorable typeの中等度リスク群における放射線療法は，腫瘍によって生命または臓器が侵される場合に限り施行する．unfavorable typeにおける放射線療法は，24週の化学療法後や，second look手術後の遺残腫瘍に対して行う．

治療成績は概して，50-70％の長期生存が期待できるが，1歳未満の方が治療成績が良い傾向にある[28-31]．（エビデンスのレベル：III，勧告のグレード：B）嶋田分類やINPC分類でunfavorable typeであったり，*MYCN* 遺伝子が増幅している症例の長期生存は50％と悪い[18, 22, 32]（エビデンスのレベル：II，勧告のグレード：A）．特に，INSS4期症例の長期生存率は，診断時年齢に大きく左右される．1才未満の症例は長期生存が期待できるが，その期待率は生物学的特性により異なり，*MYCN* 遺伝子が増幅のない症例の治癒率は90％以上であるのに対し，*MYCN* 増幅例は10％前後と不良である[33]（エビデンスのレベル：II，勧告のグレード：A）．米国で乳児神経芽腫の切除不能例または遠隔転移例を核DNA量からdiploidとhyperdiploidの二群に分けて治療し，前者の3年生存率は55％に対し，後者は治療を減弱したにも関わらず94％であり，予後因子による層別化の必要性を示した．（エビデンスのレベル：III，勧告のグレード：C）さらに，マススクリーニング発見例では，1歳未満の *MYCN* 遺伝子増幅がないINSS3期症例は，化学療法を施行しない群と施行した群で長期生存率に差がないとの報告がある[33-34]（エビデンスのレベル：IV，勧告のグレード：C）

薬剤毒性予防のため，それぞれの薬剤の総投与量を低く保つことが肝要である．実際には，先に示したstage IIIの神経芽腫患児に対する大量化学療法＋骨髄移植では，228人中治療毒性は，重篤な腎障害，心筋障害，聴力障害などは，5％以下で，4人が治療関連死したが，全員1歳以上で2人は原疾患の増悪によるものであった[7]．また，110人の転移性乳児神経芽腫患児のスタディでは，死亡した16人中13人が原疾患の再発で死亡し[23]，治療毒性は，さほど高くない．（エビデンスのレベル：III，勧告のグレード：C）

C）高リスク群の化学療法

高リスク群の化学療法の対象は

1) INSS1期または2A/2B期で *MYCN* 増幅症例
2) 1才未満のINSS 3/4期で *MYCN* 増幅例
3) 1才以上のINSS 3期で嶋田unfavorable histology，*MYCN* 増幅のいずれか一方を有する症例
4) 1才以上のINSS 4期例，1才未満でINSS 4S期かつ *MYCN* 増幅のある症例[35]

標準的な化学療法は，cyclophosphamide，cisplatin，doxorubicin，トポイソメラーゼII阻害剤が34-45％の奏効率を示したとの報告[36]などから，これらの薬剤が併用療法の基軸となっている．実際には，cisplatin，またはcarboplatinを中心に，cyclophosphamide，ifosfamide，vincristine，doxorubicin（または類似薬剤），etoposide（または類似薬剤）などを様々な用量で組合わせたレジメンが用いられ，4剤以上を併用した4-5日程度のレジメンの寛解導入率は76-93％である[38, 39]．これらのレジメンは4週ごとに繰り返され，2－5クール後に化学療法に反応した後に，原発巣をsecond look手術で切除し，その後に大量化学療法と自家幹細胞移植（骨髄移植あるいは末梢血幹細胞移植）を行う．大量化学療法は，melphalan，etopside，carboplatinの組み合わせによるHiMECレジメンが主流である．自家骨髄移植を施行した群と化学療法のみの治療群の2群のランダム比較試験で，自家骨髄移植例での長期生存率の向上が得られている[7, 40]．しかし，これらのスタディで全身放射線照射（TBI）併用と併用しな

いレジメンが使用されており，TBIの有用性やリスクについてのエビデンスは示されていない．最近では，TBIを用いないレジメンの自家移植の有効性が示唆されている．また，自家移植にパージングした骨髄を用いた方が良いかどうかの結論は得られていない．原発巣の完全摘除ができなかった症例では原発部位への放射線療法を考慮する．転移巣への放射線療法は症例により決定すべきである[41]．（エビデンスのレベル:IV，勧告のグレード:D）自家移植から回復後に，13-シスレチノイン酸の経口投与を6ヶ月間行うこともある．自家幹細胞移植後に13-シスレチノイン酸の経口投与を6ヶ月間投与の有効性が報告されている[7]（エビデンスのレベル：II，勧告のグレード：B）

本邦では，cisplatin, cyclophosphamide, vincristine, pirarubicinの5日間のレジメンを中心に，cyclophosphamideとDTIC, cyclophosphamideとpirarubicinとcarboplatin, ifosfamideとetoposideの3レジメンを組み合わせたプロトコールで治療し，*MYCN* 増幅例にはそれ以外と異なる高用量のレジメンを使用してきた．その結果，*MYCN* 遺伝子の増幅のない例の5年生存率は34.4%に対し，*MYCN* 増幅例は33.3%であり[38]，臨床評価を得ている．同様に *MYCN* 増幅を伴った症例が *MYCN* 増幅のない症例に比べ予後不良と考えられていたが[41, 42]，高用量の化学療法で差がないとの報告もある．[43]（エビデンスのレベル：IV，勧告のグレード：D）

一般の治療成績として，高リスク群の症例の長期生存率は10-40%で，強力な治療を行っても晩期再発や治療終了後5年以上での死亡例も報告されている[44, 45]．（エビデンスのレベル：II，勧告のグレード：A）

高用量化学療法に自家骨髄移植を施行した群と3クールの地固め療法を追加した群の比較では，大量化学療法＋移植群で3年無病生存率（ＥＦＳ）は34.4%と化学療法群の22.4%よりも有意に高い．さらに，その後に6ヶ月の13-シスレチノイン酸投与を追加した症例の3年ＥＦＳはより良好であった[7, 46]．（エビデンスのレベル：II，勧告のグレード：B）

転移を伴う高リスク群の症例に対し診断時に完全な外科切除を行うことの利点は明確にされていない[44, 47]．（エビデンスのレベル：IV，勧告のグレード：C）

診断時の原発巣の完全摘除が長期生存率を改善したとの報告があるが，広範切除よりも切除率を左右している腫瘍の特性に依存した結果とも考えられる[48-52]．（エビデンスのレベル：IV・勧告のグレード：D）

高リスク群で中心薬剤となるCisplatinでは，消化器症状（悪心・嘔吐，食欲不振等）がほとんど全例に起こる．また，急性腎不全等の腎障害，骨髄機能抑制，ショック（アナフィラキシー様症状），聴力低下，難聴，耳鳴，うっ血乳頭，球後視神経炎，皮質盲，脳梗塞，うっ血性心不全，まれに溶血性貧血，血栓性微小血管症，心筋梗塞，精神症状，肝障害が現れることがある．なお，フロセミドによる強制利尿を行う場合は腎障害，聴器障害が増強されることがあるので，輸液等による水分補給を十分行う．Cyclophosphamideは用量依存性に抗腫瘍効果が得られるが，大量使用時の副作用として出血性膀胱炎が知られており，その発症予防のためにメスナの使用が一般的である．さらに，腎毒性，性腺機能障害についても注意が必要である．また，Doxorubicinに特徴的である心毒性は5-10%程度の患者に出現しているが，患者に対する総投与量を最大500mg/m²に限定することにより，ある程度回避しうるものと期待できる．ただし，この心毒性は若年患者にはより高頻度に出現するというデータがある[53]ため，特に乳児患者においては総投与量をさらに減じて考慮するべきであると考えられる．また，胸部や腹部に放射線治療を受けた患者も心毒性のリスクが高いため，同様の考慮が必要である．Etoposideを含む併用化学療法による重大な晩期障害として，二次がん特に二次性白血病と骨髄異形成症候群がある．本腫瘍の化学療法は，併用療法で使用するために，骨髄抑制やその他の副作用が増強される可能性があるが，G-CSF製剤投与や輸血などの支持療法を積極的に行うことで対処が可能である．

また，高リスク神経芽腫に対する化学療法とし

て，大量化学療法＋骨髄移植療法が行われ，大量化学療法＋移植群の３年無病生存率が化学療法群よりも高かったが，血液学的な治療毒性は，初期治療中に grade3，４を 71％に認めた．治療関連死は移植群と化学療法群では 6％と 3％で有意な差は認めなかった[1]．一方，同様の治療方針であり，転移性神経芽腫に大量化学療法と自家造血幹細胞移植が行われ，７年の無増悪生存率は 29％で，造血幹細胞移植を受けた患者 29 例中の毒性死亡は４名（13%），全てが造血幹細胞移植の時期に発生しており，骨髄移植による副作用の有無については結論が出ていない[37]．（エビデンスのレベル：III，勧告のグレード：D）

現在，GM-CSF とモノクローナル抗体療法，131 I-MIBG（metaiodobenzylguanidine）によるターゲット放射線療法，複数回の幹細胞移植を伴う大量化学療法，幹細胞移植前の 131 I-MIBG 療法などが試みられている 54?61)．また，131I でラベルした神経芽腫細胞に対する抗体（抗 GD2 抗体）療法を導入したプロトコールも進行中で，観察期間の中央値が 19 ヶ月ながら 24 例中 18 例が非進行で生存中である 62)．（エビデンスのレベル：IV，勧告のグレード：D）

D）再発神経芽細胞腫（セカンドライン）の治療

再発或いは進行神経芽細胞腫の長期生存率とそれらの対する治療は，診断時の病期，再発時の腫瘍特性，再発部位とその程度，初期治療等多くの因子によって異なる．広範な再発時には，さらに集中的な治療を行っても通常予後不良である[63, 64]．一部に，再手術が奏功する症例がある．cyclophosphamide と topotecan の併用療法[50]や irinotecan の投与が再発例に試みられている[65-69]．

低リスク群腫瘍の治療後の再発腫瘍に対しては，外科的切除が可能であれば切除し，その後同様の化学療法レジメンを 12 週，残存する場合，あるいは転移巣がみられた場合は 24 週行うことが試されているが，まだその有効性は検証されていない．

中枢神経系への浸潤が 5-10％に認められる．稀に脳髄膜転移，脳内転移がみられる．神経学的症状の改善には早期発見と治療が肝要である[70, 71]．（エビデンスのレベル：III・勧告のグレード：B）

低リスク腫瘍として治療された症例の再発例は，中等量の cisplatin，cyclophosphamide，doxorubicin，etoposide による化学療法を行う．副作用防止のため，各薬剤の総投与量は低く抑えるべきである．年長児で嶋田の unfavorable histology あるいは MYCN 増幅を伴って再発した症例は，高リスク群に準じて治療を行う．

転移再発症例のうち，診断時が INSS1，2，または 4S で，１才以下での再発であれば，腫瘍の特性に準じて治療を行う．明らかに予後良好な特性で，４S 症例の転移部位で，３ヶ月以内の再発は経過観察とする[25]．（エビデンスのレベル：III・勧告のグレード：B）

３ヶ月以後の再発あるいは４S の転移部位以外の転移では，原発巣を可能な限り切除し，12-24 週の化学療法を行う[34]．（エビデンスのレベル：III，勧告のグレード：B）

INPC（嶋田）分類の unfavorable histology あるいは diploid 核型が再発，転移巣に認められれば 24 週の化学療法を行う[72]．（エビデンスのレベル：III，勧告のグレード：C）

中等度リスク症例として治療した患者の局所再発では，化学療法終了後３ヶ月以上経て，表 1，2 に示した様な各因子で予後良好特性を示す再発巣は外科的に切除する．完全に切除できなかった時は，さらに 12 週の化学療法を追加する．化学療法は cisplatin，cyclophosphamide，doxorubicin，etoposide を使用し，各薬剤の総投与量は副作用予防のため低く維持すべきである．

転移再発のうち，化学療法終了後３ヶ月以内の再発，あるいは予後不良特性を有している場合は高リスク群に準じた治療を選択する．しばしば，ifosfamide と高用量 cisplatin 併用が用いられる．幹細胞移植や 13-シスレチノイン酸の化学療法後の投与は生存期間を改善させる可能性がある[18]．

高リスク症例の再発は極めて難治であり，予後不良である[54]．新薬の治験等の考慮も必要かもしれない．

E）終わりに

小児悪性腫瘍の化学療法においては，長期無病生存を期待しうる高い有効性を期待できるが故に，成人の化学療法に比較してより強力に行われる傾向にある．このため，予想しうる副作用に十分な支持療法を行ったとしても，重篤な出血や敗血症をはじめとした重症感染症などを合併する危険が回避出来ない場合があり，合併症死に至る症例が少数ながら存在する．よって，本剤を用いた併用療法を行う場合においては小児悪性腫瘍に対するがん化学療法を熟知している専門的な小児腫瘍専門医師が使用する，もしくは専門医師の監督下において使用されるべきである．

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