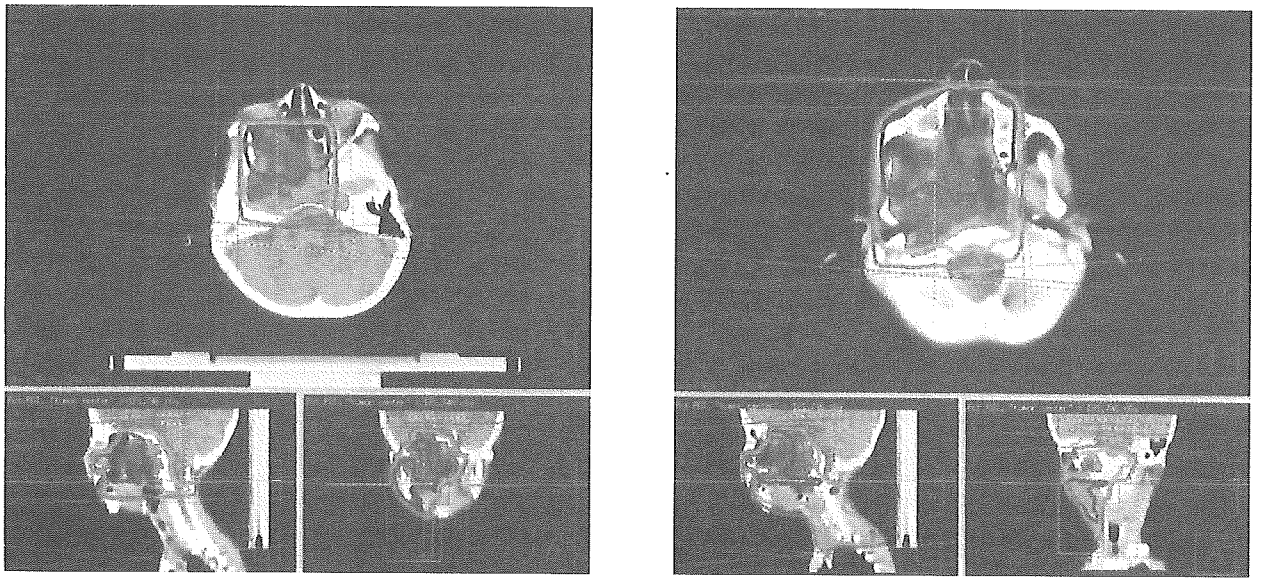

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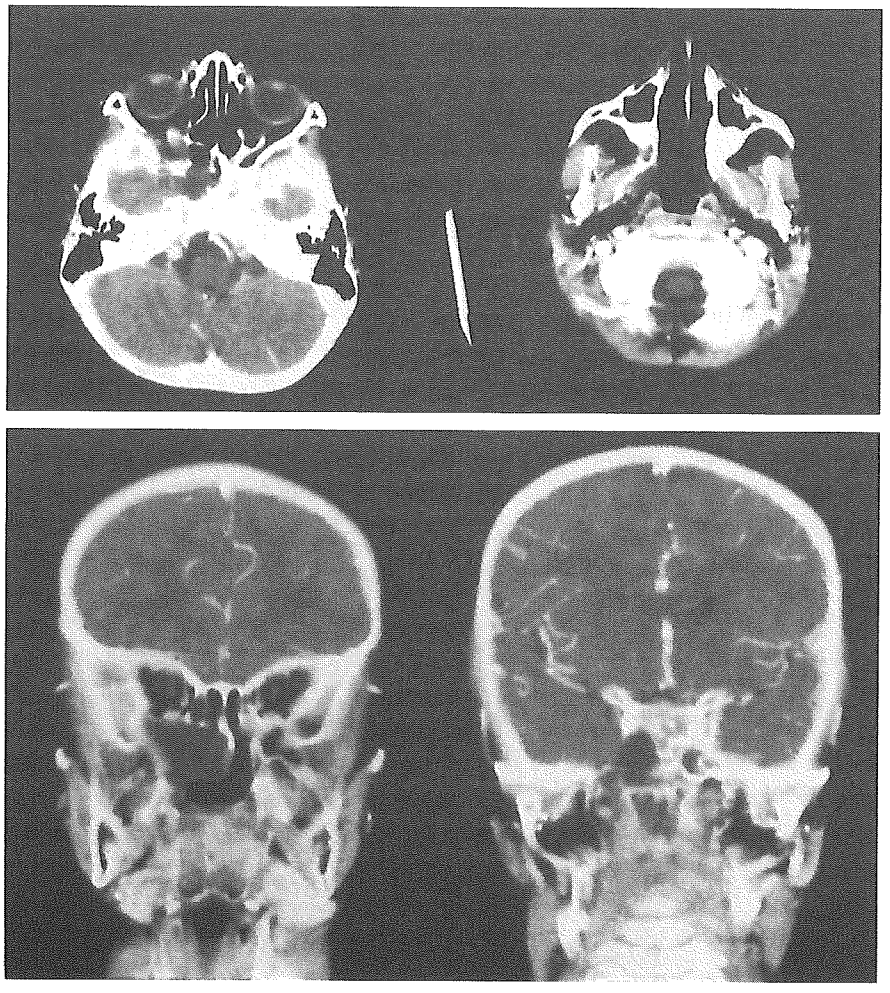

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Molecular and Developmental Biology of Neuroblastoma

Akira Nakagawara

Contents

5.1 Neural Crest Development and Neuroblastoma

Cancer has its own face reflecting the characteristics of the tissue from which it is derived. This can be demonstrated by histopathologic examination, by immunohistochemistry, and/or by in situ hybridization. Recent advances in molecular biology and genetics have also revealed that these morphological distinctions among cancers are associated with differences in gene expression profiles within tumor cell and stromal cell components. Furthermore, the patterns of gene expression unique for each cancer are dictated by genetic abnormalities which have occurred in progenitors of the specific developmental lineage. Neuroblastoma originates from the sympathoadrenal lineage, and its biology is closely related to that of normal sympathetic neurons. In this chapter, the molecular and cellular bases for the genesis and biology of neuroblastoma are summarized.

5.1.1 Genes of Neural Development and Molecular Targets of Neuroblastoma

During neural development, neural crest cells migrate and differentiate into several cell lineages, e.g., melanocytes, sensory neurons, enteric ganglion cells, and sympathetic neurons (Fig. 5.1). The first signaling molecules which trigger crest cells to differentiate or migrate are bone morphogenetic proteins (BMPs) and their receptors (Huber et al. 2002). The commitment to differentiate into sympathetic neurons is associated with the transient expression of (a) basic helix-loop-helix transcription factors, e.g., *MASH1* (a proneural gene homologous to *drosophila achaete-*

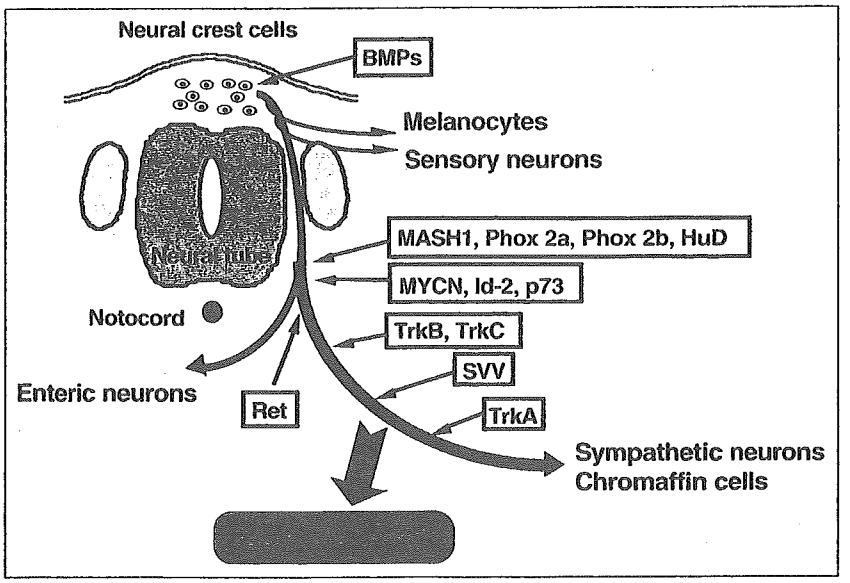


Figure 5.1

Neural crest cells originate from the sympathoadrenal lineage of neural crest. The bone morphogenetic protein (BMP) signals mainly participate in the early stage of differentiation of neural crest cells. *MASH1* (*hASH1*) may function as one of the key transcription factors which define the direction of differentiation to sympathetic neurons. The other important nuclear factors, e.g. Phox2a, Phox2b, HuD, *MYCN*, Id2, and p73, may also be involved in the cell fate determination. Some of those genes are often upregulated or amplified in aggressive neuroblastomas (Nakagawara 2004). At the stage of terminal differentiation of sympathetic neurons followed by programmed cell death, the signals through neuronal tyrosine kinase receptors, e.g., TrkB, TrkC, and TrkA are necessary sequentially and/or in a form of crosstalk. The many genes involved in regulation of neuronal terminal differentiation or programmed cell death are often expressed at high levels in favorable neuroblastomas.

scute), *HES1*, *MYCN*, HIF1α and HuD, (b) homeobox genes, e.g., Phox2a and Phox2b, and (c) p73 (a family member of the tumor suppressor gene p53; Nakagawara 2004). Several lines of investigation support the importance of these genes. *MASH1* null mice lack sympathetic ganglion cells (Guillemot et al. 1993). Notch signaling, through its intracellular domain translocation into the nucleus, stimulates the transcriptional activation of the *HES1* and *HES5* genes whose products in turn inhibit transcription of the *MASH1* gene (Radtke and Raj 2003). *MYCN* is indispensable for the normal neural development. It induces Id2 which is a negative regulator of *HES1* and pRb, a retinoblastoma suppressor (Lasorella et al. 2000). p73 knockout mice also show abnormalities in cell survival in both the nervous and immune systems (Yang et al. 2000). Gene targeting of HIF2α disturbs the catecholamine metabolism in sympathetic neurons (Tian et al. 1998). All these genes regulate each other in an orchestrated manner to drive the correct differentiation of neural crest cells into sympathetic neurons.

Further downstream, terminal differentiation to mature sympathetic cells is strongly regulated by the signaling of neurotrophin family members and their receptors (Nakagawara 2001, 2004). In addition, other genetic aberrations associated with neuroblastoma have been mapped to specific genomic regions or genes well known to be important in regulating the normal development of neurons (Nakagawara 2001, 2004). It seems obvious that a relationship should exist between the genetic or biological targets of neuroblastoma and the key molecules involved in the normal development of neural crest cells.

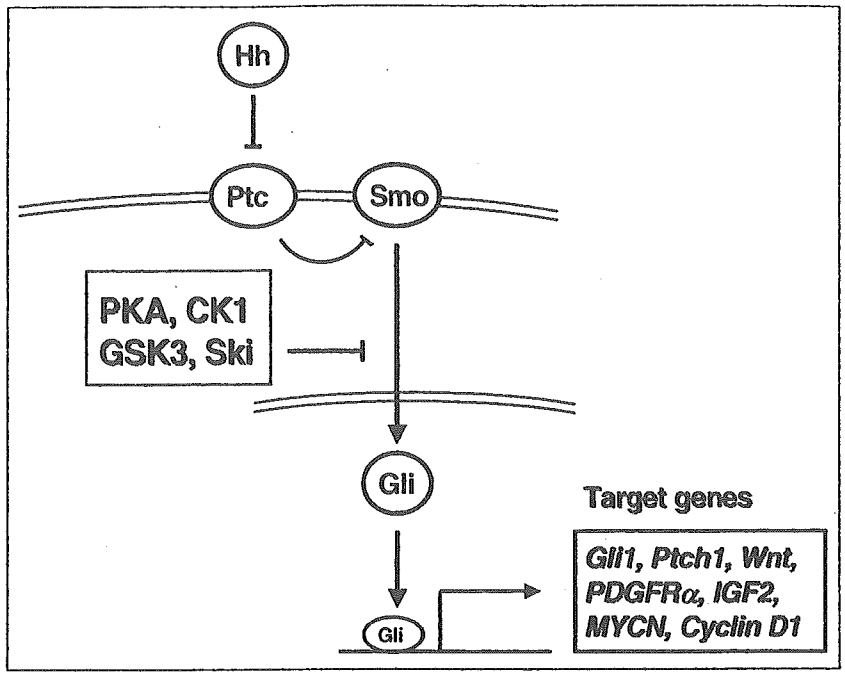


Figure 5.2
Hedgehog-Gli signaling in neural development and tumorigenesis. Sonic hedgehog (Hh) signaling activates Gli transcription factors which then induce the target genes important for regulating neural differentiation as well as neuronal tumorigenesis. They include *MYCN*, *cyclin D1*, *IGF2*, and *PDGFRα*, all of which are known to be players characterizing neuroblastoma biology. *T-bars* show inhibitory interactions. *Arrows* show positive interactions.

5.1.1.1 Bone Morphogenetic Proteins

Bone morphogenetic proteins (BMPs), members of the transforming growth factor-β (TGF-β) superfamily, may be the first signal that defines the early phase of differentiation and migration of neural crest cells during development (Oppenheim 1991). The ligand-dependent activation of BMP receptors transduces its signal into the nucleus through the sequential activation of Smad signaling molecules by phosphorylation. Although the role of BMPs in neuroblastoma has long been elusive, Nakamura et al. (2003) have recently reported that SH-SY5Y and RTBM1 neuroblastoma cell lines are responsive to BMP2 leading to growth arrest and differentiation. Of interest, BMP treatment also induces the downregulation of p53 family members including p53 and p73, as well as their target gene, $p21^{WAF1}$. In contrast, a similar cyclin-dependent kinase inhibitor, $p27^{KIP1}$, is markedly induced at the protein level by downregulation of Skp2, a component of its E3 ubiquitin ligase complex. BMP is also a direct transcriptional target of retinoic acid which induces neuroblastoma differentiation (see Chap. 15; Rodriguez-Leon et al. 1999). The DAN family members are inhibitors of BMP, and are also expressed in neuroblastomas (Enomoto et al. 1994). The DAN gene itself, which is mapped to chromosome 1p36, is a transcriptional target of BMP (Nakamura et al. 2003; Shinbo et al. 2002), suggesting that the BMP signaling network may be important in the differentiation and survival of neuroblastoma (Nakamura et al. 2003). The role of other important signals which function during neuronal development, including Sonic Hedgehog (Shh) and Wnt, is less well known in neuroblastoma. Interestingly, the Shh downstream signaling molecule, Gli, can transactivate *MYCN* and cyclin D1 (Altaba et al. 2004) (Fig. 5.2).

5.1.1.2 *MASH1/hASH1*

Achaete-Scute homolog-1 (*MASH1* in rodents and *hASH1* in humans) is a basic helix-loop-helix transcription factor which plays an important role in the early development of neural and neuroendocrine progenitor cells (Ball 2004). Helix-loop-helix proteins include achaete-scute homologs, E proteins, *MYCN*, *Math*, *NeuroD*, *neurogenin*, *Id*, and *HES*. Targeted disruption of *MASH1* in mice has led to the absence of

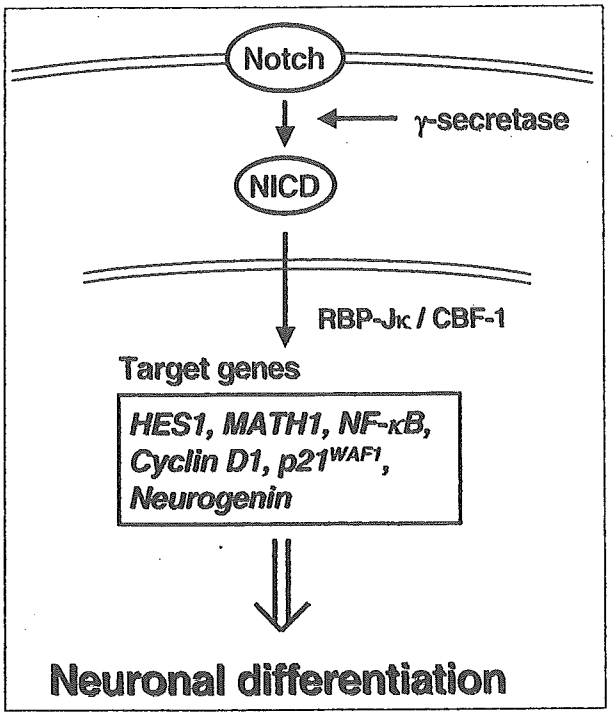


Figure 5.3
Notch signaling transactivates gene expression to induce neuronal differentiation. Binding of the ligand delta to its receptor notch triggers intramembrane proteolytic cleavage by γ-secretase. This results in the release of the notch intracellular domain (NICD), which then translocates to the nucleus where it associates with the CSL family of DNA binding proteins and transactivates gene expression. The target genes include *HES1, MATH1, NF-κB, cyclin D1, p21*, and *neurogenin*. *HES1* then inhibits transactivation of *MASH1 (hASH1)*.

sympathetic neurons, suggesting the important role of *MASH1* in sympathetic differentiation (Guillemot et al. 1993). *MASH1* is transiently induced during neural development to promote neuronal cell differentiation; however, high *hASH1* expression persists in neuroblastoma tumors and cell lines (Soderholm et al. 1999; Ichimiya et al. 2001). Retinoic acid treatment decreases the expression of *hASH1* and induces neurite extension (Ichimiya et al. 2001). *hASH1* also directly represses the expression of PACE4, a mammalian subtilin-like proprotein convertase that activates TGF-β-related proteins (e.g., BMPs) in neuroblastoma cell lines (Yoshida et al. 2001). The Notch signaling pathway also plays a key role during neuronal development (Axelson 2004). One of the important regulators of *hASH1* is a basic HLH protein, *HES1* (Fig. 5.3). *HES1* is regulated, at least in part, by Notch signaling and is induced at the transcription level. *HES1* directly binds to the promoter of *hASH1* and inhibits its transcriptional activation. A constitutively active form of Notch could block neurite extension during the induced differentiation of human neuroblastoma cells, possibly by inhibiting *hASH1* through the induction of *HES1* (Radtke and Raj 2003).

5.1.1.3 Phox2a and Phox2b

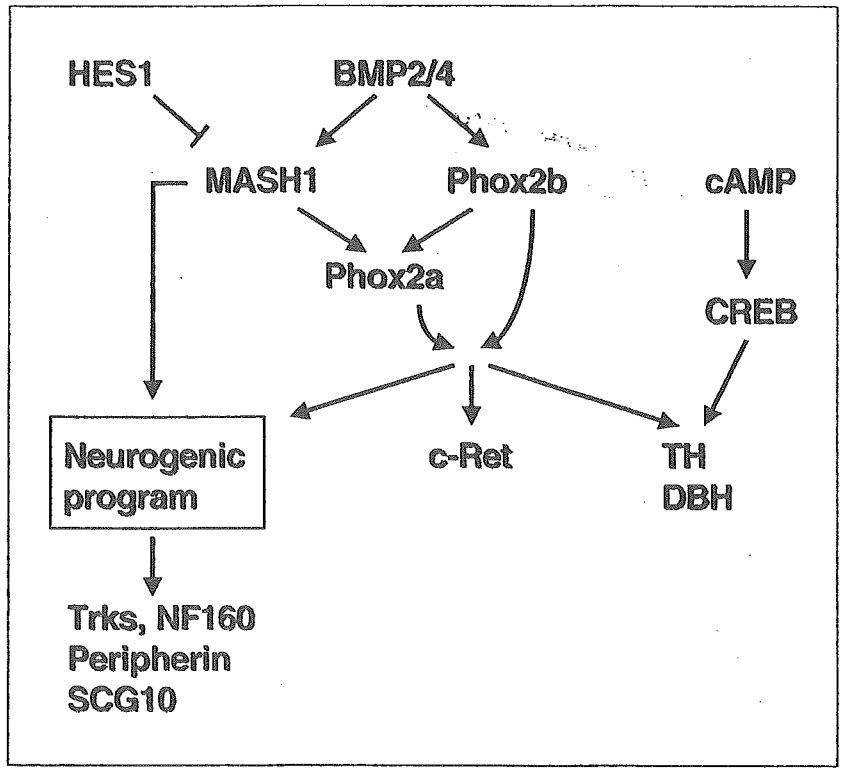
Phox2a and Phox2b are paired-like homeodomain transcription factors with complete conservation in their homeodomain. They are specifically expressed in noradrenergic neurons and activate the tyrosine hydroxylase and dopamine-β-hydroxylase genes (Schneider et al. 1999; Stanke et al. 1999; Ernberger 2000). While the expression of Phox2a is regulated by *MASH1*, Phox2b is not (Lo et al. 1999) (Fig. 5.4). The genetic disruption of either Phox2a or Phox2b gene de-monstrated that both genes are essential for the development of autonomic neural crest derivatives (Morin et al. 1997; Pattyn et al. 1999). Interestingly, Trochet et al. (2004) reported that the Phox2b gene was mutated in a family case of neuroblastoma and in a neuroblastoma patient with Hirschsprung's disease.

5.1.1.4 Id

Id proteins generally function as inhibitors of differentiation and as positive regulators of proliferation in neuronal development (Lavarone and Lasorella 2004). Id is a protein with the helix-loop-helix domain without a basic region and forms heterodimers with bHLH proteins, e.g., *MASH1* and *HES1* to inhibit their transactivation function (Massari and Murre 2000). In pediatric cancers, *MYC* oncoproteins and EWS-Ets fusion proteins are targeted to induce Id2 which in turn inhibits Rb and other target proteins including bHLH proteins, Ets and Pax. In neuroblastoma, *MYCN* has been shown to induce Id2 which stimulates cell proliferation by inhibiting Rb function (Lasorella et al. 2000).

Figure 5.4

Regulatory network controlling sympathetic neuron development. BMP2 and BMP4 are required for the expression of *MASH1* and *Phox2b*. *HES1*, induced by notch signaling, inhibits expression of *MASH1*. *MASH1* and *Phox2a* are genetically upstream of *Phox2a* and *Phox2b* is genetically upstream of *Gata3*. Expression of tyrosine hydroxylase (TH) and dopamine β-hydroxylase (DBH) depends on *MASH1*, Phox2b and Gata3. Cyclic AMP also controls expression of TH and DBH. Phox2a and Phox2b may affect induction or maintenance of *MASH1* expression. *MASH1*, Phox2a and Phox2b regulate the downstream neurogenic program, leading to terminal differentiation of sympathetic neurons by inducing the genes, e.g., Trks, NF160, peripherin, and SCG10.



5.1.1.5 *MYCN*

MYCN is a member of the group of *MYC*-box genes, and its product is a bHLH protein (Schwab et al. 2003). *MYCN* is transiently expressed during normal neural development and defines the direction of neuronal differentiation. *MYCN* is frequently amplified in advanced-stage neuroblastoma (Schwab et al. 1983, 1984; Brodeur et al. 1984; Seeger et al. 1985), and the biology of high-risk neuroblastoma is influenced by the subsequent overexpression of *MYCN* oncoprotein and its targets including telomerase and those functioning in ribosome biogenesis and protein synthesis (Mac et al. 2000; Boon et al. 2001).

5.2 Molecular Bases of Differentiation and Programmed Cell Death

5.2.1 Molecular Aspect of Spontaneous Regression

It is well known that some subsets of neuroblastoma can regress spontaneously. One of the most important hints to understand the mechanism of spontaneous regression is age of the patient at the onset of neuroblastoma. Regression rarely occurs when the tumor is found in patients over 1 year of age. The dramatic regression of the stage 4s tumor after its rapid growth usually occurs within 6 months after birth; therefore, it is plausible that epigenetic regulations, timed with the development of sympathetic neurons, might also control neuroblastoma regression. It is well known that massive death of sympathetic neurons is induced during the perinatal period – a process called developmentally regulated neuronal programmed cell death following deprivation of tar-

get tissue-derived neurotrophins (Oppenheim 1991). This same death mechanism appears to be conserved in primary neuroblastomas found in infants, leading to the induction of their spontaneous regression (Nakagawara 1998b).

5.2.2 Neurotrophic Factors and Their Receptors

5.2.2.1 Neurotrophins and Their Receptors in Neuroblastoma

The neurotrophin family of growth factors consists of nerve growth factor (NGF), brain-derived neurotrophic factor (BDNF), neurotrophin-3 (NT-3), and neurotrophin-4/5 (NT-4/5; Huang and Reichardt 2003). The corresponding high-affinity neurotrophin receptors with tyrosine kinase activity have been identified as TrkA, TrkB, and TrkC (Snider 1994) (Fig. 5.5 a, b). TrkA is a preferred receptor for NGF, TrkB for BDNF and NT-4/5, and TrkC for NT-3. All of the neurotrophins also bind similarly to a lower-affinity neurotrophin receptor $p75^{NTR}$, a member of the tumor necrosis factor receptor (TNFR)/Fas family (Snider 1994). The targeted disruption of neurotrophins and their receptors has demonstrated that NGF/TrkA signaling supports the survival and differentiation of sympathetic and sensory neurons responsive to temperature and pain, while BDNF/TrkB, NT-4/TrkB, and NT-3/TrkC signaling supports those of sensory neurons responsive to tactile stimuli and motor and sensory neurons responsive to limb movement and position, respectively (Klein 1994). These results suggest that neural development and maintenance of the neural network are spatiotemporally controlled by neurotrophin signaling with or without some redundancy in both peripheral and central nervous systems.

In neuroblastoma, high levels of TrkA are expressed in subsets of tumors with good prognosis, often showing spontaneous regression (Nakagawara et al. 1992, 1993; Suzuki et al. 1993; Kogner et al. 1993). Such tumors usually occur in patients under 1 year of age, and their DNA ploidy is aneuploid. A very limited amount of NGF may be supplied from stromal cells, e.g., Schwannian cells and fibroblasts, which at least partly regulate the differentiation and programmed cell death of neuroblastoma cells (Nakagawara 1998a). On the other hand, TrkA expression is strongly downregulated in tumors with aggressive behavior that usually possess amplification of the *MYCN* oncogene and allelic loss of chromosome 1p36 (Nakagawara et al. 1992, 1993). TrkB is preferentially expressed in aggressive neuroblastomas together with its preferred ligands BDNF and NT-4/5 which stimulate in an autocrine/paracrine manner, conferring an enhanced malignant phenotype to the tumor cells (Nakagawara et al. 1994; Matsumoto et al. 1995). TrkC is expressed in favorable neuroblastomas at variable levels (Yamashiro et al. 1996), but its preferred ligand, NT-3, is nearly undetectable by RT-PCR in primary neuroblastomas (Nakagawara 1998a); thus, in regressing neuroblastomas, tumor cells expressing the TrkA receptor may be dependent on a limited amount of NGF supplied from stromal cell. In the presence of NGF the cells mature, whereas they will die in the absence of this ligand (Nakagawara 1998a,b); however, in clinically aggressive neuroblastomas, the TrkA is downregulated and the downstream signaling cascades are disturbed, and these cells utilize the BDNF or NT-4/TrkB autocrine system for efficient growth. Neurotrophin signaling may also regulate tumor metastasis (Matsumoto et al. 1995), proliferation (Matsumoto et al. 1995), and angiogenesis (Canete et al. 2000). The role of $p75^{NTR}$ in neuroblastoma is unclear. The $p75^{NTR}$ receptor is expressed in both neuroblastoma cell lines (Azar et al. 1990) and primary neuroblastomas (Nakagawara et al. 1993). Interestingly, the expression levels of $p75^{NTR}$ mRNA are significantly higher in favorable neuroblastomas (stages 1, 2 and 4s) as compared with the advanced stage tumors, especially those with *MYCN* amplification (Nakagawara et al. 1993).

5.2.2.2 Neurotrophin Signaling in Neuroblastoma

In a rat pheochromocytoma cell line PC12, differentiation signals by NGF may be mediated through the tyrosine phosphorylation of the Trk receptor and through the subsequent activation of Shc/Grb2/SOS, Ras, Raf, MEK, and ERKs, while survival signals in the same cells may be transduced through the direct

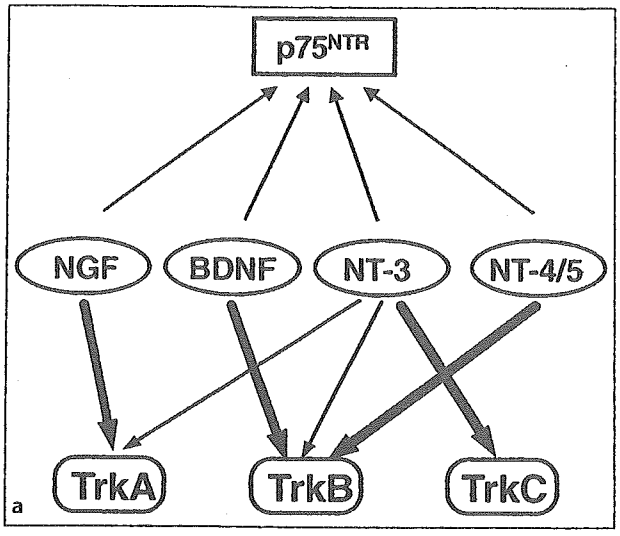
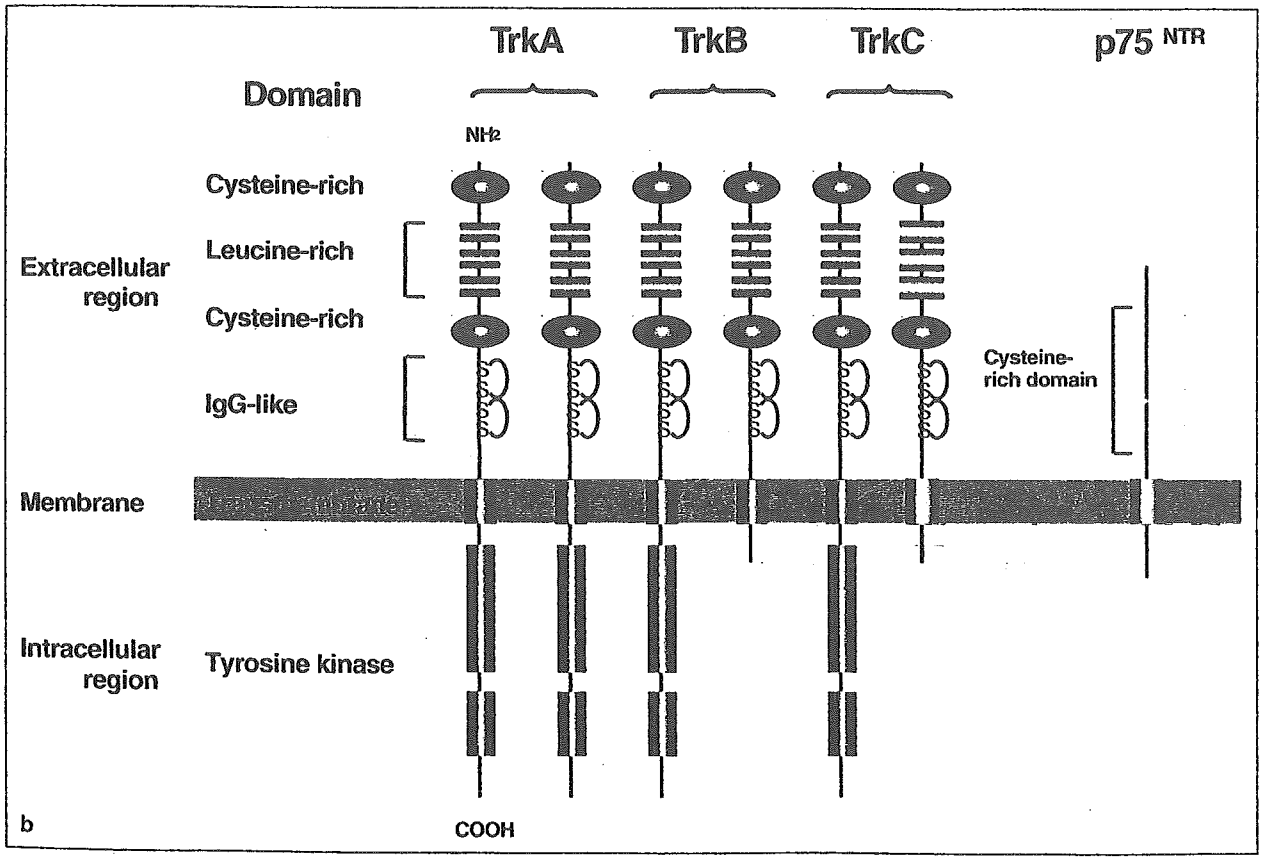


Figure 5.5 a, b

Neurotrophins and their receptors. a TrkA is a preferred high-affinity receptor for NGF, TrkB for BDNF and NT-4/5, and TrkC for NT-3. All of the neurotrophins also bind similarly to a lower affinity neurotrophin receptor, $p75^{NTR}$. b The structures of neurotrophin family receptors. The extracellular domains of TrkA, TrkB and TrkC have high structural similarity. The intracellular domain of Trks possesses tyrosine kinase activity. TrkB and TrkC receptors have truncated forms which lack the tyrosine kinase domain. The low affinity receptor, $p75^{NTR}$, has a short intracellular region containing the death domain and belongs to the Fas/TNFR family of the receptors.



activation of PI3-kinase which in turn activates downstream molecules, e.g., Akt and Bad (Klesse and Parada 1999). On the other hand, in normal sympathetic neurons, the activation of PI3-kinase is mediated not by the tyrosine phosphorylation of the receptor but by the Ras activation which promotes neuronal survival, suggesting that the Trk intracellular signaling pathway might be deregulated in cancer cells. This is also the case in neuroblastoma. In the neuroblastoma cell lines with a single copy of *MYCN*, NGF can induce differentiation when exogenous TrkA is overexpressed (Eggert et al. 2000). In the cell lines with *MYCN* amplification, however, the NGF-stimulated TrkA receptors which were overexpressed cannot normally activate downstream signaling molecules, resulting in unresponsiveness to the ligand. Furthermore, it is surprising that BDNF/TrkB signaling appears to be functioning in the same cells by promoting survival (Nakagawara et al. 1994; Hishiki et al. 1998), although the signaling pathway might be different from that of sympathetic neurons (Klesse and Parada 1999).

5.2.2.3 GDNF Family Receptors

Neurotrophic factors of the glial cell line-derived neurotrophic factor (GDNF) family, which include GDNF, artemin and neurturin, are secreted by neuroblastoma cells as well as stromal cells and activate their receptor complex composed of Ret tyrosine kinase and the GFRα co-receptors expressed in neuroblastoma cells (Hishiki et al. 1998; Ichikawa et al. 2004). In contrast to NGF/TrkA and BDNF/TrkB, however, the GDNF/Ret/GFRα autocrine system is functioning in both favorable and unfavorable neuroblastomas to enhance the survival and differentiation of tumor cells (Hishiki et al. 1998).

5.2.2.4 Other Factors and Receptors

Neuroblastoma cells express other growth factors and receptors. Both pleiotrophin (PTN) and midkine (MK) are factors in the same family with neurotrophic function (Kadomatsu et al. 1990; Li et al. 1990; Kadomatsu and Muramatsu 2004). PTN is expressed significantly at high levels in favorable neuroblastomas, while MK is highly expressed in almost all neuroblastomas with a tendency to be expressed at high levels in tumors in advanced stages (Nakagawara et al. 1995). Neuroblastoma also expresses many other receptors, e.g., fibroblast growth factor receptor (FGFR; Schweigerer et al. 1991), insulin-like growth factor (IGFR; El-Badry et al. 1991), DCC (deleted in colon cancer) (Reale et al. 1996), and neuronal leucine-rich repeat receptors (NLRRs; Hamano et al. 2004), as well as a novel plasma membrane enzyme ECEL1, which is significantly highly expressed in favorable neuroblastomas (Kawamoto et al. 2003). The biological significance of these factors and receptors in neuroblastoma are not currently known.

5.2.3 Functional Role of p53 Family Genes

Recent lines of evidence suggest that both the p53 tumor suppressor protein and its related protein p73 are involved in the induction of programmed cell death and growth arrest in neuronal cells (Pozniak et al. 2000). p73 is a recently identified candidate tumor suppressor gene mapped to chromosome 1p36.2, a frequently deleted region in many human cancers including neuroblastoma and oligodendroglioma (Ichimiya et al. 1999; Billon et al. 2004). In cultured neonatal sympathetic neurons, p53 protein levels are increased in response to NGF withdrawal as well as $p75^{NTR}$ activation, and it functions downstream of c-Jun NH_2-terminal kinase (JNK) and upstream of Bax to induce apoptosis (Aloyz et al. 1998) (Fig. 5.6). Indeed, in $p53^{-/-}$ mice, naturally occurring sympathetic neuron death is inhibited. Pozniak et al. (2000) have also reported that p73 is primarily present in developing neurons as ΔNp73, an NH_2-terminally truncated isoform, whose level is decreased when sympathetic neurons undergo apoptosis after NGF withdrawal, and that p53 becomes activated to be pro-apoptotic. In contrast to the truncated form of p73, full-length p73 has induced neuronal differentiation in a mouse neuroblastoma cell line N1E115 (Laurenzi et al. 2000). These data suggest that the neuronal apoptosis induced by NGF withdrawal is at least partly regulated by a reciprocal balance between levels of pro-apoptotic p53 and anti-apoptotic ΔNp73.

Figure 5.6

A model of signaling pathway for survival and death in sympathetic neurons regulated by NGF. NGF depletion may induce activation of JNK/p53 pathway which could be modified by p73/ΔNp73 regulatory system. $p75^{NTR}$ activation, which sends signals of both survival and death, may also regulate downstream p53/p73/ΔNp73 pathway.

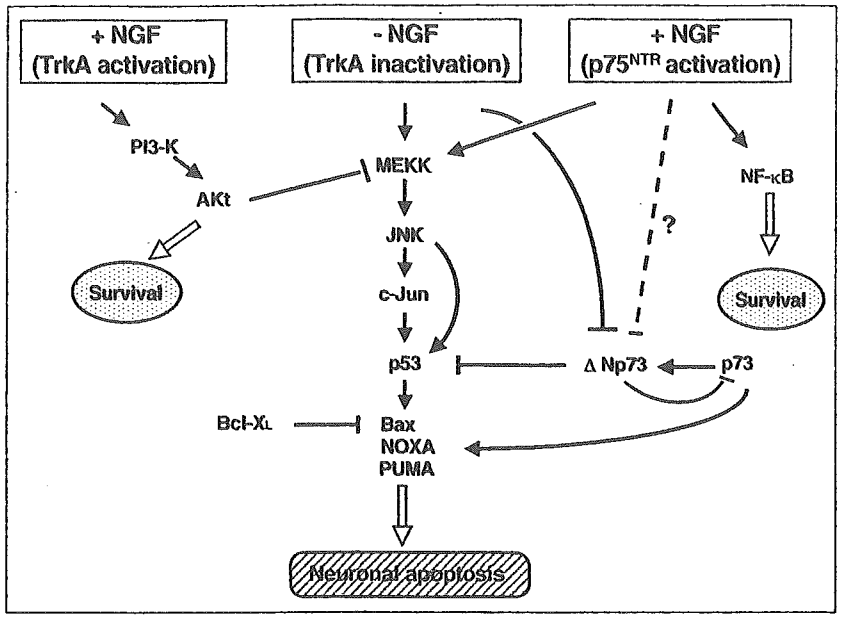
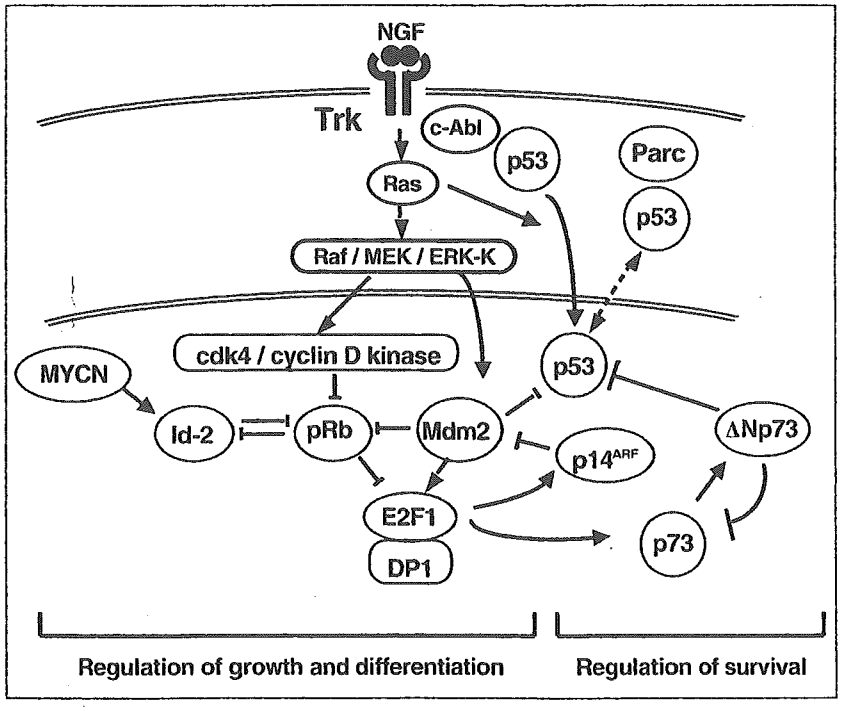


Figure 5.7

A possible signaling pathway regulating growth, differentiation and survival in neuroblastoma cells or sympathetic neurons. The NGF-triggered autophosphorylation of TrkA tyrosine kinase receptor induces activation of Ras/MAPK pathway which in turn regulates nuclear pRB and Mdm2. In some poor-outcome neuroblastomas, p53, which is shuttling between cytosol and nucleus, is trapped in the cytosol by Parc, an anchoring protein of p53. *MYCN* induces expression of *Id-2* whose protein product in turn inhibits pRB. E2F1 negatively regulated by pRB directly induces expression of p73. p73 is regulated by ΔNp73 in a negative autoregulatory manner (Nakagawa et al. 2002), and ΔNp73 also inhibits p53.



The importance of p53 and p73 has also been emphasized by the important observation that, in cultured neuroblastoma and other cancer cells, p73 directly transactivates the ΔNp73 gene by binding to its promoter after treating the cells with genotoxic reagents, e.g., cisplatin (Nakagawa et al. 2002). The induced ΔNp73 protein in turn interacts with either wild-type p53 or TAp73 and inhibits their proapoptotic function; thus, ΔNp73 can act as an oncogene and as an inhibitor of wild-type p53 and TAp73. The presence of this autoinhibitory feedback loop among p53, TAp73, and ΔNp73 may at least in part explain why there is no mutation of the p73 gene in cancers.

p53 is associated with TrkA via the proto-oncogene product c-Abl as an adaptor or bridging molecule, suggesting that it may also play a role in Trk signaling (Yano et al. 2000) (Fig. 5.7). The activation of Ras by NGF stimulation of the TrkA receptor induces p53 nuclear translocation and growth arrest in PC12 cells (Hughes et al. 2000). The c-Ha-Ras gene could be a target of p53, and protein products induce a positive feedback loop by activating $p14^{ARF}$ which counteracts the negative feedback loop mediated by mdm2 (Deguin-Chambon et al. 2000). These observations strongly suggest that p53 and p73 tumor suppressors function in neurotrophin signaling and modulate the growth, differentiation, and apoptosis of neurons.

In neuroblastoma and some other human cancers, wild type p53 is often localized in the cytoplasm (Moll et al. 1995). Although the regulatory mechanism of cellular localization of p53 and p73 is still unknown, activated Ras in NGF/TrkA signaling stimulates the nuclear translocation of p53 and leads to growth arrest by the induction of $p21^{WAF1}$ in PC12 cells (Hughes et al. 2000). Furthermore, some fractions of recurrent neuroblastomas and neuroblastoma cell lines acquire mutation of the p53 gene (Tweddle et al. 2001).

5.2.4 Apoptotic Signals in Neuroblastoma

To date, the spontaneous regression of neuroblastoma, has occurred only *in vivo*. Although this makes the analysis difficult, there are some important reports. An anti-apoptotic protein, Bcl-2, is expressed in primary neuroblastomas and neuroblastoma cell lines. The expression levels of Bcl-2 and Bcl-X_L are high in aggressive tumor cells but are low in regressing cells (Ikeda et al. 1995; Ikegaki et al. 1995). Caspase-1 and caspase-3 are expressed at significantly higher levels in favorable neuroblastomas (Nakagawara et al. 1997), and caspase-8 is silenced in aggressive neuroblastomas by the methylation of its promoter as one of mechanisms (Teitz et al. 2000). Silencing of caspase-8 is observed in 25–35% of primary neuroblastomas with a high frequency in more aggressive tumors (Teitz et al. 2000; Eggert et al. 2001; van Noesel et al. 2003). Survivin, a member of the inhibitors of apoptosis protein (IAP), is mapped to the long arm of chromosome 17. In neuroblastoma, survivin is highly expressed in high-risk tumors, and its overexpression inhibits cellular apoptosis (Islam et al. 2000). Kitanaka et al. (2002) have recently reported an interesting observation that "autophagy" may be involved in the regression of neuroblastoma cells.

5.3 Conclusions

Development of neuroblastoma may be triggered by a genetic event(s) that leads to chromosome and/or the genomic DNA abnormalities such as amplification of the *MYCN* gene and deletions or gains in chromosomal regions including 1p, 11q, and 17q. Together with other epigenetic mechanisms of gene activation or gene silencing, they affect gene and protein expression which in turn deregulate cellular signaling. In neuroblastoma the normal biology of developing neuronal cells and cancer biology appear to overlap. A further understanding of the mechanisms involved in the transformation of progenitors or the stem cells into neuroblastoma with significant cellular heterogeneity may provide clues for the development of novel therapeutic strategies for this often aggressive lethal disease.

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特　集

小児固形腫瘍の治療戦略

3. 神経芽腫の手術—これまでとこれから

筑波大学臨床医学系小児外科

金子　道夫

キーワード　神経芽腫，機能温存，臨床試験，グループスタディ

I. 内容要旨

筑波大学では1985年以降，乳児神経芽腫にも進行神経芽腫にも軽減された手術を一貫して施行してきた．85年以降のマススクリーニング発見乳児例40例では，非手術の1例を除き原発巣切除＋リンパ節サンプリングを施行し，全例無病生存，手術による合併症は全く認められない．一方，進行神経芽腫には厚生省班研究プロトコールで治療を行い，初期治療終了後，次に引き続く化学療法に影響を与えないよう，原発巣切除と2cm以上のリンパ節切除・サンプリングを行い，ほぼ全例に術中照射を併用した．これまで20例にこのような手術を施行し，局所再発は4例で，stage 4の5年生存率は70%，10年生存率は62%と良好であった．局所再発が死因となった症例はなかった．現時点では系統的リンパ節郭清を伴う徹底した切除が手術の主流であるが，2005年より局所治療を超大量化学療法終了後，すなわち治療の最後に行う治療方式の臨床試験が行われる．手術も軽減化コンセプトで施行することとなった．化学療法と局所治療を分けて施行し，お互いの干渉を避けるこのプロトコールコンセプトにより，局所治療の進行神経芽腫全体の治療に占める意義が明らかにされる可能性がある．

II. はじめに

神経芽腫の治療は限局性腫瘍には手術が，転移性腫瘍には化学療法で原発巣・転移巣，特に後者をコントロール後に腫瘍全摘とリンパ節郭清が行われ，施設によりその後に放射線治療や幹細胞移植を伴った抗癌剤大量療法が行われている．我が国では限局性腫瘍であっても遺残腫瘍がある場合には化学療法が行われてきたが，最近の欧米での限局性腫瘍に対する治療方針は多少の遺残腫瘍があっても，手術のみで90%以上の長期生存が得られており，手術のみで十分であると報告されている．1985年に全国的に施行開始された神経芽腫マススクリーニングは2004年に中止となったが，その理由として進行症例の減少，少なくとも神経芽腫の死亡例の大幅な減少が得られなかったこととに加え，発見された神経芽腫患者に行われた治療が結果的に過大となった可能性が指摘された．そこで，現時点での神経芽腫治療の問題点特に外科治療の問題点とこれからの解決法について述べる．

III. 神経芽腫の手術の現状と問題点

1　限局性腫瘍と乳児神経芽腫での問題点

乳児神経芽腫では進展症例は少なく，手術可能であることが多い．しかし，椎間孔から脊柱管内に広範囲に進展したり，stage 3では腹部の主要血管を取り巻いて摘出困難な場合も少なからずある．しかし，治療成績はstage 1，2のみでなく，stage 3もMYCN増幅がない限りほぼ100%近い．乳児例ではマススクリーニング症例を中心にグループスタディが行われ，まずstage 1，2で治療の軽減がはかられた．さらに最近はstage 3でも化学療法の軽減化スタディが進行中である．乳児期の治療成績が良いのは腫瘍自体がもつ特性によるところが大きく，治療の要素は相対的に少ない．これらの患者群に全摘を目指した過大な手術をする必要性は乏しい．できるだけ小さな侵襲で原発腫瘍を切除する，

PAST AND FUTURE ROLE OF SURGERY IN NEUROBLASTOMA
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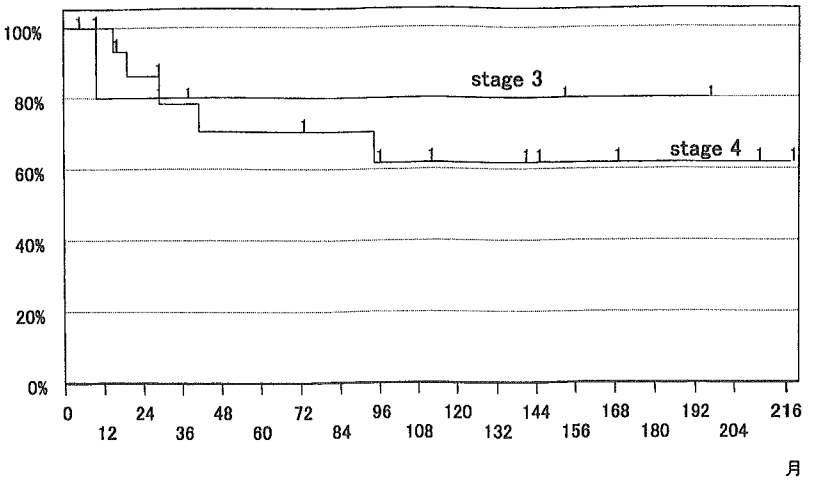

図　病期3，4　手術例の生存曲線

それ以上の手術が必要かどうかは疑わしい．stage 1，2に関しては全摘(通常容易)，stage 3でも簡単な切除でよいと考えられるが，前向き臨床試験が必要である．筑波大学では1985年以降40例のマススクリーニング症例に対し，当初から原発腫瘍切除＋リンパ節サンプリングという手術方針で，化学療法は乳児グループスタディに従って行った[1]．その結果，腹腔動脈を取り囲むstage 3で化学療法を先行して腫瘍がほぼ消失した非手術例1例を除く39例に手術が行われた．腎はすべて温存され，再発・再手術を認めず，全例後障害なく無病生存している．

非マススクリーニング例では頸部縦隔の2例が気道圧迫で手術を行い，うち1例が急速な再増殖による気道圧迫で窒息し，7年後に肺炎で死亡した．3カ月以下の急速進展例では手術を含め治療に難渋する症例がある．しかし，乳児期の神経芽腫の手術を含めた治療の基本は，治療し過ぎないことであろう．

2　進行症例に対する手術　—筑波大学の経験を中心に—

腹部原発の進行神経芽腫は原発巣とリンパ節が一塊となり大動脈とその主要分岐を取り巻くように進展し，全摘はきわめて困難である．いわゆる癌の根治手術は不可能である．化学療法により腫瘍が縮小すると易出血性であった腫瘍は硬化して出血は少なくなるが非常に硬くなり，脈管からの剝離は難しく，血管の外膜層で剝離するのがもっとも容易で，腫瘍の「全摘」に近い手術が可能である．現在，我が国でも欧米でもこのような手術が行われている．しかし，それでも全摘は困難で手術による障害も多くなる．

筑波大学では85年から厚生労働省班研究プロトコールに従って化学療法を行い，少なくとも3クール以降，多くは6クールを終了してから手術を行っている．術中照射を併用し，手術法は原発巣全摘，リンパ節は主要な血管を損傷しないようにしてなるべく切除，90年頃からは大きいものをサンプリングするにとどめた．手術した22例での結果は，大きなリンパ節が摘出困難な2例で，骨転移などが遠隔再発したときに残存リンパ節の増大がみられた．また，照射野外からの再発が手術野まで進展した例など，結局4例で「局所再発」が認められた．術後2年以上の観察で腫瘍死は6例，stage 4の5年生存率70%，10年生存率が61%であった(図)．「局所再発が」死亡の原因となった症例はなかった．腫瘍の腎進展で無機能となった1例を除き，全例で両側腎が温存され，2例の腸閉塞を除き合併症，後障害を認めていない．また，術中照射による椎体，脊髄の障害もない．

IV. 進行神経芽腫に対する適切な手術をめざして

欧米でも進行例に系統的リンパ節郭清を行う意義を疑問視する外科医は少なからず存在する．これまで，適切な局所治療の臨床試験は困難とされていたが，2005年から開始される進行神経芽腫に対する臨床試験では局所治療を治療の最後におき，手術の方式も統一した．これにより，局所治療の意義を明らかにし，さらに，治療の最後に摘出した腫瘍の病理組織と再発との関連

を見ることができるプロトコールとした．以下にそのプロトコールの外科治療の部分を掲載する．

1　外科治療

神経芽腫の診断・治療において外科治療の果たす役割は生検による組織採取にとどまらず，化学療法や放射線療法とならび根治的治療に関わる積極的な役割を担っていることは本腫瘍を扱う治療医の多くが認めるところである．しかし，進行神経芽腫に対する外科治療の実際は個々の症例やそれぞれの外科医によりばらつきがあり，わが国では進行神経芽腫自体の症例数が多くないこともあり，エビデンスに基づいた同腫瘍の外科治療指針を示すことができない．従って，そのエビデンスを作ることもこのプロトコールの目的とするところである．

神経芽腫では発生部位やリスク分類に応じた綿密な治療戦略を立てることが重要である．従って，治療前の腫瘍生検は従来から必須とされてきた．一方，high-risk 群に分類される1歳以上の病期3，4の神経芽腫は，化学療法で縮小が得られても完全摘除が非常に難しく，厳密には完全摘除は不可能といっても誤りではない．しかし，多くの施設でより高い根治性を目指して可能な限り切除を行うことに努力してきた[2)~5)]．神経芽腫の進展様式からいって，完全切除を目指す手術は血管およびその周囲の神経組織，リンパ管の損傷を伴い，術後腹部主要臓器の血行障害，腸管の運動障害，術後の大量リンパ瘻，呼吸不全の問題を常に伴っていた[6)7)]．特に，腎の血行障害は化学療法剤の変更，減量，さらに引き続いて施行される大量化学療法のリスクを大きく左右する．手術のこれらの問題は術後化学療法の開始時期の遅れ，投与抗癌剤の減量，腎障害など，術後治療のばらつきの大きな要因になり，適切な治療法を明らかにする臨床試験の遂行を妨げる大きな要因となった．

いくつかの施設では術中照射法や術後照射と組み合わせることにより，より侵襲の少ない手術とする治療方針をとり，それにより局所再発率を高めることはないとの報告が見られる[8)~10)]．局所療法を可能な限り同一化して，プロトコール全体での治療の揺らぎを小さくした．

2　外科治療に関する一般的事項

1）生検

治療に先立って組織学的診断と同時に腫瘍の生物学的特性の評価や遺伝子検索のため，凍結保存などが行えるよう可能な限り十分量の組織の採取を行う．すなわち少なくとも 1cm 角相当の腫瘍を採取することが望ましく，針生検による腫瘍採取は本ガイドラインでは推奨しない．以下省略．

2）原発巣摘除と機能温存

進行神経芽腫は治療成績がまだ不良とはいえ，5年生存率は 40% に達する．従って，局所再発の頻度を上げることなく機能温存を考慮することは重要である．また，治療中の合併症を少なくし，治療を予定通り完遂するためにも短期合併症を防止することは治療成績向上に資すると考えられる．以下に進行神経芽腫の外科治療ガイドラインを掲げる．

3）摘出術ガイドライン

1）原発巣に関して

原発部位に関わらず，原則として周囲臓器をできるだけ温存して原発巣を全摘出する．原発巣と一塊になったリンパ節は原発巣とともに切除を目指す．

1-1　副腎，後腹膜原発

肝・腎に関しては，手術時に viable と見られる浸潤がある場合は，部分合併切除を行う．

1-1-2　機能のある腎は温存する．腎血管を巻き込んでいて剝離が困難な場合，腫瘍被膜内切除にて腎血管を温存し，腎合併切除を極力避ける．腎動脈の攣縮にはキシロカインを浸したガーゼで包み，攣縮を軽減しつつ手術を続行し，腎温存に努める．

1-1-3　広範な腎実質浸潤がある場合には，腎合併切除をする．腎合併切除を行っても，腫瘍全摘出困難な場合は，腎を温存して，できるだけ腫瘍切除を行う．

1-1-4　腹腔動脈や上腸間膜動脈などの腹部大動脈からの主要な血管を巻き込んでいて剝離が困難な場合は，腫瘍被膜内切除にて血管を温存してできるだけ腫瘍を切除するものとする．

1-1-5　脾臓への直接浸潤，あるいは，脾動静脈を巻き込んでいる場合，5歳以上の症例では，脾合併切除による腫瘍摘出を行ってもよいが，5歳未満の症例では，脾温存によるできるかぎりの腫瘍切除とする．

縦隔・頸部・仙骨前については省略．

2）リンパ節に関して

2-1-1　原則として系統的リンパ節郭清は行わないものとする．

2-1-2　転移リンパ節と思われる 2.0cm 以上のリンパ節は切除する．それ以下の大きさであっても肉眼・触診上で viable と見られる腫瘍があると考えられるリンパ節は切除する．

2-1-3　2.0cm 以上のリンパ節の腫大したリンパ節が

手術時にない場合，治療前に転移の見られた部位のリンパ節サンプリングを行う．

3) 外科登録と central review

本プロトコールに従い治療を行い生検以上の手術を行った場合，担当の外科医は手術記録と外科登録用紙(原発部位，病期，初回手術の内容などを記載)を手術後10日以内に事務局あてに送付する．

V. おわりに

神経芽腫治療における手術や放射線治療の意義付けは非常に困難であった．そこでこれら局所治療を最後に行う厳密な臨床試験により，局所治療の意義と局所残存腫瘍の再発に対する意義を明らかにすることができれば画期的なことと考えられる．その後に，局所治療の時期・方法を randomized clinical trial ができれば，進行神経芽腫治療における局所治療の意義はより一層明らかになる．

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PAST AND FUTURE ROLE OF SURGERY IN NEUROBLASTOMA

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The significance of surgery in the treatment of neuroblastoma remains unresolved. Moderate surgical resection has been employed in both infantile and advanced neuroblastoma since 1985 at the University of Tsukuba Hospital. Resection of the original tumor accompanied by lymph node sampling was performed in 39 cases of infantile neuroblastoma detected by mass screening. The patients are alive without tumor and with no treatment sequelae. In 20 patients with advanced neuroblastoma aged 1 year or older, the same surgery was performed with intraoperative radiation after more than 5 cycles of induction chemotherapy. Four patients experienced local recurrence with recurrence of remote metastases which were the cause of patient death. The overall survival rate of patients with stage 4 neuroblastoma was 70% at 5 years and 62% at 10 years. A clinical trial in which local therapy will be performed after myeloablative chemotherapy will be carried out from 2005 to evaluate separately the efficacy of chemotherapy and local therapy avoiding their direct interference. This will elucidate the value of local therapy and also moderate surgical resection in the treatment of advanced neuroblastoma.

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症　例

BNPが多剤併用化学療法における心筋毒性の評価に有用であった2幼児例

Elevated BNP is a useful predictor of cardiac dysfunction during treatment of $_{98}A_3$ protocol in two patients with stage 4 neuroblastoma.

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要　旨

$_{98}A_3$プロトコールを遂行する上で心毒性は大きな制約条件となりえる．BNPは心不全のマーカーとしてだけでなく心毒性の早期評価にも有効である．我々が経験した2例において，進行神経芽腫に対する$_{98}A_3$プロトコールの遂行には，心筋毒性の早期評価が重要であり，心電図・心エコー検査に加えてBNPの定期的な測定と，小児循環器専門家との密接な共同診療が必須であった．

Key words：ナトリウム利尿ペプチド，心毒性，神経芽腫，$_{98}A_3$，小児
brain natriuretic peptide, cardiotoxicity, neuroblastoma, $_{98}A_3$, children

I　はじめに

進行神経芽腫研究グループの$_{98}A_3$プロトコールの有効性は明らかであるが[1)]，プロトコールを遂行する上で骨髄抑制，腎機能障害，心機能障害といった，治療関連毒性をいかにコントロールし治療を完遂するかが課題となっている．

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BNP(brain natriuretic peptide)は心機能のマーカーとして特に成人領域で臨床的に良く用いられている[2)]．それだけでなくアントラサイクリン系薬剤などによる心毒性の早期評価のための生化学的マーカーとして有用であるとの報告が，近年主に成人領域でみられる[3,4,5,6)]．心電図や心エコーに加えて，BNPを心毒性の早期評価に用いて$_{98}A_3$ regimenに続いて自家末梢血幹細胞移植(PBSCT)を完遂することができた進行神経芽腫の2症例の治療経過を報告する．

症　例

【症例1】6歳男児．【主訴】活気不良，腹部腫瘤．【現病歴】1か月前頃から活気・食欲が低下し，1