に見出された数種類の遺伝子の機能解析やがんとの関連の検討も今後の研究課題である。

E. 結論

高転移性肺癌細胞株における HIF-1α mRNA の高発現には、ROS-PI3K-Akt/PKB-PKC シグナル伝達経路が関わっていることが判明した。また、NEDL1 を含む数種類の遺伝子が新たに低酸素応答遺伝子であることが明らかになった。

F. 健康危険情報

特に無し。

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H. 知的財産権の出願・登録状況

1. 特許取得

なし

2. 実用新案登録

なし

3. その他

なし

厚生労働科学研究費補助金（第３次対がん総合戦略研究事業）
分担研究報告書

マウスモデルを用いた個体発生と発がんに関連する遺伝子の解析

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研究要旨 Runx1、2、3 遺伝子座において、転写状態の変化にしたがって、ポリコーム群タンパクである Ring1B 結合パターンが顕著に変化することが明らかになった。また、Ring1B の標的遺伝子の候補として 15 の遺伝子座を同定し、それらについてゲノムタイリングアレイの作成を行った。一方、Ring1B 欠失細胞を用いた解析では、Ring1B がヒストン H2A をモノユビキチン化する E3 リガーゼであることを示した他，B 前駆細胞の増殖に必須であることを明らかにした。

A．研究目的

Runx ファミリーとポリコーム群遺伝子産物の相互作用機序を明らかにする。染色体免疫沈降法を用いて、リンパ球におけるポリコーム群結合部位のライブラリを作成する。マウス・ポリコーム群である RYBP と Ring1B と様々なポリコーム群結合タンパクの発がんと細胞周期制御への寄与を解明する。

B．研究方法

本年度の業務目標に従い、以下に示すような目的をもって委託業務にあたった。①ポリコーム群複合体による Runx ファミリーの転写制御機序とその生物学的意義を明らかにする。②染色体免疫沈降法を用いて、ポリコーム群複合体結合部位のライブラリを作成し、標的遺伝子群を系統的に明らかにする。③マウス・ポリコーム群である RYBP と Ring1B のコンデイショナルアリルを用いて、RYBP と Ring1B の発がんと細胞周期制御における機能を明らかにする。④ポリコーム群結合タンパクである Homeoprotein Interacting Kinase (HIPK)-1, 2, 3 および Topoisomerase 1/p53-binding Ring finger protein (Topors)を欠損したマウスにおける異常を系統的に明らかにする。

C．研究結果

① ポリコーム群複合体による Runx ファミリーの転写制御機序とその生物学的意義の解明

マウス胎児を用いた染色体免疫沈降法を用いたポリコーム群複合体の標的遺伝子スクリーニングにより、Runx1、2、3 遺伝子座がその標的でありえることを、昨年度までに明らかにしてきた。今年度は、ポリコーム群の結合が、実際の Runx ファミリーの発現とどのように相関しうるかを、胎児軟骨細胞、胃粘膜を用いて解析した。Runx1 の発現は、胎生 12.5 日では、上腕骨原基では見られないが，13.5 日から見られる。上腕骨原基における Runx1 遺伝子座への Ring1B 結合パターンを解析したところ、転写状態の変化にしたがって、その結合パターンが顕著に変化することが明らかになった。転写が抑制された状態では、Runx1 遺伝子座の様々な領域に結合していたものが、転写の活性化に応じて、プロキシマルプロモーター領域に限局されていくことが明らかになった。Runx２遺伝子座においても、転写が活性化されている時期には、プロキシマルプロモーター領域に Ring1B の結合は限局されていた。また、Runx３遺伝子座においては、同様の傾向は軟骨原基において観察されただけでなく、胃粘膜においても観察された。別

のポリコーム群タンパクである Phc2 も Runx 1および Runx 3 遺伝子座に結合していたことから、Runx ファミリーの転写に、ポリコーム群複合体が寄与している可能性が強く示唆された。

② 染色体免疫沈降法を用いたポリコーム群複合体結合部位のライブラリの作成と標的遺伝子群の系統的検索

Ring1B の標的遺伝子の候補として 15 の遺伝子座を同定し、それらについてゲノムタイリングアレイの作成を行った。

③ マウス・ポリコーム群である RYBP と Ring1B のコンデイショナルアリルを用いて、RYBP と Ring1B の発がんと細胞周期制御や個体発生過程における機能の解析

Ring1B、Mel18、Mph1 を含むポリコーム群複合体は分化した ES 細胞において不活性 X 染色体に結合していることを明らかにした。不活性 X 染色体には、モノユビキチン化されたヒストン H2A が同様に結合し、ES 細胞分化の時間軸にそったヒストン H2A モノユビキチン化のプロフィールは、ポリコーム群タンパクの結合プロフィールとよく一致した。Ring1B は、RING フィンガータンパクであり、このドメインはユビキチン E3 リガーゼによく見られる構造であることから、Ring1B コンディショナルノックアウトマウスから Ring1B 欠失 ES 細胞を作成し、その細胞におけるヒストン H2A モノユビキチン化のレベルを調べた。その結果、Ring1B 欠失 ES 細胞では、ヒストン H2A モノユビキチン化レベルは顕著に低下し、そこに Ring1B を強制発現させることで、そのレベルが回復することが明らかになった。すなわち、Ring1B がヒストン H2A をモノユビキチン化する E3 リガーゼである可能性が強く示された。Ring1B および構造的に類似した Ring1A の両方を欠失した胎児性繊維芽細胞では、X 染色体上のヒストン H2A モノユビキチン化はほとんど見られなくなったことから、X 染色体不活性化への寄与も強く示唆された。

④ ポリコーム群結合タンパクである Homeoprotein Interacting Kinase (HIPK)-1, 2, 3 および Topoisomerase 1/p53-binding Ring finger protein (Topors)欠損マウスの表現型の解析

HIPK1,2,3 および Topors の単独変異マウスではいずれも顕著な異常は現れないことを明らかにした。

D. 考察

ポリコーム群と Runx ファミリーの機能的な相関が明らかにされた。ポリコーム群タンパク複合体による細胞周期制御、特に、細胞増殖と老化を制御することが示されており、がん抑制遺伝子である *Ink4a* の転写制御を介することが強く示唆されてきたが、本研究は新たな経路の存在を示唆するものであると考えられる。今後，この転写制御機序がどのような生物学的意義を有しているのかを、コンディショナル変異マウスを用いて明らかにしていく必要がある。また、ポリコーム群複合体のクロマチンに対する新たな触媒活性を明らかにした。ヒストンH2A のモノユビキチン化は 20 年以上以前から報告されているが、それを触媒する活性、また、転写へのインパクトは久しく明らかにされていなかった。

E. 結論

本研究により、ポリコーム群複合体によるヒストンH2A のモノユビキチン化が、ヘテロクロマチン形成に重要なインパクトを有していることが示唆された。

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CHAPTER 15

Neural crest development and neuroblastoma: the genetic and biological link

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Abstract: Neuroblastoma is one of the most common pediatric solid tumors originating from the sympathoadrenal lineage of neural crest. The tumor shows extremely different clinical phenotypes such as spontaneous regression on one hand and aggressive growth on the other hand. The different biological behavior of neuroblastoma appears to be determined by the genetic abnormalities including amplification of *MYCN* oncogene, DNA ploidy and some allelic imbalances. However, the spontaneous regression of neuroblastoma mimics the programmed cell death normally occurring in developing sympathetic cells expressing both TrkA tyrosine kinase A and $p75^{NTR}$ neurotrophin receptor. Indeed, TrkA expression is the most important factor related to the induction of tumor cell differentiation and/or programmed cell death because without its expression spontaneous regression of neuroblastoma never occurs. Thus, the enigmatic clinical behaviors of neuroblastoma are strictly linked to the molecular mechanism of neural crest development.

Keywords: neuroblastoma; NGF; TrkA; $p75^{NTR}$; *MYCN* oncogene; MYCN oncoprotein; stem cells

Neuroblastoma, a neural crest tumor in childhood

Neuroblastoma is an embyonic tumor originating from the sympathoadrenal lineage of neural crest and one of the most common solid tumors found in children (Bolande, 1974). Its incidence is about 1/8000 births and there is no significant difference among U.S., Europe and Japan. However, after beginning the mass screening to test the urine for the levels of catecholamine metabolites (VMA and HVA) in Japan in 1985 (Sawada et al., 1984), the incidence of neuroblastoma has almost doubled without decreasing the number of the sporadic tumors (Bessho, 1996). This strongly suggested the actual presence of '*in situ* neuroblastoma', which was first proposed by Beckwith and Perrin (1963), during the development of sympathetic neurons in human fetuses. They described the detection of '*in situ* neuroblastoma' in developing human embryos at the incidence of more than 40 times that of sporadic neuroblastomas, but most of them regressed spontaneously. Therefore, it is highly possible that we detect a part of the '*in situ* neuroblastomas' by mass screening, most of which otherwise regress without giving any therapy. However, at this moment it is unclear whether the regression of *in situ* neuroblastoma is due to the developmentally regulated programmed cell death of neuronal cells.

The sporadic neuroblastomas clinically found are divided into several subsets according to the clinical behavior, biological markers and genetic abnormalities (Brodeur, 2003). One of the most important clinical factors is the patient's age. The tumors found in the patients under one year of age are usually favorable and take a good clinical course to cure. On the other hand, many of the tumors symptomatically

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found in the patients over one year of age are poor prognostic and eventually kill the patients. Among the biological markers so far found, expression of the TrkA tyrosine kinase A receptor, as well as p75 neurotrophin receptor ($p75^{NTR}$) expression, is the most important indicator of prognosis (Nakagawara et al., 1992, 1993). TrkA is a high-affinity receptor for nerve growth factor (NGF), and $p75^{NTR}$ is its low-affinity receptor. The high levels of TrkA expression are strongly associated with favorable prognosis, whereas its decreased levels are significantly correlated with poor prognosis (Nakagawara et al., 1993). The important genetic markers include DNA ploidy, amplification of the *MYCN* oncogene and an allelic loss of the distal region of chromosome 1p (1p36) (Westermann and Schwab, 2002; Brodeur, 2003). Contrary to the other cancers, neuroblastomas with hyperdiploid karyotype show a good prognosis, while those with *MYCN* amplification and/or deletion of chromosome 1p36 are strongly associated with poor prognosis. The combination of these strong prognostic indicators segregates the subsets of neuroblastoma with different clinical behavior.

Figure 1 shows three types of neuroblastoma subset. Fig. 1 (left) demonstrates a stage 1 tumor originated from the adrenal gland in a patient under one year of age. The tumor is well encapsulated without metastasis. This type of neuroblastoma usually expresses high levels of TrkA and shows triploid DNA pattern with a single copy of *MYCN*. It clinically regresses spontaneously but very slowly. The baby in Fig. 1 (middle) is the patient with stage 4s neuroblastoma. The immature tumor cells occupy

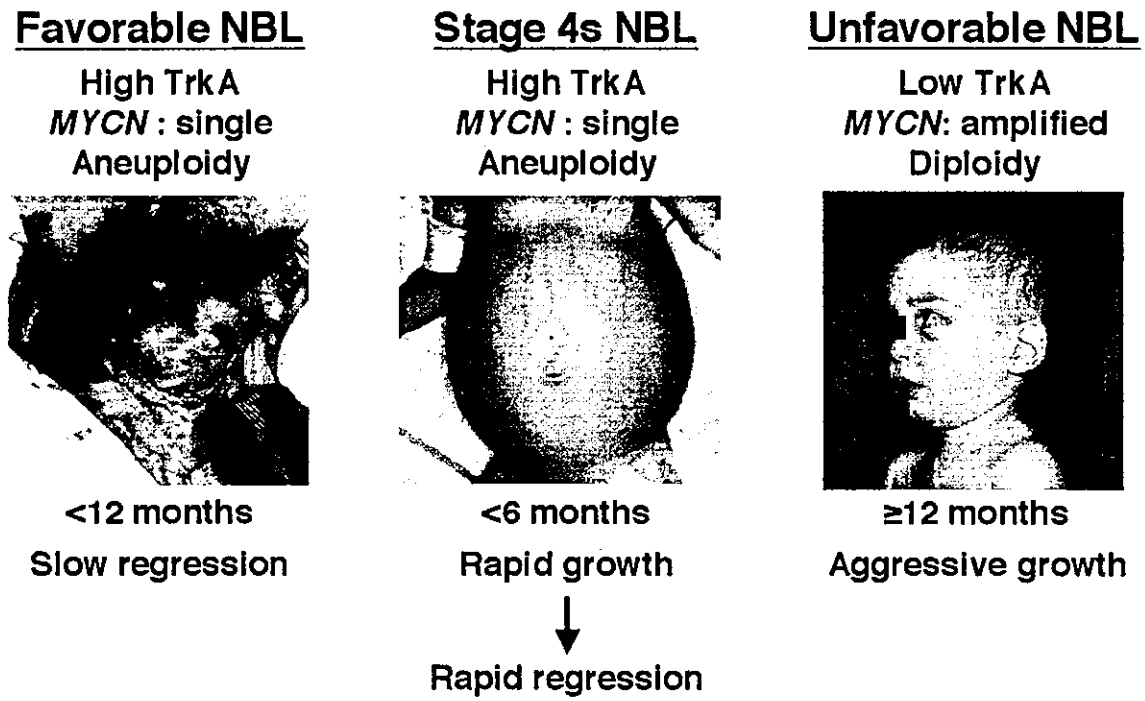

Fig. 1. Three distinct subset of human neuroblastomas with different biology and clinical behavior. Left: stage 1 neuroblastoma in a 7-month-old patient. The tumor originated from the right adrenal gland is small and well encapsulated. This kind of neuroblastoma usually regresses spontaneously. Middle: stage 4s neuroblastoma in a one-month-old baby. The primary tumor is located at the left adrenal gland. The liver is extremely enlarged and occupied by the tumor cells. The neuroblastoma cells are also positive in the bone marrow. The abdominal distension often oppresses the diaphragm to induce dyspnea. In a typical stage 4s neuroblastoma, the rapid tumor growth suddenly stops and starts to regress spontaneously. Right: stage 4 neuroblastoma in a 3-year-old boy. The tumor cells originated from the adrenal gland metastasize to long bones, skull and orbita with protrusion of the eye. The tumor cells show low TrkA expression, amplification of *MYCN*, diploid karyotype and deletion of the distal region of chromosome 1p.

the adrenal gland, liver and bone marrow (sometimes even skin), and rapidly grow at an early clinical stage. However, one day the tumor cells suddenly stop growing and start to regress spontaneously. This seems like just a miracle. The stage 4s tumor also shows high TrkA expression, triploidy and no amplification of *MYCN*. In contrast, the advanced stage of neuroblastoma shown in Fig. 1 (right) usually occurs in the patient over one year of age and metastasizes to bones and distant lymph nodes and eventually kill the patient. In this type of neuroblastoma, TrkA expression is strongly downregulated, the DNA ploidy pattern is diploid, and *MYCN* is amplified.

Genetic abnormalities of neuroblastoma

Neuroblastoma has many types of genetic abnormalities including chromosomal aneuploidy, gene amplifications, deletions, mutations, and deregulated DNA methylations. However, the pattern of the genetic aberration is different among the subsets, especially between those with favorable and unfavorable prognosis (Westermann and Schwab, 2002; Brodeur, 2003). The tumors with a tendency to regress spontaneously usually have triploidy but few abnormalities in the genome. On the other hand, the tumors with aggressive growth show a diploid or tetraploid karyotype, frequent amplification of *MYCN* oncogene, and chromosomal deletion of 1p36. The frequent gain of the chromosome 17q is reported to be associated with poor prognosis, however, it is also commonly observed in the tumors with favorable prognosis (Tomioka et al., 2003). The loss of heterozygosity at the chromosome 11q23 is reported to be frequent in the intermediate type of neuroblastoma in advanced stages with a single copy of *MYCN* and variable levels of TrkA expression (Guo et al., 1999). Thus, the subsets with different clinical behavior may be defined by the combination of the genomic aberrations.

Molecular and biological bases of neuroblastoma

Figure 2 shows a scheme of migration of the developing neural crest-derived cells, which segregate into several lineages such as melanocytes, sensory neurons, enteric neurons, and sympathetic ganglion cells. However, neuroblastoma never occurs in the other tissues than sympathetic ganglion or adrenal medulla. This suggests that the genetic events to cause neuroblastoma occur after the cell fate determination directing to sympathetic differentiation. The most likely candidate molecule to decide the direction of sympathetic differentiation at this moment is a basic helix-loop-helix transcription factor MASH1 which is transiently expressed during the neural development (Guillemot et al., 1993). In human neuroblastomas, MASH1/hASH1 is kept overexpressed (Soderholm et al., 1999; Ichimiya et al., 2001). Interestingly, induction of neuroblastoma cell differentiation in culture by treating with retinoic acid decreases the level of MASH1 mRNA. These suggest that the

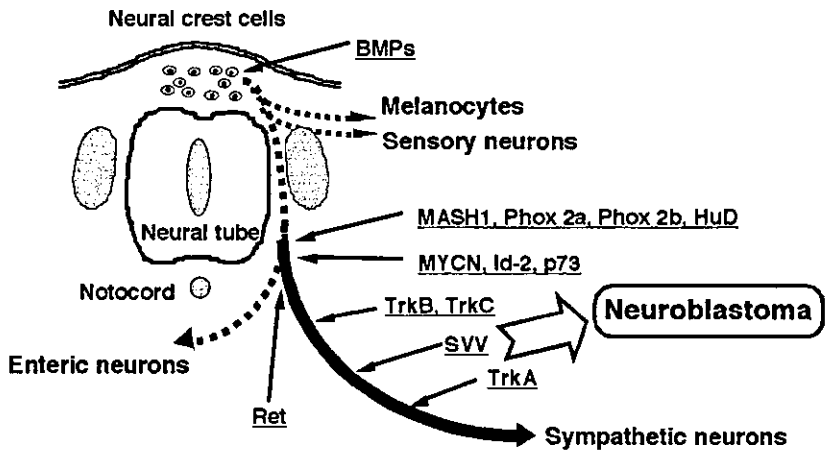

Fig. 2. Neuroblastoma occurs only from the sympathoadrenal lineage of neural crest. The important molecules regulating the sympathetic development are shown.

oncogenic events occurred in the early stage maintain the cells under arrest of differentiation by keeping the MASH1 expression at high levels, although this hypothesis must be proved. Even though the precise mechanism to regulate the oncogenic events of neuroblastoma and normal sympathetic differentiation is still elusive, it may be true that the targeting of the specific genes in such events is strictly controlled by the developmental program.

According to the accumulating evidence, it is clear that many important genes regulating normal development of sympathetic neurons are targeted to cause neuroblastoma or to modulate its biology. They include the *MYCN* gene encoding a basic helix-loop-helix transcription factor (Brodeur et al., 1984; Schwab et al., 1984), Id-2, a target of MYCN and a negative regulator of basic helix-loop-helix transcription factors (lasorella et al., 2000), MASH1 (Soderholm et al., 1999; Ichimiya et al., 2001), Phox2a and Phox2b, the homeotic proteins functioning with MASH1 (Kuno et al., unpublished data), and the downstream receptors such as Trk family members (Nakagawara et al., 1993) and Ret (Hishiki et al., 1998). These suggest that the important regulators of sympathetic differentiation are targeted to cause or maintain the cancerous status of neuroblastoma. This idea can be extended to the possible link between developmentally regulated programmed cell death of sympathetic neurons and spontaneous regression of neuroblastoma, because in both phenomena, expression of TrkA receptor is necessary (Nakagawara, 1998a, 2001). In other words, TrkA expression is almost exclusively required to induce spontaneous regression of neuroblastoma.

NGF family signaling in neuroblastoma

The NGF signals and their depletion strongly regulate survival and death of the normal sympathetic neurons, respectively. Similarly, it has recently become obvious that the NGF family signals strongly regulate the biology of neuroblastoma. Most neuroblastomas with favorable prognosis express high levels of both TrkA and $p75^{NTR}$ and functionally respond to exogenous NGF by extending neurites and promoting survival in primary culture (Nakagawara et al., 1993). The association between high levels of expression of TrkA and/or $p75^{NTR}$ and favorable outcome is statistically significant in primary human neuroblastomas. On the contrary, in aggressive neuroblastomas with *MYCN* amplification in advanced stages, expression of TrkA is extremely downregulated. The many studies about the role of Trk signaling in neuroblastoma cell lines also suggest that the intracellular TrkA signal is disturbed even though autophosphorylation of TrkA is induced by addition of NGF (Nakagawara et al., 1994). Thus, for the gain of growth advantage, the aggressive neuroblastoma cells appear to shut off the TrkA signal. Instead, they utilize a functional brain-derived neurotrophic factor (BDNF) and/or neurotrophin-4 (NT-4)/TrkB signaling system in an autocrine manner (Nakagawara et al., 1994). This BDNF/TrkB autocrine system also promotes invasion and metastasis in advanced tumors (Matsumoto et al., 1995). These suggest that spontaneous regression occurs only in neuroblastoma with high levels of TrkA expression and is induced by depletion of NGF within the tumor. The aggressive neuroblastoma cells seem to escape from the control by NGF, but to take advantage of the BDNF/TrkB autocrine loop for promotion of survival.

The family of glial cell line-derived neurotrophic factor (GDNF) mediates another important extracellular signal to regulate the survival of sympathetic neurons. Many neuroblastoma cells express the GDNF family receptors (Ret, GFRα-1, -2 and -3) and functionally respond to their ligands (GDNF, neurturin and artemin) in the primary culture (Hishiki et al., 1998). However, their expression and the responsiveness to the ligands are not associated with the disease stages or prognosis.

The other neurotrophic factors, pleiotrophin (PTN) and midkine (MK), may also be important in regulating neuroblastoma biology (Nakagawara et al., 1995). The expression of PTN is high in favorable neuroblastomas, whereas that of MK is high in all primary neuroblastomas. However, their functional roles in neuroblastoma are currently unknown.

Role of p53 and p73 in life and death of neuroblastoma

Pozniak et al. (2000) have recently reported about the crucial role of the tumor suppressor p53 and its

family member p73 in regulating survival and apoptosis during the induction of programmed cell death in mouse sympathetic neurons. Life and death of the sympathetic cervical ganglion (SCG) neurons are regulated by the balance between the levels of p53 and ΔNp73, an NH_2-terminally truncated dominant-negative form of p73. In neuroblastoma, p53 is not mutated but localized in the cellular cytoplasm especially in advanced stage tumors (Moll et al., 1995). Just recently, the anchoring molecule of p53 in the cytoplasm has been identified as Parc which is a structurally E3 ubiquitin ligase but binds to and stabilizes p53 (Nikolaev et al., 2003). It is interesting that the apoptosis-inducing stresses often trigger nuclear translocation of cytoplasmic p53 in neuroblasatoma cell lines (Ostermeyer et al., 1996).

p73 is the first family member of p53 and has occasionally been discovered as a candidate tumor suppressor of neuroblastoma (Kaghad et al., 1997). It is mapped to chromosome 1p36.2-3 which is commonly deleted in many aggressive neuroblastomas with *MYCN* amplification. The extensive mutation search has revealed that p73 is not mutated in many cancers including neuroblastoma (Ikawa et al., 1999). However, we found two mutations of the COOH-terminally located proline residues, one was somatic and the other germline. Nevertheless, most primary neuroblastomas have no mutation of p73 (Ichimiya et al., 1999).

Interestingly, in many malignant solid tumors, p73 has satisfactorily shown to be upregulated, though it has functionally the apoptosis-inducing ability like p53. We and the other investigators have recently found that p73 can bind to the *ΔNp73* proper promoter and induce transcription of which possesses the oncogenic function (Nakagawa et al., 2002). In addition, *ΔNp73* binds to both wild type p53 and p73 to suppress their apoptosis-inducing function (Nakagawa et al., 2002). These observations are very important because they might at least in part explain how the cancers without p53 mutation do develop the tumors with poor prognosis. In neuroblastoma, Casciano et al. (2002) have reported that both *p73* and *ΔNp73* are highly expressed in aggressive rather than favorable tumors.

Figure 3 shows the current summary of the signals for induction of neuronal apoptosis. Both p53 and p73 as well as ΔNp73 might be cooperatively functioning to regulate the programmed cell death of sympathetic as well as neuroblastoma cells.

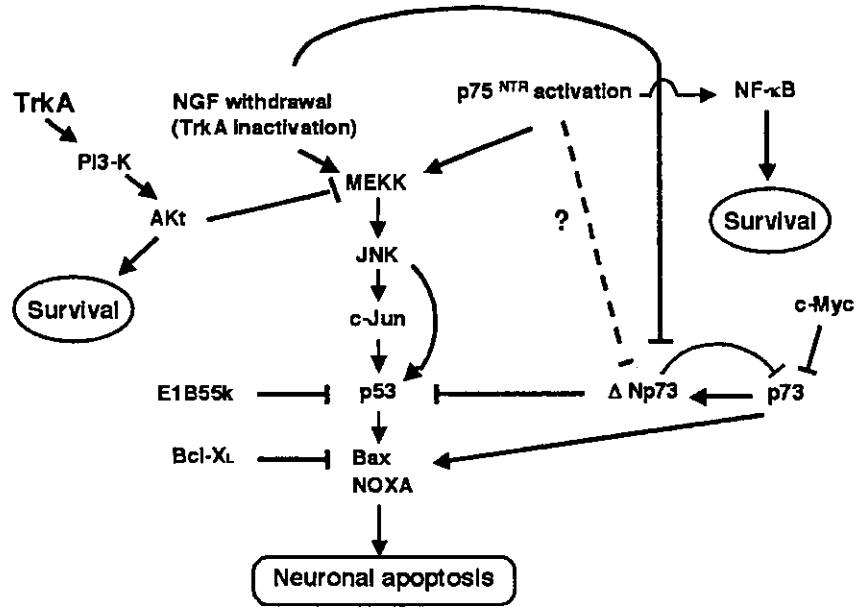

Fig. 3. Intracellular signaling of neuronal survival and apoptosis: the role of p53, p73 and ΔNp73.

Comprehensive genomics to identify the novel genes

To date, several genes functioning as landmark regulators in different subsets of neuroblastoma, such as *MYCN*, *MASH1*, *Trk*, *p53* and *p73*, have been identified. However, in the present postgenome era, we can try comprehensive approach to identify the important genes in a mass scale. For that purpose, we generated oligo-capping cDNA libraries from the primary neuroblastoma tissues of three different subsets as shown in Fig. 1. In total, we obtained 6252 gene clusters from 9729 clones randomly picked up from the cDNA libraries, among which 34% were novel genes with unknown function. The expression profiles of each subset of neuroblastoma were extremely different. By using the semi-quantitative reverse transcriptase (RT)-PCR, we have identified 757 genes differentially expressed between favorable (stage 1, high expression of *TrkA* and a single copy of *MYCN*) and unfavorable (stage 3 or 4, decreased levels of *TrkA* expression and amplification of *MYCN*) neuroblastomas. Among them, 502 are novel genes. [The results of our neuroblastoma cDNA project excluding those obtained from the stage 4s cDNA libraries were published elsewhere (Ohira et al., 2000; Ohira et al., in press).]

The expression profile of known genes was very different among the three subsets of neuroblastoma. The favorable subset frequently expressed neuronal specific genes including those related to neural differentiation, synapse, catecholamine metabolism and protein degradation. On the other hand, the unfavorable subset expressed many genes related to cell cycle control, protein synthesis and transcriptional regulation. The 4s tumor contained apoptosis-related genes, oncogenes and HLA family members which might be derived from the infiltrated lymphocytes into the tumor.

The 757 differentially expressed genes were strongly implicated in understanding of neuroblastoma biology. Of interest, vast majority of those genes was expressed at higher levels in the favorable subset as compared to the unfavorable one. The genes highly expressed in the favorable subset contained those related to neuronal differentiation, migration, cell–cell interaction, protein degradation, synaptic vesicles, catecholamine metabolism and intracellular signaling (Ohira et al., 2000; Ohira et al., in press). Most of them define the neuronal-specific phenotype and maintain the neuronal function. They also included heat shock proteins and ubiquitin/proteasome-related molecules that might sense the stress. On the other hand, only about 10% of the differential genes were expressed at high levels in the unfavorable subset. The protein products of such known genes contained many transcriptional and translational regulators including oncoproteins.

We also applied the primary culture of newborn mouse SCG neurons for screening those genes which change during the NGF-induced differentiation and/or the NGF depletion-induced apoptosis. This approach has identified 33 genes related to the former and 56 genes changeable during the latter (Isogai et al., unpublished data).

Our unique approach has identified more than several interesting genes as well as their products which include Nbla0219/BMCC1, a novel proapoptotic molecule with BCH domain, P-loop and coiled-coil domain, and Nbla0078/NEDL1, a novel E3 ubiquitin ligase with the HECT domain. The other interesting genes whose analyses have been published during our studies also include human *RIM/Nbla0761*, a Rab3-interacting molecule in the synaptic vesicles, *XCE/Nbla3145*, a new endothelin-converting enzyme and *FOG2/Nbla3139*, a coactivator of GATA transcription factor. Currently, a total of 7000 genes we cloned from the primary neuroblastomas are being fixed on the slide glass for cDNA microarray analysis.

Thus, our neuroblastoma cDNA project has provided enormous information and the gene materials for understanding of neuroblastoma biology as well as the molecular mechanism of neural crest development.

Developmental time axis and oncogenic events

Our neuroblastoma cDNA project has provided us with tremendous information about the genes expressed in different subsets with characteristic biology (Ohira et al., 2000; Ohira et al., in press). It suggested the presence of a kind of rule in the expression patterns of the subset-specific genes. Figure 4 shows the groups of genes expressed along the time axis of sympathetic neuron development. During the early

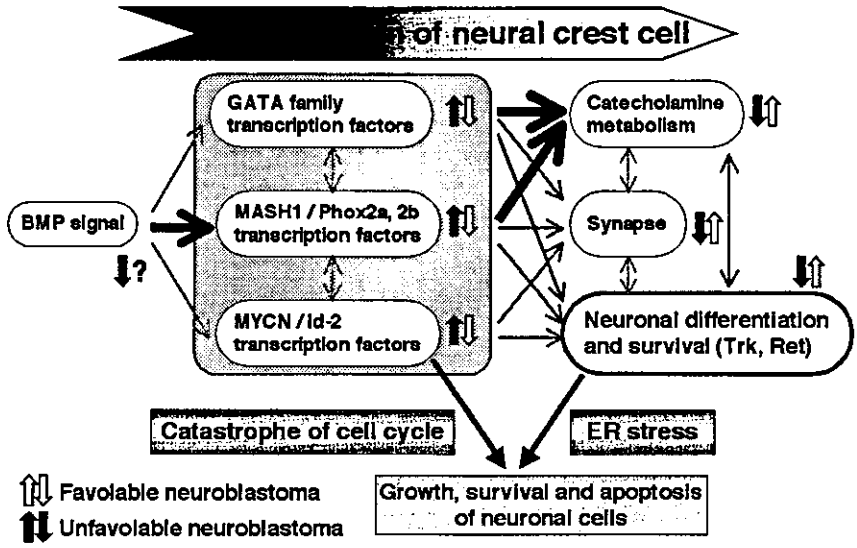

Fig. 4. The gene expression cascade along the time axis during the neural crest development, which is expected from the results obtained from the neuroblastoma cDNA project. Many transcription factors are upregulated in the unfavorable neuroblastoma, whereas the genes related to the terminal differentiation of neuron are upregulated in the favorable neuroblastoma. ER, endoplasmic reticulum.

stages of development, many transcription factors seem to function in deciding the direction of differentiation as well as regulating cell growth and survival of neural crest-derived cells. Interestingly, many genes highly expressed in unfavorable NBLs contain transcription factors and the components of their complexes. They include MYCN and Id family transcription factors that link to the regulation of Rb and p53 and regulate cell growth and apoptosis (Lasorella et al., 2000). The another basic helix-loop-helix transcription factor, MASH1, is constitutively activated in neuroblastoma, and by collaborating with Phox2a and Phox2b, it may regulate the arrest of differentiation in an unfavorable neuroblastomas (Kuno et al., unpublished data). Our neuroblastoma cDNA project has also revealed that there may be a neuronal cassette of GATA transcription factor complex that controls growth and differentiation of sympathetic progenitor cells (Ohira et al., 2003). Some molecules in this complex are upregulated in unfavorable neuroblastomas (Aoyama et al., manuscript in preparation). Thus, many important components in the transcriptional regulators appear to be highly expressed in unfavorable neuroblastomas and function to regulate the tumor cell growth or the status of de-differentiation.

On the other hand, most of a remarkable number of the genes expressed at high levels in favorable neuroblastomas encode the molecules that are necessary to maintain the neuronal function. They may be necessary for keeping catecholamine metabolism, synapse formation, neuronal cell survival, etc. We have also found many genes related to the ubiquitin-proteasome pathway and heat shock proteins in favorable neuroblastomas. They might be involved in induction of apoptosis triggered by endoplasmic reticulum stress.

Thus, the pattern of the differentially expressed genes in neuroblastoma subsets suggests the changes in the developmentally regulated gene expression along the time axis.

The hypothesis of neuroblastoma stem cells

According to the result of neuroblastoma mass screening, it may be true that most of the early stage neuroblastomas do not progress to the advanced tumors. In addition, the study of molecular mechanism linking neural development and neuroblastoma has revealed that the aggressive neuroblastoma occuring in an older patient seems to be arrested at