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Behavior of Sphingosine 1-Phosphate Receptor 4 Gene Expression in Patients with Neuroblastoma

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ABSTRACT

Sphingosine 1-phosphate receptor 4 (S1P4), which induces cellular migration and prevents apoptosis, was investigated in patients diagnosed with neuroblastoma in our study. The study included 37 neuroblastoma patients and 25 healthy children. After RNA isolation, cDNA were performed and S1P4 gene expression levels were measured in leukocytes. S1P4 gene expression levels presented as mean ± SD were high in the study group. The difference was statistically significant between neuroblastoma patients (0.0387±0.0647) and healthy children (0.0366±0.0238) for S1P4 gene expression levels (p=0.028). Patients given no chemotherapy yet and and those who already completed chemotherapy showed no significant difference statistically (p=0.886). While decreased S1P4 gene expression levels (0.0188±0.0069) were seen in patients receiving maintenance therapy, patients completed chemotherapy had increased S1P4 gene expression levels (0.0322±0.0303). The difference was meaningful (p=0.048). Although S1P4 gene expression levels (0.0310±0.0201) estimated before the beginning of chemotherapy were higher than that of maintenance phase (0.0188±0.0069), the difference was not significant (p=0.158). Higher S1P4 gene expression levels were remarkable in neuroblastoma patients. The suppression of S1P4 gene expression levels during maintenance phase and the increasing of expression in following up without chemotherapy could bring to mind that the chemotherapy could cause to decreased cell migration and/or induction of apoptosis. The effect of S1P4 on the tumor progression and the association with chemotherapy should be investigated in cancer cases.

Keywords: Neuroblastoma, Sphingosine 1-phosphate, Apoptosis, Cancer, Children

ÖZET

Nöroblastomlu Hastalarda Sfingozin 1-Fosfat Reseptör 4 Gen Ekspresyonunun Davranışı

Çalışmamızda nöroblastom tanılı hastalarda hücresel migrasyon ve apoptozisi önleyen sfingozin 1-fosfat reseptör 4 (S1P4) araştırıldı. Çalışma 37 nöroblastom hastalarını ve 25 sağlıklı çocukları içeriyordu. RNA izolasyonundan sonra cDNA oluşturuldu ve hastaların S1P4 gen ekspresyon seviyeleri ölçüldü. Ortalama±standart sapma olarak belirtilen S1P4 gen ekspresyon seviyeleri çalışma grubunda yüksekti. Sağlıklı çocuklar (0.0366±0.0238) ile nöroblastom tanılı hastalar (0.0387±0.0647) arasındaki fark S1P4 gen ekspresyon seviyeleri açısından istatistiksel olarak anlamlıydı (p=0.028). Henüz kemoterapi verilmemiş hastalar ile kemoterapisi çoktan tamamlanmış hastalar arasında belirgin fark yoktu (p=0.886). İdame tedavisi alan hastalarda azalmış S1P4 gen ekspresyon seviyeleri (0.0188±0.0069) görülürken, kemoterapisi tamamlanmış hastaların yükselmiş S1P4 gen ekspresyon seviyeleri (0.0322±0.0303) vardı. Fark anlamlıydı (p= 0.048). Kemoterapi başlanmadan ölçülen S1P4 gen ekspresyon seviyeleri (0.0310±0.0201) idame tedavisinde olanlardan (0.0188±0.0069) yüksek olmasına rağmen, fark anlamlı değildi (p= 0.158). Yüksek S1P4 gen ekspresyon seviyeleri nöroblastomlu hastalarda belirgindi. İdame fazında S1P4 gen ekspresyon seviyelerinin baskılanması ve kemoterapisiz takip edilmede tekrar yükselmesi kemoterapinin hücre migrasyonunun ve/veya apoptozisin indüksiyonunun azalmasına neden olabileceğini akla getirebilir. Kanser vakalarında S1P4'ün tümör progresyonu üzerindeki etkisi ve kemoterapi ile ilişkisi araştırılmalıdır.

Anahtar Kelimeler: Nöroblastom, Sfingozin 1-fosfat, Apoptozis, Kanser, Çocuklar

INTRODUCTION

Neuroblastoma (NBL) is the most common extracranial solid tumor, accounting for 8% to 10% of all childhood cancers. This solid tumor is derived from primordial neural crest cells which are normally found in the adrenal medulla or sympathetic ganglia. Children's Cancer Group Study (CCG) reported that the median age at diagnosis is 19 months and male/female ratio is 1.1:1.0. Although the exact etiology of neuroblastoma is unknown, genetic factors have a possible role in the pathogenesis of neuroblastoma.1 Information about the intracellular conduction pathways, which will clarify the etiology of cancer, is gaining an importance in neuroblastoma.[1,2]

Sphingosine 1-phosphate (S1P) involved in the intracellular signal transduction is composed of sphingomyelin. Ceramide formed by the hydrolysis of sphingomyelin is a common milestone of sphingolipid metabolism.[3] Ceramide is acylated by ceramidases and then sphingosine (Sph) occurs. Because high levels of sphingosine are toxic to the cells, sphingosine must be phosphorylated by sphingosine kinase to form S1P. S1P is dephosphorylated by sphingosine phosphatase and degraded irreversibly into ethanolamine phosphate and hexadecanal by S1P lyase. Intracellular levels of S1P have been shown to be maintained by the balance between the production by sphingosine kinase and the destruction by S1P lyase and S1P phosphatase.[4]

S1P is found in plasma bound to albumin and lipoprotein. The effects of S1P on the different cell types appear variable as antiapoptotic, cell adhesion, migratory, invasive and proliferative events.[5] S1P shows its effects in extracellular area by binding to five S1P receptors (S1PRs) known as S1P1, S1P2, S1P3, S1P4 and S1P5.[6] S1P4 is located in the chromosome 19p13.3 and mainly expressed in lymphoid and hematopoietic tissues.[7] S1P4 promotes cell migration,[7,8] influences cell morphology and may contribute to the fine balance of migration-stimulating.[6-9] Also S1P4 mediates immunosuppressive effects of S1P by inhibiting proliferation and secretion of effector cytokines, while enhancing secretion of the suppressive cytokine IL-10.[10]

To date, no study has been reported on a functional cellular response regulated by S1P4 in neuroblastoma patients. S1P has been highlighted to induce cellular migration[11] and to show antiapoptotic[12] properties. Understanding of the intracellular effects of ceramide and sphingosine 1-phosphate will open new doors in the treatment of cancer. Since the increasing of intracellular ceramide triggers apoptosis, the pathways increased the levels of ceramide should be discovered. The prevention of formation or accumulation of S1P will also prevent tumor growth.[13,14] We hypothesized that S1P4 which induces cellular migration and prevents apoptosis could be a prognostic factor in neuroblastoma. Therefore we aim to investigate the relationship between S1P4 gene expression levels and both clinical and laboratory features of patients with neuroblastoma.

Table 1. The clinical features of neuroblastoma patients.

	n (%)
Gender	
Girl	21 (56.8%)
Boy	16 (43.2%)
Age (month)	
<18	15 (40.54%)
≥18	22 (59.46%)
Tumor location	
Surrenal	30 (81.1%)
Mediastinum	2 (5.4%)
Paraspinal	2 (5.4%)
Mediastinum-Surrenal-Paraspinal	1 (2.7%)
Surrenal-Paraspinal	1 (2.7%)
Central Nervous System-Surrenal	1 (2.7%)
Staging	
Stage I	4 (10.8%)
Stage II	2 (5.4%)
Stage III	2 (5.4%)
Stage IV	28 (75.7%)
Stage IVS	1 (2.7%)
Surgery	
Operated	10 (27%)
Non-operated	27 (73%)
Risk	
Low	7 (18.9%)
Intermediate-favorable histology	1 (2.7%)
Intermediate-unfavorable histology	10 (27%)
High	19 (51.4%)
Relapse	
Relapse	6 (16.2%)
Primary site	3 (8.10%)
CNS	2 (5.40%)
Mediastinum	1 (2.70%)
No relapse	31 (83.8%)
The treatment status	
No chemotherapy (Stage I patients)	4 (10.9%)
At induction phase	7 (18.9%)
At maintenance phase	12 (32.4%)
Completed chemotherapy	14 (37.8%)
The last status of patients	
Survive with disease	20 (54.1%)
Survive disease-free	16 (43.2%)
Exitus	1 (2.7%)

PATIENTS AND METHODS

Patients

The study included 37 neuroblastoma patients and 25 healthy children was performed in Cukurova University Faculty of Medicine, Department of Pediatric Oncology. Parental informed consent was obtained from the patient and healthy control group. Blood samples were collected from January to April 2011. At first admission, clinical parameters of all patients such as gender, age, tumor location and operability status were recorded. Staging was developed by International Neuroblastoma Staging System (INSS) based on the clinical criteria and tumor imaging for all patients.[1] Tumors were placed by International Neuroblastoma Pathology Classification (INPC) into two broad categories of favorable and unfavorable based on analysis of the stromal component, degree of differentiation, mitosis karyorexis index, and patient age.[15] Pretreatment risk stratification, the International Neuroblastoma Risk Group (INRG) classification system, was determined by clinical factors of patient age at diagnosis, INSS stage, tumor histopathology, and DNA ploidy to assign patients to one of three distinct risk groups (low, intermediate and high).[16]

Risk-adapted chemotherapy was divided into an induction and a maintenance phase. According to Turkish Pediatric Oncology Group (TPOG)-NBL 2003 chemotherapy protocol, vincristine, cyclophosphamide/ifosfamide, cisplatin, doxorubicin, dacarbazine and etoposid were given in induction phase, and vincristine, cyclophosphamide and 13-cisretinoic acid were used during maintenance phase. After completing of chemotherapy, the existence of tumor relapse and relapse site was defined. Patient survival was recorded as living with or without disease and exitus (Table 2). S1P4 gene expression levels were measured in the different treatment periods related to the beginning of the study

Table 2. Sphingosine 1-phosphate receptor 4 gene

Primer	Length	Position	Tm	%GC	Sequence
Left	20	164-183	60	50	ccatccaaggacagcaattc
Right	19	219-237	60	63	gagtgactctgggggcttg
Amplicon (74 nt)					

Table 3. The relationship between S1P4 gene expression levels and the clinical/laboratory parameters.

Parameters	S1P4 gene expression levels			
	Minimum	Maximum	Mean±SD	p
Cases				
Study group	0.0092	0.3980	0.0387±0.0647	0.028
Control group	0.0164	0.1267	0.0366±0.0238	
Gender				
Girl	0.0132	0.3980	0.0567±0.0960	0.400
Boy	0.0092	0.0633	0.0249±0.0126	
Age (month)				
<18	0.0092	0.1359	0.0287±0.0248	0.112
≥18	0.0108	0.3980	0.0594±0.1080	
Tumor localization				
Surrenal	0.0092	0.3980	0.0427±0.0714	0.332
Non-surrenal	0.0126	0.0344	0.0215±0.0087	
Stage				
Stage I,II,III,IVS	0.0092	0.0718	0.0279±0.0195	0.789
Stage IV	0.0108	0.3980	0.0421±0.0736	
NSE				
<100 mg/dl	0.0092	0.0718	0.0286±0.0172	0.686
≥100 mg/dl	0.0108	0.3980	0.0455±0.0828	
LDH				
<1000 U/L	0.0092	0.1359	0.0322±0.0262	0.487
≥1000 U/L	0.0108	0.3980	0.0522±0.1091	
Ferritin				
0-150 ng/ml	0.0092	0.1359	0.0333±0.0277	0.141
>150 ng/ml	0.0108	0.3980	0.0465±0.0975	
Risk				
Low-intermediate	0.0092	0.1359	0.0317±0.0300	0.867
High	0.0126	0.3980	0.0452±0.0862	
Surgery				
Operated	0.0092	0.0718	0.0271±0.0172	0.869
Non-operated	0.0108	0.3980	0.0429±0.0750	
Chemotherapy				
Before chemotherapy	0.0092	0.0718	0.0310±0.0201	0.886
After chemotherapy	0.0108	0.3980	0.0428±0.0793	
Treatment status 1				
Before chemotherapy	0.0092	0.0718	0.0310±0.0201	0.158
Maintenance phase	0.0108	0.0296	0.0188±0.0069	
Treatment status 2				
Maintenance phase	0.0108	0.0296	0.0188±0.0069	0.048
Completed chemotherapy	0.0142	0.1359	0.0322±0.0303	
Relapse				
Relapse	0.0126	0.0296	0.0226±0.0059	0.908
Non-relapse	0.0092	0.3980	0.0418±0.0704	
The last status of patients				
Survive with disease	0.0108	0.3980	0.0452±0.0848	0.339
Survive with disease-free	0.0092	0.1359	0.0315±0.0286	

NSE: Neuron specific enolase LDH: Serum lactate dehydrogenase

such as first admission, maintenance phase and after completed chemotherapy in patients with neuroblastoma (Table 3).

The measurement of S1P4 gene expression

Two ml of blood was collected with tubes containing EDTA from each patient and healthy child. The separation of leukocytes was primarily done on the same day. Then RNA isolation was performed by using high pure RNA isolation kit (cat no: 11828665001, Roche Applied Science, California, USA). RNAs were directly translated into cDNAs by Roche transcriptor high fidelity cDNA synthesis kit (cat no: 05081955001). Light Cycler 480 probe master mix (cat no: 04707494001, Roche Applied Science, California, USA) with realtime ready catolog assays beta-actin gene (cat no: 05532957001, Roche Applied Science, California, USA) and realtime ready designer assays target gene S1P4 (cat no: 05583055001, Roche Applied Science, California, USA) primary probe designs were used to find the S1P4 gene expression levels. S1P4 gene expression levels of all patients were measured by using sphingosine 1-phosphate receptor 4 gene [Roche Applied Science, Universal Probe Library (UPL), California, USA] (cat no: 04689054001) via real-time PCR (Table 2).

The Measurement of Serum NSE, LDH and Ferritin Levels

Blood was collected by venipuncture, serum separated by centrifugation at 5000 rpm for 10 min and specimens were stored at -80^{0}C until the time of thawing and assay. The serum Neuron specific enolase (NSE) level was measured using an electrochemical immunoassay with double monoclonal antibodies directed against NSE (Roche Diagnostics, Florida, USA) on an Elecsys instrument. Serum values of >15 ng/ml were considered abnormal. Serum lactate dehydrogenase (LDH) was determined on photometric system performed by spectrophotometric assay on a COBAS integra 800 Analyser, Roche Diagnostics, Minnesota, USA. For data analysis, the upper limit of normal of LDH was defined as 250 IU/ml. The serum ferritin level was detected by using an automated technique based on the principle of immunoagglutination with electrochemical immunoassay (E-170 Analyser, Roche Diagnostics, Minnesota, USA). Levels of >400 ng/ml were reported as increased ferritin levels in serum. NSE, LDH and ferritin test results categorised as greater than or less than 100 ng/ml, 1000 IU/L and 150 ng/ml were correlated with S1P4 gene expression levels, respectively.[17-19]

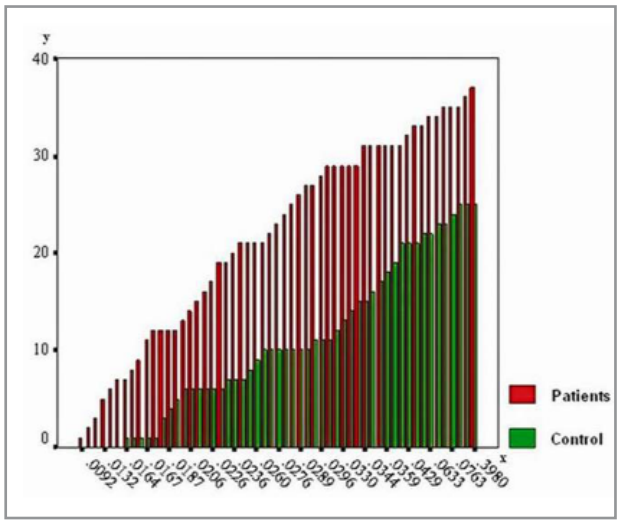

Figure 1. The distribution of S1P4 gene expression levels on x-axis and the number of patients on y-axis were presented.

Statistical Analysis

A computer program (SPSS 11.0) was used for the statistical analysis of all results. The data were presented as means ± standard deviation (SD). The statistical significance of differences between the two groups was determined by Mann-Whitney U-test. $p< 0.05$ was considered statistically significant.

RESULTS

The patients diagnosed with neuroblastoma consisted of 21 (56.8%) girls and 16 (43.2%) boys. These patients were (Mean±SD) 30.89 ± 26.04 years old. The clinical features of patients were listed in Table 1.

The mean S1P4 gene expression levels were found as 0.0387±0.0647 in patients with neroblastoma and 0.0366 ± 0.0238 in healthy children. S1P4 gene expression levels were high in patients group

(Figure 1). The difference was statistically significant (p= 0.028). S1P4 gene expression levels were almost two and a half times more in girls (0.0567±0.0960) than that of boys (0.0249±0.0126). Also patients aged 18 months and above and with surrenal localization had nearly twofold much S1P4 gene expression levels in comparision to patients aged younger than 18 months and with non-surrenal location. In addition, the increasing of S1P4 gene expression levels was found as one and half fold in patients with an inoperable, high risk and at stage IV. But the difference between the expression levels and gender, age, tumor location, stage, operation and risk status was not statistically significant. Patients who had elevated NSE, LDH and ferritin values than normal limit demonstrated increased S1P4 gene expression levels. But there was no statistically distinctive difference between S1P4 gene expression and NSE, LDH and ferritin levels (Table 3).

There was no significant difference between patients given no chemotherapy yet and and those who already completed chemotherapy for S1P4 gene expression levels (p= 0.886). While decreased S1P4 gene expression levels were seen in patients receiving maintenance therapy (0.0188±0.0069), increased S1P4 gene expression levels (0.0322±0.0303) were noticed in patients already completed chemotherapy. The difference was significant (p= 0.048). However S1P4 gene expression levels estimated before the beginning of chemotherapy (0.0310±0.0201) were approximately half fold higher than that of maintenance phase (0.0188±0.0069). The difference was not statistically remarkable between them (p= 0.158). Patients survived with disease-free had around one and half fold lower levels of S1P4 receptor gene expression (0.0315±0.0286) than that of survived without any symptoms and/or progression of neuroblastoma (0.0452±0.0848). But there was no difference prominently between those patients groups for their S1P4 gene expression levels (p= 0.339). The association between S1P4 gene expression levels and the clinical/laboratory parameters was displayed in Table 3.

DISCUSSION

The growth and metastasis of a tumor depend on various factors, such as migration, adhesion, invasion and proliferation of tumor cells, and angiogenesis. Sphingosine 1-phosphate has been highlighted to induce cellular migration[11] and to show antiapoptotic properties[12] in the literature. Many studies have been conducted in cell lines order to investigate the effects of S1P in the tumor cells. However, there has not been a study conducted yet on S1P4 receptor gene expression status in pediatric cancer cases. Although S1P and especially S1P4 have been shown to have an important role to induce cellular migration in breast cancer in a study of cell lines,[20] the immunsupressive effect of S1P4 studied by Wang et al[10] should be clarified in cancer cells as well as T cells. We aimed to investigate the behavior of S1P4 gene expression, which is one of sphingosine 1-phosphate receptors, in children with neuroblastoma.

The present study showed that S1P4 gene was much expressed in neuroblastoma patients than that of healthy children. Long et al., in their study on cell lines, reported that S1P4 induced cellular migration.[21] In the literature, there was no study clarifying the high levels of S1P4 gene expression in neuroblastoma patients. The interaction between various cancer cell types and S1P4 gene expression are described in various studies of cell lines. Van Brocklyn et al. demosntrated that S1P4 triggers estrogen-dependent tumorigenesis in human breast cancer cells and induced the invasion of human glioblastoma cells.[22] However, there has been no study on S1P4 gene expression levels neither in neuroblastoma patients nor in patients with the diagnosis of other cancer types. Therefore there is no data indicating the reference ranges of S1P4 in cancer patients. Based on the cancer cell line studies, it could be taken into account that the tendency of cellular migration and prevention of apoptosis could be predicted by increased S1P4 gene expression levels in patients with neuroblastoma in our study.

The relationship between age at diagnosis and patient outcome was reported firstly by Breslow and McCann in neuroblastoma.[23] Recent reviews by the Children's Cancer Group Study (CCG) and Pediatric Oncology Group (POG) reported that 18 months of age was distinctive for outcome accor-

ding to International Neuroblastoma Staging System (INSS).[24,25] In other author pointed that the age was less than 18 months of age at diagnosis, the outcome was favorable.[2] Since there has been no clinical study on S1P4 gene expression levels in the literature, we also could not find data on the relationship between clinical and laboratory findings of patients and S1P4 gene expression values. In our study, patients with older than 18 months of age had high S1P4 gene expression levels. Nevertheless the difference was not remarkable between high levels of S1P4 gene expression and age at diagnosis in neuroblastoma patients, the following up neuroblastoma patients aged older than 18 months with S1P4 gene expression levels could be distinctive for outcome. The staging of neuroblastoma by the INSS is clearly correlated with patient outcome and is used by all cooperative groups to stratify therapy.[26] Our patients with INSS stage 4 had high S1P4 gene expression level. Similarly, no significant difference was found between S1P4 gene expression levels and stage of the disease. Besides increased S1P4 gene expression levels could be an important clue for the patients at high stage as well as age at diagnosis.

Imbach indicated that thorax, presacral and cervical location had favorable prognosis.[2] In the present study, tumor mostly localized in surrenal and S1P4 gene expression levels were lower in patients with non-surrenal localization. Also the same author reported that low-risk groups (Stages I, II, IVS, no MYCN amplification, favorable histology and radical tumor resection) had more than 90% long-term survival.1 Our high risk patients demonstrated elevated S1P4 gene expression levels. In a retrospective review of 141 cases at Memorial Sloan-Kettering Cancer Center, gross total resection of the primary tumor correlated with improved survival.[27] S1P4 gene expression levels were high in 27 (73%) neuroblastoma patients accepted as inoperable in our investigation. Therefore it could be stated that low S1P4 gene expression levels were correlated with favorable outcome criteria such as non-surrenal localization, low risk group and resectable tumor in the current study.

A variety of serum markers have been proposed either to predict outcome or to follow the activity of disease. For example, increased serum ferritin levels may be a simple marker of rapid tumor growth and/or large tumor burden.[28] Survival is substantially worse for patients with advanced disease and high NSE.[17] Although not specific to neuroblastoma, serum LDH level has been used as a prognostic marker for neuroblastoma, and it may reflect rapid cellular turnover or large tumor burden.[18,29] However, none of these markers are used currently to predict outcome or select therapy. In the present study, we demonstrated that increased S1P4 gene expression were shown in patients with high levels of NSE, LDH and ferritin. Possibly it could be implied that S1P4 gene expression might be used as a prognostic marker in neuroblastoma patients. Nevertheless clinical and laboratory studies on larger patient series will be needed to investigate whether S1P4 gene may play an important role in the prognostic consideration of neuroblastoma patients.

French et al demonstrated that the using of various chemotherapeutics, such as cytosine arabinoside, vincristine and daunorubicin and ionizing radiation induces apoptosis by causing the inhibition of SphK enzyme which forms S1P and the accumulation of ceramide.[30] We have found no literature data showing the effect of chemotherapeutic drugs on S1P4 gene expression in the clinical follow-up of pediatric cancer cases. When we examined the relationship between S1P4 gene expression levels and chemotherapy, any meaningful correlation was not found between newly diagnosed neuroblastoma patients received no induction chemotherapy yet and patients already given chemotherapy. It could be thought that few patients (4 patients at stage 1 and 9 patients enrolled before induction chemotherapy) might be responsible for this result in our study.

There has also not yet been literature data about how the types and duration of chemotherapeutic drugs affect S1P4 gene expression levels. In our study, we performed a comparison between the expression levels of 12 patients who were on the maintenance treatment after receiving induction chemotherapy and 14 patients who finished chemotherapy and followed up in outpatient clinic. Cyclophosphamide, vincristine, carboplatin, etoposide, dacarbazine, melphalan, and 13 cis retinoic acid are used for a period of 6 months during the maintenance therapy. The mean S1P4 gene expression levels estimated in patients who had not yet re-

ceived chemotherapy were high compare to the maintenance phase. Additionally S1P4 gene expression levels of patients on maintenance therapy were found to be lower than that of patients completed chemotherapy. Therefore during maintenance therapy, S1P4 gene expression levels were found to be suppressed with chemotherapy. Reincrease of S1P4 gene expression levels in patients whom chemotherapy was discontinued and who were followed up in outpatient clinic could be attributed to the elimination of this suppression with the cessation of chemotherapy. In other words, when we considered the effects of S1P4 in cell line studies, it could be postulated that chemotherapy might inhibit S1P and conduct the progression of apoptosis in neuroblastoma patients. Prospective studies should be performed for more detailed information regarding how chemotherapic agents and duration of their use affect S1P4 gene expression levels in clinical practice.

S1P4 gene expression levels of patients who continued to live with the tumor without a progression were found to be higher than that of survived without disease. Still S1P4 gene had been over expressing in patients who had tumor with any symptom. High levels of S1P4 gene expression could be a warning biological indicator whether tumor would show a progression later in patients survived with disease. Therefore it could be advisable that patients with neuroblastoma should be followed up closely in terms of increased S1P4 gene expression values in outpatient clinic.

Our study has some limitations. First, few neuroblastoma patients could be investigated. Second, because of our limited laboratory facilities some prognostic factors determined in neuroblastoma such as MYCN amplification and chromosomal aberrations could not be determined and correlated with S1P4 gene expression levels.

CONCLUSION

As the genetic and biologic characterization of neuroblastoma at diagnosis becomes more comprehensive, S1P4 gene expression should be explored in neuroblastoma patients in a controlled research setting. Our study is the first study that S1P4 gene was much expressed in neuroblastoma patients. Based on the information that S1P4 has been shown to induce cellular migration and prevent apoptosis in cell line studies, S1P4 could have a prognostic value in neuroblastoma patients. The demonstration of suppressed S1P4 gene expression levels by chemotherapy re-emphasizes the importance of chemotherapy in the treatment of cancer. Nevertheless, clinical studes on a large group of patients are needed to definitely prove the effects of S1P4 on the tumor progression and the efficacy of chemotherapy in cancer cases. Moreover gene expression studies of the other sphingosine 1-phosphate receptors should be performed and their behavior in cancer cases should be investigated.

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