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Klinik Araştırma

C-KIT expression in pediatric tumors: What is hidden beneath the tip of the iceberg?

Pediatrik tümörlerde C-KİT ekspresyonu: Buzdağının altında saklı olan ne?

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SUMMARY

Objective: C-kit protein is a member of the type III receptor tyrosine kinase family. Although c-kit is believed to have a pathogenetic role in gastrointestinal stromal tumors (GIST), it is expressed by several other tumors. The aim of this study is to evaluate c-kit expression in pediatric tumors.
Methods: C-kit expression was retrospectively evaluated by immunohistochemical method in 205 pediatric tumors. A chi-square test was used to analyze the c-kit expression in different tumor groups.
Results: Expression of c-kit is demonstrated in 9.8% of our pediatric tumor cases. C-kit is mostly expressed in Wilms tumor (in 9 cases of 32). Three of the 26 rhabdomyosarcoma cases were positive for c-kit. In three of 7 cases with hepatoblastoma, 2 of 3 cases of three inflamatory fibrous tumor, one of two nasopharingeal carcinomas, one epitheloid sarcoma, hepatocellular carcinoma and pancreatic pseudopapillary tumor c-kit was positive.
Conclusion: Since Wilms tumor, rhabdomyosarcoma, hepatoblastoma, and nasopharingeal carcinomas express c-kit, this marker may represent a new suitable therapeutic target for these pediatric tumors.

Key words: c-kit, pediatric tumor

ÖZET

Amaç: C-kit, tip 3 reseptör tirozin kinaz ailesini üyesi bir proteindir. Gastrointestinal stromal tümör patogenezinde rolü olduğuna inanılmakla birlikte, birçok başka tümörde de eksprese edilmektedir. Bu çalışmanın amacı pediatrik tümörlerde c-kit ekspresyonunu değerlendirmektir.
Yöntemler: C-kit ekspresyonu immün histokimyasal boyamalarla 205 pediatrik tümörde retrospektif olarak değerlendirilmiştir. Farklı tümörlerdeki c-kit ekspresyonunun değerlendirilmesinde ki-kare testi kullanılmıştır.
Bulgular: Pediatrik tümör olgularının %9,8'inde c-kit ekspresyonu gözlenmiştir. En yoğun c-kit ekspresyonu gözlenen tümör Wilms tümörüdür (32 olgunun 9'unda). Yirmi altı rabdomyosarkom olgusunun 3'ünde c-kit pozitiftir. Yedi hepatoblastomun 3'ünde, 3 inflamatuvar fibröz tümörün 2'sinde, 2 nazofarinks karsinomunun birinde, 1 epiteloid sarkomda, 1 hepatosellüler karsinomda ve 1 pankreatik psödopapiller tümörde c-kit pozitif bulunmuştur.
Sonuç: Ekspresyon saptanan Wilms tümörü, rabdomyosarkom, hepatoblastom ve nazofarenks karsinomunda; pediatrik tümörlerdeki tedavi protokolleri için, c-kit yeni bir hedef oluşturabilir.

Anahtar kelimeler: C-kit, pediatrik tümör

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INTRODUCTION

The c-kit antibody labels the transmembrane tyrosine kinase receptor CD117/c-kit. The proto-oncogene c-kit belongs to the class III receptor kinase family including colony stimulating factor 1 and the platelet-derived growth factor receptors type A and B. It encodes the stem cell factor receptor. It is localized on human chromosome 4. The receptor is activated by dimerisation, substrate phosphorylation, autophosphorylation, receptor internalisation, activation of protein kinases and phospholipases and transcription of different protoonkogenes [1-7]. Mutation in the c-kit gene leads to ligand-independent phosphorylisation causing tumor growth and progression. Even in the absence of proximal transforming events, signaling of tyrosine kinases may contribute to survival advantage of the transformed cells. Although c-kit is believed to have a central pathogenetic role in gastrointestinal stromal tumors, it is expressed by several other tumors, including mastocytosis, mast cell leukemia, acute myelogenous leukemia, melanoma, ovarian, breast, and small-cell lung carcinoma (SCLC).

Results of recent clinical studies have suggested the promising therapeutic impact of imatinib in the treatment of CML and GIST. Imatinib mesylate and other KIT-targeted agents may have therapeutic potential for malignancies other than GISTs, which are also subjected to a KIT-mediated oncogenic drive. Effects of imatinib on c-Abl, c-Kit, and PDGFR kinase activities were demonstrated. These effects are also reported on pediatric tumors such as neuroblastoma, Ewing sarcoma [7-10] and on a few pediatric solid tumors [11]. The aim of this study is to examine c-kit expression in pediatric tumors including neuroblastoma, lymphoma, Wilms tumor, rhabdomyosarcoma, fibrous tumor, gynecologic tumors, hepatoblastoma and some rare tumors.

MATERIALS and METHODS

We examined c-kit expression in 205 pediatric tumors to verify its putative expression. Since this study was performed on archive files, no ethics approval was required C-kit expression was retrospectively evaluated by immunohistochemical method in 205 pediatric tumors, and 12 GIST as the control group diagnosed between 1995-2004 at Pathology Laboratory of Dr Behcet Uz Children Research Hospital. The clinical properties such as age, sex, prognosis, stages of the disease states were not included in this study. The 12 adult GIST cases used as a comparison control group were collected from authors' archive files. Immunohistochemistry: Five micrometer - sections on polylisine coated slides of formalin- fixed, and paraffin-embedded well-preserved tissue blocks of tumors (one block for each case) were used for immunhistochemical (IHC) study. IHC staining for KIT (CD117) was performed using a 1: 200 dilution of the rabbit polyclonal antibody A4502 (DAKO, USA) by SAB method. Pretreatment of tissues for heat-induced epitope retrieval was applied in 0.001mol/L EDTA solution (pH 8.5) for 20 minutes in a microwave (400 watt). Incubation time with primary antibody was 60 minutes. Control slides of the product were used as a positive control of the method. For each tissue sample, the percentage of positive cells was estimated. Intensive or focal cytoplasmic and/or membranous staining as GIST was considered as positive. Samples were scored as negative when no immunoreactive tumor cells were observed.

Statistical Analysis: All statistical analyses were performed using SPSS program. Incidences and descriptive characteristics were evaluated. Chi-square test was used to analyze the c-kit expression in different tumor groups. The significance was set at $p<0.05$.

RESULTS

All of the control GIST cases were strongly and diffusely positive. Expression of c-kit was demonstrated in 9.8% of our pediatric tumors. The frequen-

Table 1. c-kit Positivity in various pediatric tumors

	n	Percent	c-kit	c-kit n
Wilms tumor	32	15,6	Po/neg	9/23
Rhabdomyosarcoma	26	12,7	Po/neg	3/23
Hepatoblastoma	7	3,4	Po/neg	3/4
Inflammatory fibrous tumor	3	1,5	Po/neg	2/1
Nasopharingeal carcinoma	2	1,0	Po/neg	1/1
Epitheloid Sarcoma	1	,5	positive	1
Pancreatic pseudopapillary tumor	1	,5	positive	1
Neuroblastoma	27	13,2	negative	27
NonHodgkin Lymphoma	17	8,3	negative	17
Hodgkin Lymphoma	16	7,8	negative	16
Pnet/Ewing Sarcoma	8	3,9	negative	8
Fibroma	8	3,9	negative	8
Endodermal sinus tumor,ovarian	7	3,4	negative	7
Infantile fibrosarcoma	6	2,9	negative	6
ganglioneuroma	4	2,0	negative	4
Langerhans cell histiositosis	4	2,0	negative	4
Granulosa cell tumor, ovarian	3	1,5	negative	3
Mature CysticTeratoma, ovarian	3	1,5	negative	3
Immature Teratoma, sacrococsigeal	3	1,5	negative	3
Astrocytoma	2	1,0	negative	2
Clear cell sarcoma, kidney	2	1,0	negative	2
Dysgerminoma, ovarian	2	1,0	negative	2
retinoblastoma	2	1,0	negative	2
Adrenal cortical carcinoma	1	,5	negative	1
ependymoma	1	,5	negative	1
fibromatosis	1	,5	negative	1
Hemangioendotelioma	1	,5	negative	1
Calcifying fibrous tumor, soft tissue	1	,5	negative	1
Carcinoid tumor, appendix	1	,5	negative	1
Mesenchimal hamarthoma, liver	1	,5	negative	1
Malignant meningioma	1	,5	negative	1
Medulloblastoma	1	,5	negative	1
Granular cell tumor	1	,5	negative	1
Nodulary Hydradenoma	1	,5	negative	1
Neurofibroma	1	,5	negative	1
Osteocondroma	1	,5	negative	1
Osteosarcoma	1	,5	negative	1
Malignant rabdoid tumor	1	,5	negative	1
Rabdomyoma	1	,5	negative	1
Mucinous adenocarcinoma, rectum	1	,5	negative	1
Renal cell carcinoma	1	,5	negative	1
Thyroid papillary carcinoma	1	,5	negative	1
Total	205	100,0	Po/neg	20/185

cy of positivity of c-kit in pediatric tumors is shown in Table 1. c-kit is mostly expressed in Wilms tumor (9/32; 28.1%, p=0.0001). The expression was mainly observed in the epithelial component (Figure 1). Three out of 26 cases with rhabdomyosarcoma were positive for c-kit (11.5%, p=0.765) (Figure 2), two of them being spindle cell variant. C-kit positivity was also detected in cases with hepatoblastoma (3/7;

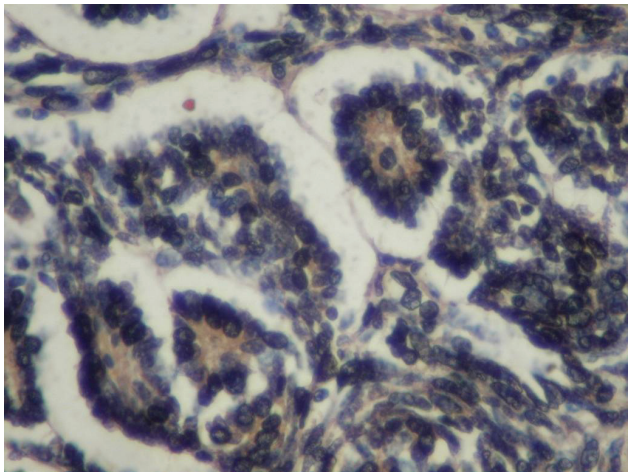

Figure 1. c-kit positivity in Tubulary areas of Wilms tumor (DABx400).

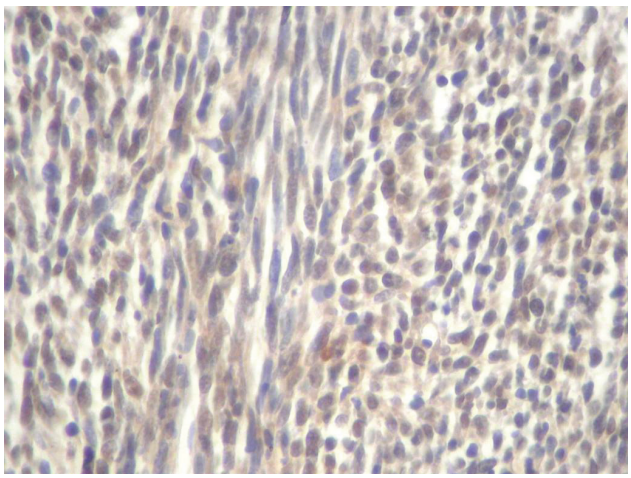

Figure 2. c-kit positivity in a spindle cell rhabdomyosarcoma (DABx200).

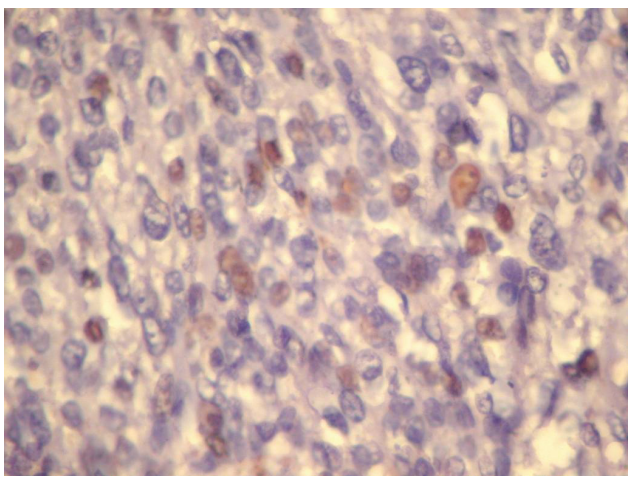

Figure 3. c-kit positivity in hepatoblastoma (DABx400).

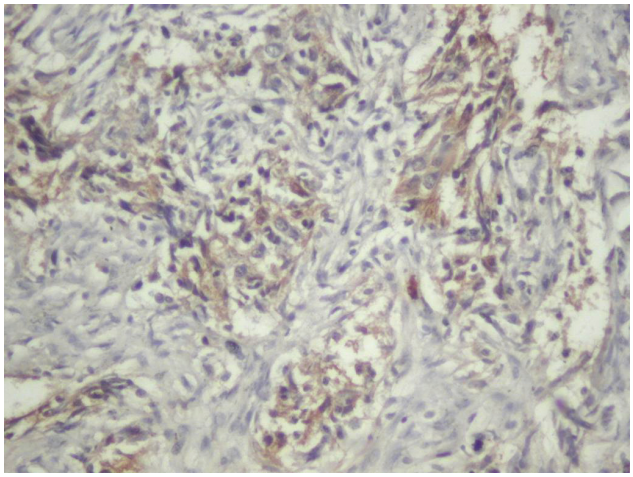

Figure 4. c-kit positivity in nasopharingeal carcinoma (DABx200).

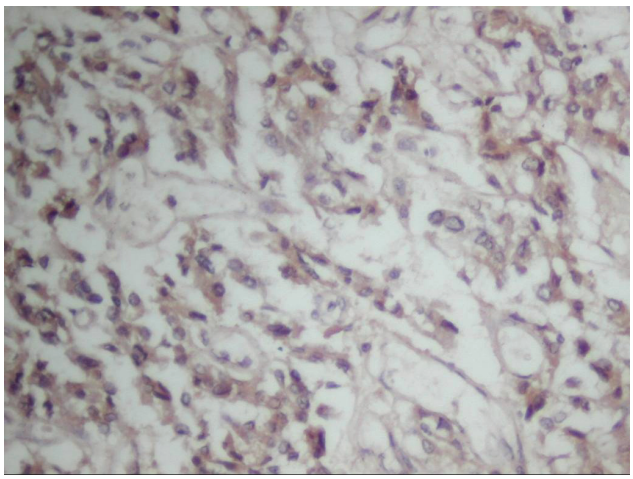

Figure 5. c-kit positivity in pancreatic pseudopapillary tumor (DABx200).

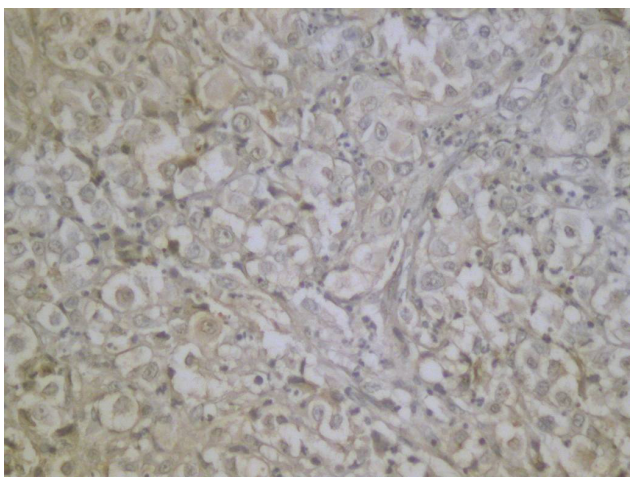

Figure 6. c-kit positivity in epithelioid sarcoma (DABx200).

42.9%, p=0.003), (Figure 3), inflammatory fibrous tumor (2/3) , nasopharyngeal carcinoma (1/2) (Figure 4), pancreatic pseudopapillary tumor (n=1) (Figure 5) and epitheloid sarcoma (n=1) (Figure 6). No c-kit expression was observed in cases with neuroblastoma, non-Hodgkin lymphoma, Hodgkin lymphoma, PNET/Ewing sarcoma, fibroma, ovarian endodermal sinus tumor, infantile fibrosarcoma, ganglioneuroma, Langerhans cell histiocytosis, ovarian granulosa cell tumor, ovarian mature cystic teratoma, sacrococcygeal immature teratoma, astrocytoma, clear cell sarcoma, renal, ovarian dysgerminoma, retinoblastoma, adrenal cortical carcinoma, ependymoma, and fibromatosis.

DISCUSSION

KIT tyrosine kinase activity has been linked to the genesis of GIST. Rubin et al. reported that all forms of GIST (benign, borderline, and malignant) demonstrated elevated levels of KIT tyrosine kinase activity, whereas 92% of them showed a mutant c-kit gene [1,2]. Inhibition of KIT by the small-molecular agent renders considerable response rates in patients with metastasized malignant GIST. After that c-kit expression status and its effect on proliferation and apoptosis [12-15] has been widely studied in other cancer types and normal tissues [16]. In a series of sixty small-cell lung carcinoma Boldrini et al found expression of c-kit in about 40% of the samples. Two mutations in exon 9 and three mutations in exon 11 were found. They concluded that the expression of c-kit and its mutational status failed to appear relevant or have a significant impact on survival [15]. C-kit expression has been infrequently detected in breast cancer [17], hepatoblastoma [18], and medulloblastoma [19], it was absent in Burkitt lymphoma [20].

Hornick and Fletcher reported very low percentages (6%) of KIT expression in 365 different types of soft tissue tumors, most of them being focally and weakly stained. They also claim that high percentages found in previous studies were more often associated with high background (false positive) staining possibly due to inappropriate staining methods and types f primary antibodies used [21]. Studies about c-kit expression in pediatric tumors are slowly accumulating as case reports or series, and even phase 1 studies conducted with tyrosine kinase inhibitors [22-25].

C-kit is variably expressed in Ewing sarcoma detected by using either monoclonal or polyclonal antibodies. Detection of c-kit expression in Ewing sarcoma has been improves with the use of antigen retrieval methods [7]. At present there is no evidence suggesting that KIT expressing tumors can benefit from STI-571 therapy. It has been suggested that the response rate to STI-571 may be dependent on the presence and type of KIT mutations in the tumor cells [17].

In our series no expression was observed in cases with neuroblastoma, non-Hodgkin lymphoma, Hodgkin lymphoma, PNET/Ewing sarcoma, fibroma, ovarian endodermal sinus tumor, infantile fibrosarcoma, ganglioneuroma, Langerhans cell histiocytosis, ovarian granulosa cell tumor, ovarian mature cystic teratoma, sacrococcygeal immature teratoma, astrocytoma, clear cell sarcoma, renal, ovarian dysgerminoma, retinoblastoma, adrenal cortical carcinoma, ependymoma, and fibromatosis. Scarce number of these tumor or lesion groups in our investigation may not be enough to claim that these tumours do not use stem cell/c-kit pathway and would not be responsive to targeted treatment, still our data will give information for further investigation planning. Proliferation, cell survival, differentiation, migration and homing processes that included in c-kit signaling pathway are the main properties of many tumors but these signaling pathways are also affected by many other factors.

Further studies are required to investigate the expression and the possible beneficial effects of imatinib mesylate in KIT positive Wilms tumor, rhabdomyosarcoma especially spindle cell variant, hepatoblastoma, inflammatory fibrous tumor, nasopha-

ryngeal carcinoma and epitheloid sarcoma and also the prognostic role of c-kit in these pediatric tumor groups.

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