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Friend leukemia virus integration-1 (FLI-1) expression in gastrointestinal stromal tumors

Gastrointestinal stromal tümörlerde Friend leukemia virüs integration-1 (FLI-1) ekspresyonu

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Background/aims: *Friend leukemia virus integration-1 expression has been shown in a variety of tumors, including vascular tumors, desmoplastic small round cell tumor, Merkel cell carcinoma, and lymphoblastic lymphoma, in addition to Ewing's sarcoma/primitive neuroectodermal tumor. The aim of the current study was to examine Friend leukemia virus integration-1 protein expression in a series of gastrointestinal stromal tumors and also to assess if Friend leukemia virus integration-1 has any role in the disease process. It is the first study analyzing Friend leukemia virus integration-1 expression in gastrointestinal stromal tumors in the English literature.* ***Methods:*** *A tissue microarray block containing 52 cases of gastrointestinal stromal tumors was done. Immunohistochemical analysis was performed for Friend leukemia virus integration-1 polyclonal antibody.* ***Results:*** *Immunohistochemically, Friend leukemia virus integration-1 was negative in all cases.* ***Conclusions:*** *Friend leukemia virus integration-1 can be expressed in a variety of tumors, and is helpful in making the diagnosis of Ewing's sarcoma/primitive neuroectodermal tumor. We think that this protein is not expressed in gastrointestinal stromal tumors and it is not a part of the pathogenesis of this disease.*

Key words: Friend leukemia virus integration-1, Ewing's sarcoma/primitive neuroectodermal tumor, gastrointestinal stromal tumor

Amaç: *Friend leukemia virüs integration-1 ekspresyonunun, "Ewing" sarkoma/primitif nöroektodermal tümör yanında vasküler tümörler, desmoplastik küçük yuvarlak hücreli tümör, Merkel hücreli karsinoma ve lenfoblastik lenfoma gibi çeşitli tümörlerde bulunduğu gösterilmiştir. Bu çalışmanın amacı, gastrointestinal stromal tümörlerde Friend leukemia virüs integration-1 protein ekspresyonunu ve bu tümörlerin gelişimindeki rolünü araştırmaktır. Bu çalışma, gastrointestinal stromal tümörlerde Friend leukemia virüs integration-1 ekspresyonunu araştıran İngilizce literatürdeki ilk çalışmadır.* ***Yöntem:*** *52 vaka içeren doku mikroarrey bloğu hazırlanmıştır. Friend leukemia virüs integration-1 poliklonal antikoru kullanılarak kesitler üzerinde immunohistokimyasal inceleme yapılmıştır.* ***Bulgular:*** *İmmunohistokimyasal olarak Friend leukemia virüs integration-1 tüm vakalarda negative bulunmuştur.* ***Sonuç:*** *Friend leukemia virüs integration-1 çeşitli tümörlerde eksprese olabilir ve "Ewing" sarkoma/primitif nöroektodermal tümör tanısı için yardımcıdır. Bulgularımıza dayanarak, bu proteinin gastrointestinal stromal tümörlerde eksprese olmadığını ve bu hastalığın patogenezinde yer almadığını düşünüyoruz.*

Anahtar kelimeler: Friend leukemia virüs integration-1, Ewing sarkoma/primitif nöroektodermal tümör, gastrointestinal stromal tümör

INTRODUCTION

Ewing's sarcoma/primitive neuroectodermal tumor (EWS/PNET) is a member of the small, blue, round cell tumors that occur in bone and soft tissue. Approximately 80% of EWS/PNET cases have a characteristic balanced chromosomal translocation, t(11;22) (q24;q12), which includes the N-terminal transactivation domain of the EWS gene and the C-terminus DNA-binding domain of the Friend leukemia virus integration-1 (FLI-1) gene (1-3). The EWS/FLI-1 fusion proteins resulting from this type of translocation behave as aberrant transcriptional regulators and play an essential role in the development of EWS/PNET (4, 5). FLI-1 antibody, a polyclonal antibody directed against the C-terminus of the FLI-1 gene, has been proposed as a diagnostic immunohistochemical marker, combined with CD99, in differentiating EWS/PNET from the other small round cell tumors (6-8). FLI-1 is also expressed by hematopoietic cells and endothelial cells in normal tissues. FLI-1 expression has been shown in a variety of tumors, including vascular tumors, desmoplastic small round cell tumor, Merkel cell carcinoma, and lymphoblastic lymphoma, in addition to EWS/PNET (7-10).

Gastrointestinal stromal tumors (GISTs) are the most common mesenchymal tumors of the digesti-

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ve tract that may show spindle, epithelioid, or mixed type of cellular morphology, in contrast with EWS/PNET. The diagnosis of GISTs is dependent on clinical and morphological features, supported by KIT immunoreactivity and/or the presence of activating KIT mutations. A minority (4-15%) of GISTs fail to express detectable KIT protein, and may have a mutation of PDGFRA (11-13). The histopathological diagnosis in KIT-negative cases may be very difficult. For this reason, some other markers may be used for diagnostic purposes. With the development of specific targeted therapy (imatinib mesylate) for GISTs, it is crucially important to discriminate GISTs from the other lesions in their differential diagnosis (14).

The aim of the current study was to examine FLI-1 protein expression in a series of GISTs and also to assess whether or not FLI-1 has any role in the disease process. It is the first study analyzing FLI-1 expression in GISTs.

MATERIALS AND METHODS

The cases, obtained from the archives of our department between 1996-2001, showing typical clinicopathological and immunohistochemical features of GISTs were included in this study. A tissue microarray (TMA) block containing 52 cases of GISTs was built. In the TMA block, each tumor was represented by two cores using a tissue arrayer (Beecher Instruments; Silver Spring, MD, USA). Cores (0.6 mm) were taken from paraffin-embedded tumors. The following parameters were recorded for each case: age and gender, primary tumor location, morphological type, risk group, and immunohistochemical features.

Immunohistochemistry

Immunohistochemistry was performed for FLI-1 polyclonal (C-19) antibody (clone sc-356, 1:150; Santa Cruz, CA, USA) on tissue sections by using Ventana Automated Immunostainer. Positive and negative control tissues were put into the TMA block. Nuclear staining in the lymphocytes and endothelial cells was considered as positive internal control. The cases staining less than 5% of tumor cells were scored as negative.

RESULTS

Thirty-two of 52 cases were (61.5%) male. The mean age was 59.5 years (range: 26 to 90). The most frequent location of the lesion was the stomach (n=26, 51%), followed by small bowel (n=16, 31%), omentum (n=7, 14%), colon (n=1, 2%), and pelvis (n=1, 2%). In one case, the exact intra-abdominal site was not specified.

Twenty-six cases (51%) were classified as high risk, 11 (21.5%) as intermediate risk, 11 (21.5%) as low risk and 3 (6%) as very low risk. One case could not be classified because of the inadequate clinical information regarding tumor size. The main morphological type of the tumor was spindle type (n=33, 63.5%), followed by epithelioid type (n=13, 25%) and mixed type (n=6, 11.5%) (Figure 1A-C).

Immunohistochemically, FLI-1 was negative in all cases (Figure 1D,E). Only endothelial cells and lymphocytes were stained. To compare the FLI-1 staining profile, the previous immunohistochemical features of the cases at the time of diagnosis were recorded. CD117, CD34 and SMA positivity were seen in 45 cases (86.5%), 35 cases (67%) and 37 cases (71%), respectively. Very few cases were positive for desmin (n=3, 6%) and for S-100 (n=2, 4%).

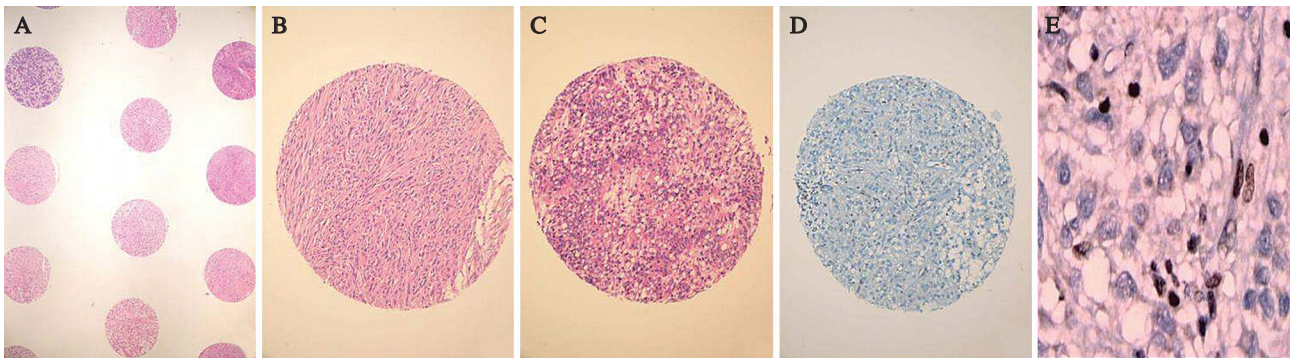

Figure 1. A. Hematoxylin and eosin (H&E) stained tissue microarray sections of the GIST cases (x40). **B, C.** Spindle cell and epithelioid morphology were noted (H&E x200, x200). **D, E.** FLI-1 negativity of the cases with an internal positive control (x200, x1000).

DISCUSSION

Up to 80% of EWS/PNET are characterized by the chromosomal translocation, t(11;22) (q24;q12), which results in the EWS/FLI-1 fusion protein. This chimeric protein is located in the nucleus and induces malignant transformation in NIH3T3 cells (15-17). A polyclonal antiserum against the C-terminus of the FLI-1 protein has been shown to be a useful immunohistochemical marker in discriminating EWS/PNET from other round cell tumors (8). Histologically, EWS/PNET is composed of uniformly undifferentiated small round cells with vesicular nuclei and a small cytoplasm within a sparse intercellular stroma. The cellular origin of EWS/PNET is still debated. Endothelial and neuroectodermal origins have been postulated (19-21). Riggi et al. (22) showed that the primary bone marrow-derived mesenchymal progenitor cells display permissiveness for EWS-FLI-1-mediated transformation and generate tumors that display hallmarks of EWS/PNET. They proposed that mesenchymal progenitor cells may constitute a candidate environment from which EWS/PNET originate.

GISTs are specific mesenchymal tumors occurring in the GI tract but also in the omentum and mesentery. They originate from the interstitial cells of Cajal or their stem-cell-like precursors (11,23). These cells form a complex network providing the regulation of intestinal motility in the GI tract. Morphologically, most of the GISTs appear as spindle cell tumors, but epithelioid or mixed spindle-epithelioid cell appearance may occur. Epithelioid GISTs, similar to spindle cell lesions, tend to have uniform round-to-ovoid nuclei with vesicular chromatin. This histological feature may cause diagnostic confusion with epithelial, melanocytic or other neoplasms. More than 80% of GISTs display mutations in KIT or PDGFRA receptor tyrosine kinase proteins sporadically, but familial GISTs are also present. The end result of activating mutations of KIT and PDGFRA is increase in cellular proliferation and decrease in apoptosis, ultimately leading to neoplasia (11,24,25). The major diagnostic feature of GIST is positivity for the KIT (CD117) receptor tyrosine kinase, observed in more than 95% of GISTs. GISTs are also variably positive for CD34, smooth muscle markers and rarely desmin and S-100 protein. Protein kinase theta, a downstream effector in the KIT signaling pathway activated following KIT activation, and DOG1, a new gene encoding for a protein of unknown function, seem to be expressed in GISTs irrespective of mutation type (26,27). The diagnosis of GISTs is dependent on clinical and morphological features, supported by KIT immunoreactivity and/or the presence of activating KIT mutations.

FLI-1 has been proposed as a diagnostic immunohistochemical marker, combined with CD99, in differentiating EWS/PNET from the other small round cell tumors (6-8). FLI-1 is also expressed by hematopoietic cells and endothelial cells in normal tissues (18). FLI-1 expression has been shown in a variety of tumors, including vascular tumors, desmoplastic small round cell tumor, Merkel cell carcinoma, and lymphoblastic lymphoma, in addition to EWS/PNET (7-10,28). Mhawech-Fauceglia et al. (29) analyzed the expression pattern of FLI-1 in a large series of benign and malignant solid tumors. They also tested this antibody on the spindle cell mesenchymal tumors that may enter the differential diagnosis of GIST. In their series, all cases of leiomyosarcoma, synovial sarcoma, fibrosarcoma, and malignant peripheral nerve sheath tumors were negative for FLI-1. In our study, we did not find FLI-1 expression in GIST cases. According to the clinical presentation, cellular morphology and immunohistochemical or molecular findings, the differentiation of GIST from EWS/PNET is generally not a diagnostic problem. But GISTs can display unusual morphological features, such as signet ring cell, rhabdoid, paraganglioma-like, or poorly differentiated morphology in the unusual sites (30-32), such that they may be confused with other sarcomas. We also know that EWS/PNET may be histologically indistinguishable, particularly when poorly differentiated or in the setting of a small biopsy (8).

In conclusion, FLI-1 can be expressed in a variety of tumors and is helpful in making the diagnosis of EWS/PNET, but it seems that it is not expressed in GISTs or in the mesenchymal tumors that should be differentiated from GISTs. This finding could be helpful in differentiating these two entities in the setting of a limited amount of tissue biopsy from a tumor showing poorly differentiated morphology.

REFERENCES

1. Turc-Carel C, Aurias A, Mugneret F, et al. Chromosomes in Ewing's sarcoma. I: an evaluation of 85 cases of remarkable consistency of t(11;22)(q24;q12). Cancer Genet Cytogenet 1988; 32: 229-38.
2. Turc-Carel C, Philip I, Berger MP, et al. Chromosome study of Ewing's sarcoma (ES) cell lines. Consistency of a reciprocal translocation t(11;22)(q24;q12). Cancer Genet Cytogenet 1984; 12: 1-19.
3. Zucman J, Delattre O, Desmaze C, et al. Cloning and characterization of the Ewing's sarcoma and peripheral neuroepithelioma t(11;22) translocation breakpoints. Genes Chromosomes Cancer 1992; 5: 271-7.
4. Arvand A, Denny CT. Biology of EWS/ETS fusions in Ewing's family tumors. Oncogene 2001; 20: 5747-54.
5. May WA, Lessnick SL, Braun BS, et al. The Ewing's sarcoma EWS/FLI-1 fusion gene encodes a more potent transcriptional activator and is a more powerful transforming gene than FLI-1. Mol Cell Biol 1993; 13: 7393-8.
6. Nilsson G, Wang M, Wejde J, et al. Detection of EWS/FLI-1 by immunostaining: an adjunctive tool in diagnosis of Ewing's sarcoma and primitive neuroectodermal tumor on cytological samples and paraffin-embedded archival material. Sarcoma 1999; 3: 25-32.
7. Llombart -Bosch A, Navarro S. Immunohistochemical detection of EWS and FLI-1 proteins in Ewing sarcoma and primitive neuroectodermal tumors: comparative analysis with CD99 (MIC-2) expression. Appl Immunohistochem Mol Morphol 2001; 9: 255-60.
8. Folpe AL, Hill CE, Parham DM, et al. Immunohistochemical detection of FLI-1 protein expression: a study of 132 round cell tumors with emphasis on CD99-positive mimics of Ewing's sarcoma/primitive neuroectodermal tumor. Am J Surg Pathol 2000; 24: 1657-62.
9. Folpe AL, Chand EM, Goldblum JR, et al. Expression of FLI-1, a nuclear transcription factor, distinguishes vascular neoplasms from potential mimics. Am J Surg Pathol 2001; 25: 1061-6.
10. Llombart B, Monteagudo C, Lopez-Guerrero JA, et al. Clinicopathological and immunohistochemical analysis of 20 cases of Merkel cell carcinoma in search of prognostic markers. Histopathology 2005; 46: 622-34.
11. Hirota S, Isozaki K, Moriyama Y, et al. Gain-of-function mutations of c-kit in human gastrointestinal stromal tumors. Science 1998; 279: 577-80.
12. Lee JR, Joshi V, Griffin JW Jr, et al. Gastrointestinal autonomic nerve tumor: immunohistochemical and molecular identity with gastrointestinal stromal tumor. Am J Surg Pathol 2001; 25: 979-87.
13. Miettinen M, Lasota J. Gastrointestinal stromal tumors (GISTs): definition, occurrence, pathology, differential diagnosis and molecular genetics. Pol J Pathol 2003; 54: 3-24.
14. Demetri G, van Mehren M, Blanke CD, et al. Efficacy and safety of imatinib mesylate in advanced gastrointestinal stromal tumors. N Engl J Med 2002; 347: 472-80.
15. Thompson AD, Teitell MA, Arvand A, et al. Divergent Ewing's sarcoma EWS/ETS fusions confer a common tumorigenic phenotype on NIH3T3 cells. Oncogene 1999; 18: 5506-13.
16. May WA, Arvand A, Thompson AD, et al. EWS/FLI1-induced manic fringe renders NIH 3T3 cells tumorigenic. Nat Genet 1997; 17: 495-7.
17. Arvand A, Welford SM, Teitell MA, et al. The COOH-terminal domain of FLI-1 is necessary for full tumorigenesis and transcriptional modulation by EWS/FLI-1. Cancer Res 2001; 61: 5311-7.
18. Mager AM, Grapin-Botton A, Ladjali K, et al. The avian FLI gene is specifically expressed during embryogenesis in a subset of neural crest cells giving rise to mesenchyme. Int J Dev Biol 1998; 42: 561-72.
19. Ewing J. Diffuse endothelioma of bone. Proc New York Pathol Soc 1921; 21: 17-24.
20. Cavazzana J.S, Miser JS, Jefferson J, et al. Experimental evidence for a neural origin of Ewing's sarcoma of bone. Am J Pathol 1987; 127: 507-18.
21. Noguera R, Navarro S, Peydro-Olaya A, et al. Patterns of differentiation in extraosseous Ewing's sarcoma cells. An in vitro study. Cancer 1994; 73: 616-24.
22. Riggi N, Cironi L, Provero P, et al. Development of Ewing's sarcoma from primary bone marrow-derived mesenchymal progenitor cells. Cancer Res 2005; 65: 11459-68.
23. Kindblom LG, Remotti HE, Aldenborg F, et al. Gastrointestinal pacemaker cell tumor (GIPACT). Gastrointestinal stromal tumors show phenotypic characteristics of the interstitial cells of Cajal. Am J Pathol 1998; 153: 1259-69.
24. Kitamura Y, Hirota S. Kit as a human oncogenic tyrosine kinase. Cell Mol Life Sci 2004; 61: 2924-31.
25. Corless CL, Fletcher JA, Heinrich MC. Biology of gastrointestinal stromal tumors. J Clin Oncol 2004; 22: 3813-25.
26. Duensing A, Joseph NE, Medeiros F, et al. Protein kinase C theta (PKCtheta) expression and constitutive activation in gastrointestinal stromal tumors (GISTs). Cancer Res 2004; 64: 5127-31.
27. West RB, Corless CL, Chen X, et al. The novel marker, DOG1, is expressed ubiquitously in gastrointestinal stromal tumors irrespective of KIT or PDGFRA mutation status. Am J Pathol 2004; 165: 107-13.
28. Rossi S, Orvieto E, Furlanetto A, et al. Utility of the immunohistochemical detection of FLI-1 expression in round cell and vascular neoplasm using a monoclonal antibody. Mod Pathol 2004; 14: 547-52.
29. Mhawech-Fauceglia P, Herrmann FR, Bshara W, et al. Friend leukemia integration-1 expression in malignant and benign tumors: a multiple tissue microarray analysis using polyclonal antibody. J Clin Pathol 2007; 60: 694-700.
30. Suster S, Fisher C, Moran CA. Expression of bcl-2 oncoprotein in benign and malignant spindle cell tumors of soft tissue, skin, serosal surfaces, and gastrointestinal tract. Am J Surg Pathol 1998; 22: 863-72.
31. Richmond JA, Mount SL, Schwarz JE. Gastrointestinal stromal tumor of the stomach with rhabdoid phenotype: immunohistochemical, ultrastructural, and immunoelectron microscopic evaluation. Ultrastruct Pathol 2004; 28: 165-70.
32. Miettinen M, Sobin LH, Lasota J. Gastrointestinal stromal tumors of the stomach: a clinicopathologic, immunohistochemical, and molecular genetic study of 1765 cases with long-term follow-up. Am J Surg Pathol 2005; 29: 52-68.