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Expression of Insulin-Like Growth Factor 1 Receptor in Primary Breast Cancer: Immunohistochemical Analysis

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Insulin-like growth factor-1 receptor (IGF-1R) has been implicated in regulation in tumor growth. The results of previous studies performed by radioimmunoassay are conflicting, and the prognostic significance of IGF-1R expression in primary breast cancer is still controversial. IGF-1R expression was evaluated in formalin-fixed, paraffin-embedded tissue of 210 primary breast cancer patients by using anti-IGF-1R antibody. The clinicopathologic variables and 5-year disease-free survival were studied, and their correlations between IGF-1R expressions were investigated. IGF-1R overexpression was observed in 43.8% of tumors. IGF-1R overexpression had no correlation with prognosis or with other clinicopathologic parameters, such as age, tumor size, nodal status, histologic grade, hormone receptor status, and human epidermal growth factor 2 status. Though its prognostic value in breast cancer is limited, immunohistochemical evaluation of IGF-1R by using this monoclonal antibody may be useful in translational research using archived material. HUM PATHOL. 35:1537-1542. © 2004 Elsevier Inc. All rights reserved.

Key words: Insulin-like growth factor 1 receptor, immunohistochemistry, primary breast cancer, prognostic marker.

Abbreviations: IGF-1R, insulin-like growth factor-1 receptor; IGFBPs, IGF-binding proteins; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2; IHC, immunohistochemistry; DFS, disease-free survival; RIA, radioimmunoassay.

IGF-1R is a glycosylated heterotetramer composed of 2 extracellular α-subunits and β-subunits that have intrinsic tyrosine kinase activity with 70% homology to the insulin receptor.[1] IGF-1R mainly mediates the effect of insulin-like growth factors (IGFs), which are potent mitogens that regulate cell proliferation, differentiation, and protection from apoptosis.[2] The clinical and epidemiologic data suggest that the levels of IGF-1 or IGF binding proteins (IGFBPs) in the serum are related to the risk of solid tumors such as breast, prostate, endometrial, ovarian, and colon cancer.[3]

IGF-1R has been found to be significantly expressed and highly activated in breast cancer, and its prognostic and predictive value in clinical samples are of interest.[4-8] There are several methods to measure IGF-1R expression: radioimmunoassay, Western blotting, and immunohistochemistry (IHC). Immunohistochemical evaluation is the most simple and the easiest to perform. To date, there are several commercially available anti-IGF-1R antibodies, but there are no established scoring methods for IGF-1R expression in formalin-fixed, paraffin-embedded tissue. We herein report the prognostic significance of IGF-1R overexpression as determined by IHC on archive materials of consecutive primary breast cancer patients when evaluated by the intensity of membrane staining. We also investigated its correlation with various clinicopathologic factors.

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MATERIALS AND METHODS

Patients

This study was performed on 276 consecutive primary breast cancer patients who underwent surgery or biopsy at National Cancer Center Hospital from January to December 1997. From the cases, 268 paraffin-embedded formalin fixed tissues were obtained. Thirteen stage IV breast cancer patients, 9 patients with malignancy of other origin, 7 metachronous bilateral breast cancer patients, 4 synchronous breast cancer patients, and cases impossible for evaluation in invasive component such as ductal carcinoma in situ were excluded from analysis. Thus immunohistochemical staining was performed on 210 invasive carcinomas.

Pathology

Tumor size, number of axillary lymph node metastasis, histologic type, and histologic grade according to Nottingham combined histologic grading were noted.

Immunohistochemistry

IHC was performed for estrogen receptor (ER), progesterone receptor (PR), human epidermal growth factor 2 (HER2), and IGF-1R on 4-μm-thick serial sections from formalin-fixed, paraffin-embedded tissue.

Monoclonal antibodies 1D5 (DAKO) and 1A6(DAKO) were used for ER and PR IHC, respectively, according to the recommended staining protocol by the manufacturer. It was scored to be positive when $\geq$10% of the cancer cells were

TABLE 1. Scoring of Insulin-Like Growth Factor-1 Receptor Expression According to Intensity of Membrane Staining

Score	Pattern of Immunohistochemical Staining in Invasive Component
0	No staining observed or staining observed in <10% of tumor cells.
1+	A faint or barely perceptible membrane staining in >10% of tumor cells. The cells are only stained in part of their membrane.
2+	A weak to moderate complete membrane staining in >10% of tumor cells.
3+	A strong complete membrane staining in >10% of tumor cells.

stained. Herceptest (DAKO) was used for HER2 assay as described elsewhere, and (2+) and (3+) was defined as overexpression.[9]

The primary antibody for IGF-1R used in this study (clone 24-31) is a mouse monoclonal antibody that is specific for α-subunit of human IGF-1R.[10] Paraffin sections were retrieved in distilled water at 95°C for 40 minutes. Then the sections were incubated with the anti-IGF1R antibody for 30 minutes and were rinsed in EnVision plus (DAKO) for 30 minutes. The reaction product was made visible after incubation in diaminobenzidine for 10 minutes.

Human normal colon mucosa and breast cancer–cultured cellblock was used as positive control. The IGF-1R expression in human colon mucosa was defined as (1+), and we scored (2+), (3+) according to the intensity of the membrane-staining within invasive component in accordance to scoring of HER2 by HercepTest (Table 1, Fig 1) at magnification of ×100 to ×200. When there was heterogeneity in IGF-1R staining within a tumor, the highest score was applied regardless of its area among the tumor.

Statistical Analysis

The results were statistically evaluated by SAS software (version 8.2; SAS Institute Inc, Cary, NC).

Disease-free survival (DFS) was calculated from the date of surgical excision of the primary tumor to the date of recurrence or last follow-up. Prognostic information was masked to the pathologists responsible for evaluation of biologic markers. DFS was calculated for all 210 cases. DFS curves were computed by the Kaplan-Meier method. Correlation between IGF-1R expression and various clinicopathologic factors were analyzed by using Fisher's exact test. Univariate analysis of DFS was performed with the use of log-rank test. *P* values of less than 0.05 were considered to be statistically significant.

RESULTS

Characteristics of the Patients

The median age of study population was 53 years (range, 25-83). The median diameter of invasion was 2.2 cm (range 0.1 to 14.0). The majority of the histologic type was invasive ductal carcinoma. About half of the cases were node negative. The number of cases with Nottingham combined histologic grade 1, 2, and 3 were 12, 37, and 137 cases, respectively.

ER and PR was positive in 154 (73.3%) and 98 (46.7%) tumors. HER2 overexpression was seen in 36 tumors (17.1%; 2+: 2.9%, 3+: 14.2%). See Table 2 for a summary of data on patient characteristics.

IGF-1R Immunohistochemistry

IGF-1R was localized to epithelial compartment including normal breast epithelium, ductal carcinoma in situ, and invasive carcinoma (Fig 1). A weak to moderate (ie, (1+) or (2+)) staining was observed in normal duct epithelium. The majority of invasive carcinoma showed both cytoplasmic and membrane staining. There was heterogeneity of staining inside the same tumor: sporadic or patchy, focal, and diffuse pattern. Heterogeneity of IGF-1R staining was observed in 61 (29%) of 210 cases. Though this intratumoral heterogeneity made scoring difficult in some cases, immunohistochemical staining of IGF-1R was stable and reproducible. The number of cases of IGF-1R score 0, 1+, 2+, 3+ was 24 (11.4%), 94 (44.8%), 25 (11.9%), and 67 (31.9%), respectively.

IGF-1R Expression in Association With Various Clinicopathologic Parameters

There was no correlation between IGF-1R expression and age, size of invasion, presence or absence of axillary lymph node metastasis, and histologic grade. ER, PR, and HER2 status also did not correlate with IGF-1R expression. See Table 3.

Univariate Analysis

The median follow-up period was 5.0 years. The 5-year DFS was significantly better among patients with positive ER expression, and negative HER2 overexpression (Table 4). The patients with invasion less than 2 cm, negative axillary lymph node and positive PR expression had a trend of better prognosis, though it did not reach statistical significance. IGF-1R expression status did not correlate with DFS (Fig 2).

DISCUSSION

We tested the prognostic significance of IGF-1R overexpression on formalin-fixed, paraffin-embedded tissue and found no correlation between IGF-1R expression in primary tumor and 5-year DFS. Because this monoclonal antibody is specific[10] and prognostic value of other known biologic markers was validated within this patient population, we conclude that IGF-1R overexpression has no impact on prognosis of breast cancer in this study. This result is concordant with the Foekins et al[4] report, in which IGF-1R was evaluated in 214 primary breast cancer by ^{125}I-IGF radioimmunoassay (RIA).

Estimates of the proportion of IGF-1R expression that have been derived from previous studies, mostly performed by RIA, vary from 39% to 93%.[5-8] This range of positivity may be due to the sensitivity of RIA, because strong membrane staining of 2+ and 3+ was seen

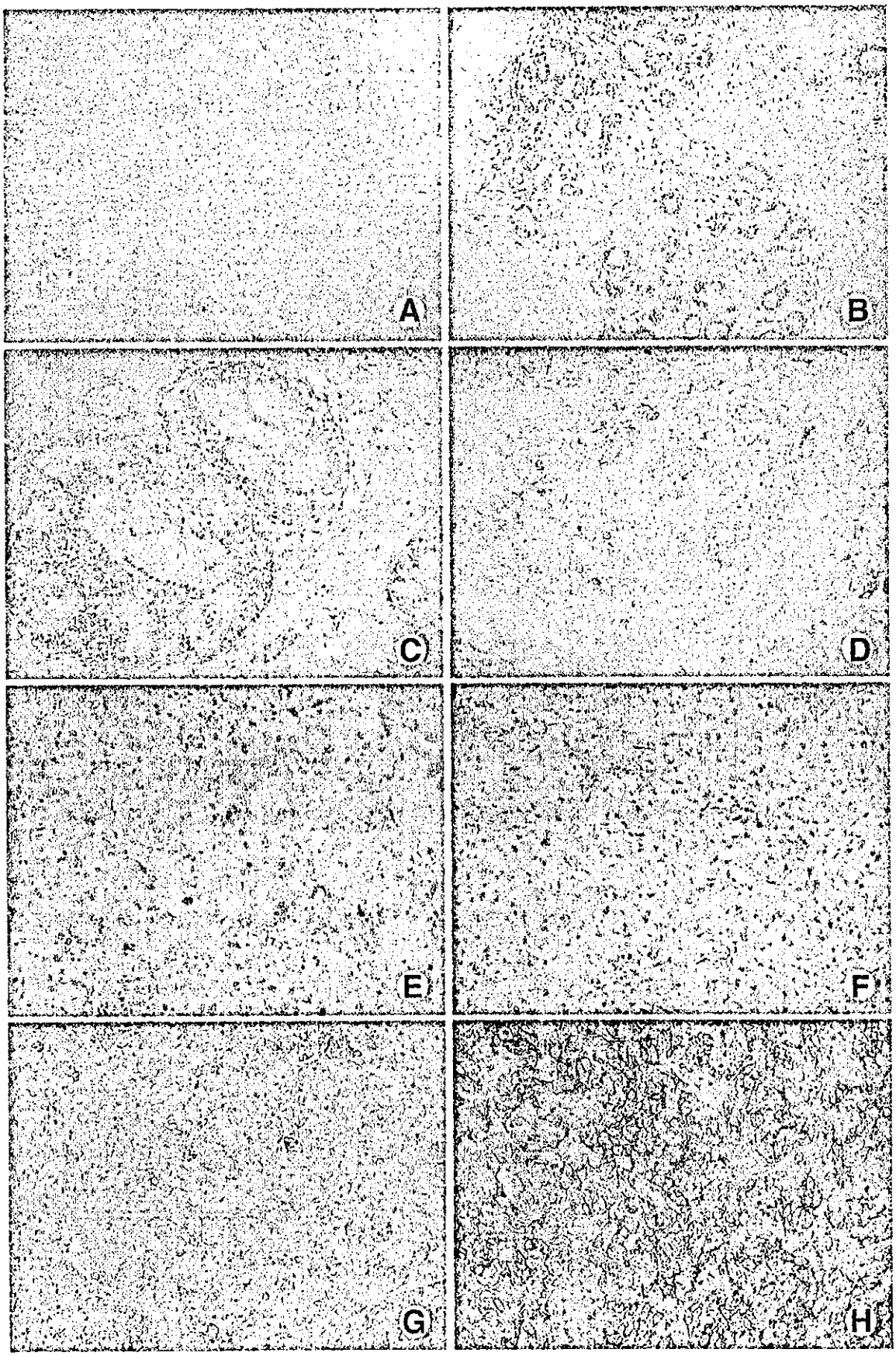

FIGURE 1. Immunohistologic staining of insulin-like growth factor-1 receptor in (A and B) normal epithelium, (C and D) ductal carcinoma in situ, and invasive ductal carcinoma (E-H). IGF-1 receptor expression was scored according to area and intensity of membrane staining (E: score = 0, F: 1+, G: 2+, H: 3+; original magnification, ×100).

TABLE 2. Characteristics of the Patients and Tumors

Parameters	Data
Total	210
Age in yr, range (median)	25-82 (51)
Size of invasion in cm, range (median)	0.1-14.0 (2.2)
Histologic type	
Invasive ductal carcinoma	19
Invasive lobular carcinoma	7
Others	6
Histologic grade	
Grade 1	10
Grade 2	80
Grade 3	120
Axillary lymph node status	
Positive	95
Negative	112
Unknown	3
ER	
Positive	154
Negative	56
PR	
Positive	98
Negative	112
HER2	
0-1	174
2	6
3	30
IGF-1R	
0	24
1	94
2	25
3	67

NOTE. Data are n unless otherwise indicated.

Abbreviations: ER, estrogen receptor, PR, progesterone receptor; HER2, human epidermal growth factor 2; IGF-1R, insulin-like growth factor-1 receptor.

in 43.8%, whereas almost 90% of invasive carcinoma showed moderate staining (scores 1, 2, and 3) in our observation. Happerfield et al[11] reported the localization of IGF-1R staining in benign and malignant fresh-frozen tissue by using monoclonal antibody α-IR3 and found high-intensity labeling in all normal mammary epithelium with an intensity that matches that of carcinomas. They observed membrane, cytoplasmic, and mixed staining patterns, which was concordant with our observation. We scored IGF-1R expression according to the intensity of membranous staining, but the role of cytoplasmic IGF-1R has yet to be clarified.

TABLE 3. Correlation Between Various Factors and IGF-1R IHC score (0/1 vs. 2/3)

Parameters	IHC Score 0/1+	IHC Score 2+/3+	Odds Ratio (95% CI)	Fisher's Exact Test (*P*)
Lymph node status			1.347 (.776-2.337)	.3268
Positive	49	46		
Negative	66	46		
Age (yr)			.932 (.536-1.620)	.8878
<50	51	41		
≥50	67	51		
ER			1.165 (.627-2.165)	.6413
Positive	85	69		
Negative	33	23		
PR			1.174 (.680-2.028)	.5800
Positive	53	45		
Negative	65	47		
HER2			1.032 (.501-2.125)	1.000
0-1	98	76		
2-3	20	16		

Abbreviations: IGF-1R, insulin-like growth factor-1 receptor; IHC, immunohistochemistry; ER, estrogen receptor; PR, progesterone receptor; HER2, human epidermal growth factor 2.

TABLE 4. Univariate Analysis of DFS by Various Clinicopathologic Parameters

Parameters	5-yr DFS (%)	*P* Values
Lymph node status		0.0670
Positive	68.4	
Negative	79.5	
Age (yr)		
<50	78.3	0.6194
≥50	71.2	
Size of invasion (cm)		0.0667
<2.0	84.3	
≥2.0	66.4	
ER		0.0290
Positive	77.3	
Negative	66.1	
PR		0.1269
Positive	83.7	
Negative	66.1	
HER2		0.0483
0-1	78.4	
2-3	47.2	

Abbreviations: DFS, disease-free survival; ER, estrogen receptor, PR, progesterone receptor; HER2, human epidermal growth factor 2.

There are several other reports discussing the prognostic value of IGF-1R expression determined by RIA in primary breast cancer. Findings are contradictory: Foekins et al found no relationship between IGF-1R levels,[4] whereas Bonneterre et al[6] and 2 other groups reported IGF-1R as a favorable prognostic factor.[7,8] Because sensitivity of RIA has wide discrepancy as mentioned earlier, further studies by IHC are warranted.

Ouban et al[12] showed the overexpression of IGF-1R by using anti-IGF-1R polyclonal antibody toward the β-subunit of the human IGF-1R in variety of human carcinomas. Bhatavdekar et al[13] suggested that IGF-1R–negative tumor with concomitant hyperprolactinemia might indicate unfavorable prognosis in advanced colorectal cancer. Some data show prevalence of serum or tumor IGF-BP3 within clinical outcome in malignancy, such as breast and prostate cancer.[14,15] In Ewing sarcoma, there was a trend toward increased survival in a high IGF-BP3 to IGF-1 ratio.[16] Because biology of IGF-1R is regulated by a complex endocrine and paracrine system that involves various humoral and local factors, we should take into account those multiple factors that may affect IGF-1R in future studies.

In this study, there was no correlation between IGF-1R expression and ER, PR, or HER2 expression. In previous clinical studies in breast cancer, IGF-1R expression has been reported to have positive correlation with ER expression.[17] However, ER was not necessarily coexpressed in IGF-1R–overexpressed cells in serial sec-

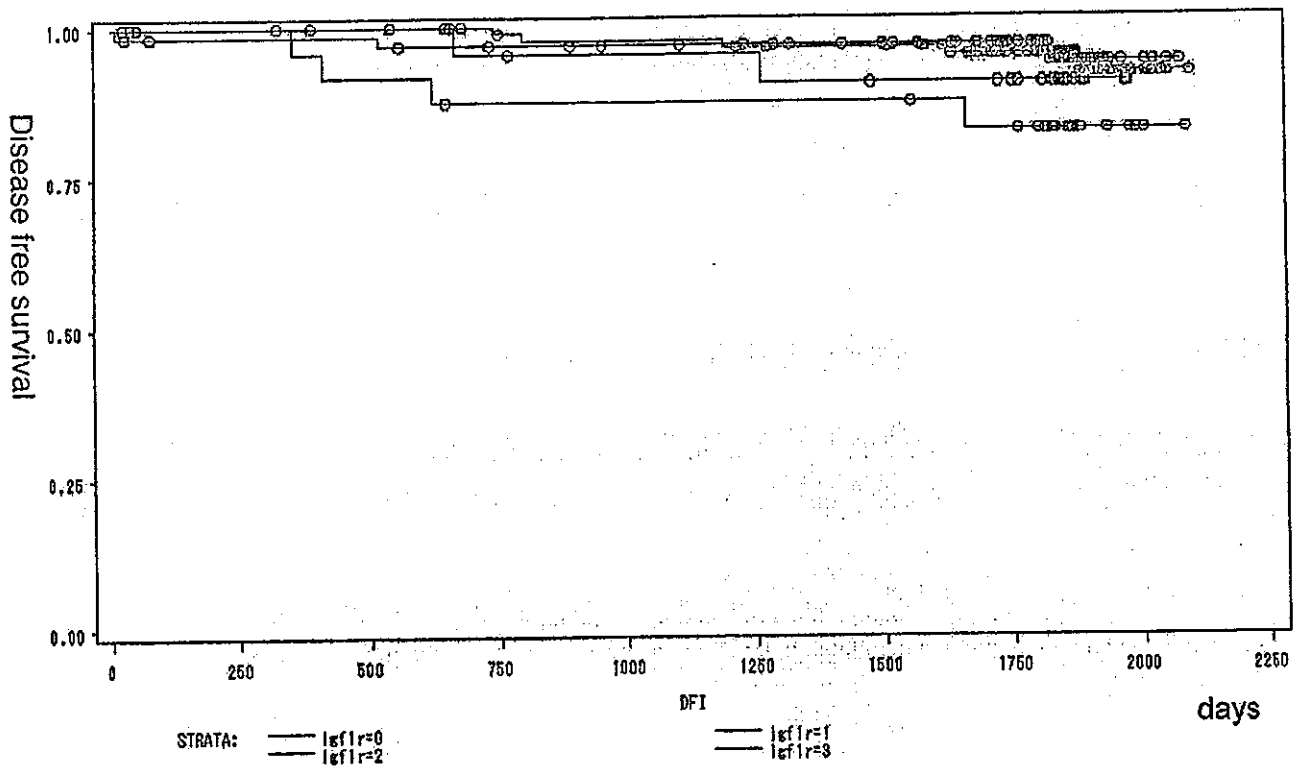

FIGURE 2. Disease-free survival curves for patients group according to Insulin-like growth factor-1 receptor expression.

tions in our study. In cellular experiments, there are growing evidences that support reciprocal interaction between estrogens and IGF-1R or between IGF-1 and ER.[18] In terms of HER2, Balana et al[19] suggested existence of hierarchical interaction between IGF-1R and HER2 in regard to HER2 phosphorylation. Multiple signaling pathways are involved in regulation of breast cancer proliferation, apoptosis and metastasis. Technologies such as cDNA array may be useful in understanding the role of IGF pathways in breast cancer.[20]

Though impact of IGF-1R expression on prognosis seems to be limited, IHC is a clinically useful tool for examining protein expression in archive materials. It also resolves the issues of localization and heterogeneity within the tissue. Moreover, blockade of IGF signaling pathway represents an attractive targeted therapy. Preclinical studies of IGF-1R targeted therapy, such as antisense strategies, have shown promising anti-tumor effect, and some are currently under clinical trials.[21-23] Determination of IGF-1R expression by IHC has potential in clinical use in selecting a particular subset of patients that may benefit from IGF-1R targeted therapy.

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Re: Insulinlike growth factor 1 receptor: predictive factor in breast cancer patients treated with trastuzumab?

To the Editor,

The mechanism of resistance to trastuzumab, either inherent or acquired, is critical in improving the prognosis of HER2-overexpressing metastatic breast cancer patients, but there is limited knowledge from in vitro experiments. Testing a preclinical hypothesis in clinical samples is crucial.

We recently tested the relationship between insulinlike growth factor-1 receptor (IGF-1R) protein expression and the efficacy of trastuzumab in hormone-resistant, chemotherapy-naive, metastatic breast cancer patients, with a hypothesis that IGF-1R overexpression might correlate with trastuzumab resistance [1]. IGF-1R expression was immunohistochemically measured in 26 formalin-fixed paraffin-embedded tissue specimens from primary or metastatic lesions of patients treated with single-agent trastuzumab using a mouse monoclonal antibody clone 24-31 [2]. As a result, patients with higher IGF-1R expression demonstrated a trend toward longer duration of trastuzumab therapy but did not correlate with the clinical response to trastuzumab. Although limited by the nature of a small retrospective study, the result suggests that IGF-1R protein expression itself may not be the major determinant of the resistance to, or efficacy of, trastuzumab in HER2-overexpressing tumors.

Lu et al [3,4] demonstrated in human breast cancer cell lines that an increased level of IGF-1R signaling adversely interferes with the action of trastuzumab on cell growth and that IGF-1R blockade can restore its sensitivity. They observed a dose-response enhancement of trastuzumab-induced growth inhibition by the addition of IGF-BP3, which physiologically interferes with the ligand-receptor interaction of IGF-1 [3]. On the other hand, Nahta et al [5] reported that down-regulation of p27(kip1) may be associated with trastuzumab resistance in breast cancer cells. p27(kip1) is a distal downstream effecter of growth factor pathways including EGFR, HER2, and IGF-1R pathways. It is possible that both upstream regulations of IGF-1R and downstream cross-talk with other signaling pathways are involved in trastuzumab resistance. As Altundag et al [6] state, further studies are necessary to clarify the role of the IGF-1R axis in the biology and therapeutics of HER2-overexpressing breast cancer.

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CASE REPORT

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Second primary osteosarcoma with rosette-like structure in a patient with retinoblastoma

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Abstract A Japanese male patient developed bilateral retinoblastomas at the age of 1 year, but remained continuously disease-free after enucleation of the left eye and radiation therapy to the right eye. He noticed a painless hard mass around the right temporal bone when he was 25 years old. Biopsy specimen showed a small multinodular proliferation of tumor cells with prominent rosette-like structures. Eosinophilic material with focal mineralization was seen in the center of the rosettes. Immunostaining of the tumor cells showed positive reactions for epithelial membrane antigens CD 56 and CD 99. The patient was treated with systemic chemotherapy, and the tumor partially diminished. It is well known that a few osteosarcomas show a rosette-like appearance with production of osteoid in the center, but this is the first case of second primary osteosarcoma with prominent rosette-like features.

Keywords Second primary osteosarcoma · Retinoblastoma · Rosette · Diagnosis

Introduction

Retinoblastoma is a malignant neoplasm in childhood occurring in 1/15,000 to 1/30,000 live births. Improved treatment for retinoblastoma together with early diagnosis has resulted in the cure of an increasing number of patients [17]. It has long been established that children affected by retinoblastoma have a high risk for the development of second primary malignant lesions. The majority of these second primary malignancies are osteosarcomas and soft-tissue sarcomas [1, 11, 14].

Second primary sarcomas after retinoblastoma may be related to prior radiation therapy for the original retinoblastoma [5, 6, 12]. In patients previously irradiated for bilateral or familial unilateral retinoblastoma, 70% of second malignancies have occurred within the radiation field, while 30% occurred outside the field of radiation [1]. Differential diagnosis between these second primary sarcomas and recurrent retinoblastoma may be problematic [19].

Recently, we encountered a 25-year-old Japanese man with a second primary osteosarcoma within the prior radiation field. Peculiarly, this lesion showed rosette-like features that have not been described in previous literature in a second primary osteosarcoma. We, therefore, present here a case requiring special attention during the differential diagnosis of osteosarcoma with rosette-like features from recurrence of retinoblastoma.

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Clinical history

A Japanese male patient was diagnosed with bilateral retinoblastomas at the age of 1 year and underwent enucleation of the left eye and radiation therapy with 49 Gy to the right eye. He had remained continuously disease-free after the treatments. Both his father and mother had a history of retinoblastoma. When he was 25 years old, he noticed a painless hard mass around the right temporal bone. Axial computed tomography showed a well mineralized mass (arrow) on the temporal bone associated with focal subarachnoid hemorrhage (arrowheads, Fig. 1). Axial T1-weighted magnetic resonance (MR) images showed a mass of iso signal intensity relative to white matter. On T2-weighted MR images, the tumor showed heterogeneous and high signal intensity relative to white matter. Multiple fluid levels suggesting the presence of intratumoral hemorrhage were also observed (arrows, Fig. 2A, B). Laboratory data, including peripheral blood count, alkaline phosphatase, and C-reactive protein, were within the normal range. Clinical

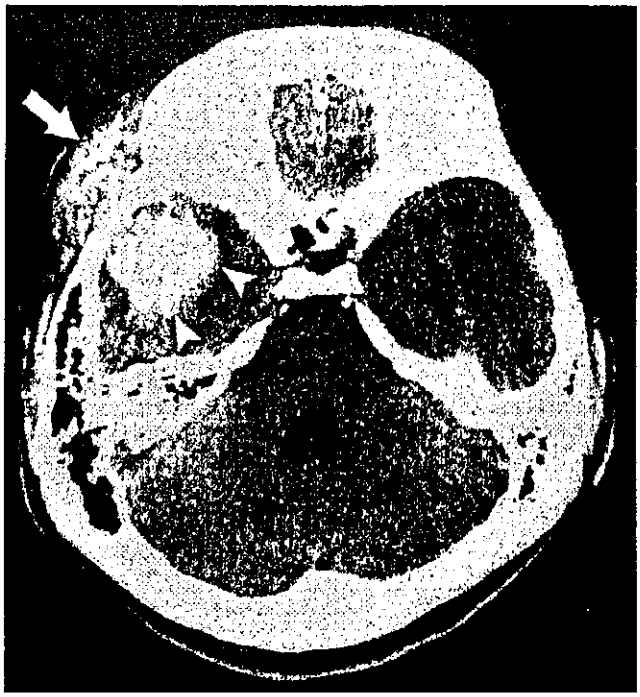

Fig. 1 Axial cranial computed tomography showing a well-mineralized tumor (*arrow*) around the right temporal bone associated with focal subarachnoid hemorrhage (*arrowheads*)

and radiological diagnosis was second primary osteosarcoma after retinoblastoma. A biopsy was performed, and the diagnosis of second primary osteosarcoma with rosette-like features was established. The patient was treated with systemic chemotherapy of high-dose methotrexate, cisplatinum, and adriamycin in March 2001, and the tumor size partially decreased. A surgery of the tumor was not performed, since the tumor was still not resectable after the chemotherapy. The patient has been alive with disease for 43 months after the treatment without any sign of regrowth of the tumor or distant metastases.

Materials and methods

The biopsy specimen was fixed in 10% formalin, embedded in paraffin, and sectioned. One of the 4-mm-thick sections was stained with hematoxylin and eosin. The other serial sections were examined using the labeled streptavidin–biotin method with appropriate use of positive and negative controls throughout, after pretreatment with heat-induced epitope unmasking in a 10-mM citrate buffer, pH 6.0, in an autoclave at 121°C for 10 min. The primary antibodies were applied as follows: cytokeratin (clone AE 1/3, 1:100, Dako, Glostrup, Denmark), CD99 (clone O-13, 1:50, Signet, Dedham, MA), epithelial membrane antigen (EMA, clone E29, 1:100, Dako) and CD56 (clone N-CAM; NCC-Lu-243, 1:200, Nihonkayaku, Tokyo, Japan). In the evaluation of immunostaining for AE1/3, CD99, EMA, and CD56, when there was only homogeneous staining, along with staining of the cell membranes or cytoplasm at the same intensity and pattern as that in the adjacent normal epithelium, the finding was assessed as 2+ positive, while heterogeneous or focal staining was assessed as 1+.

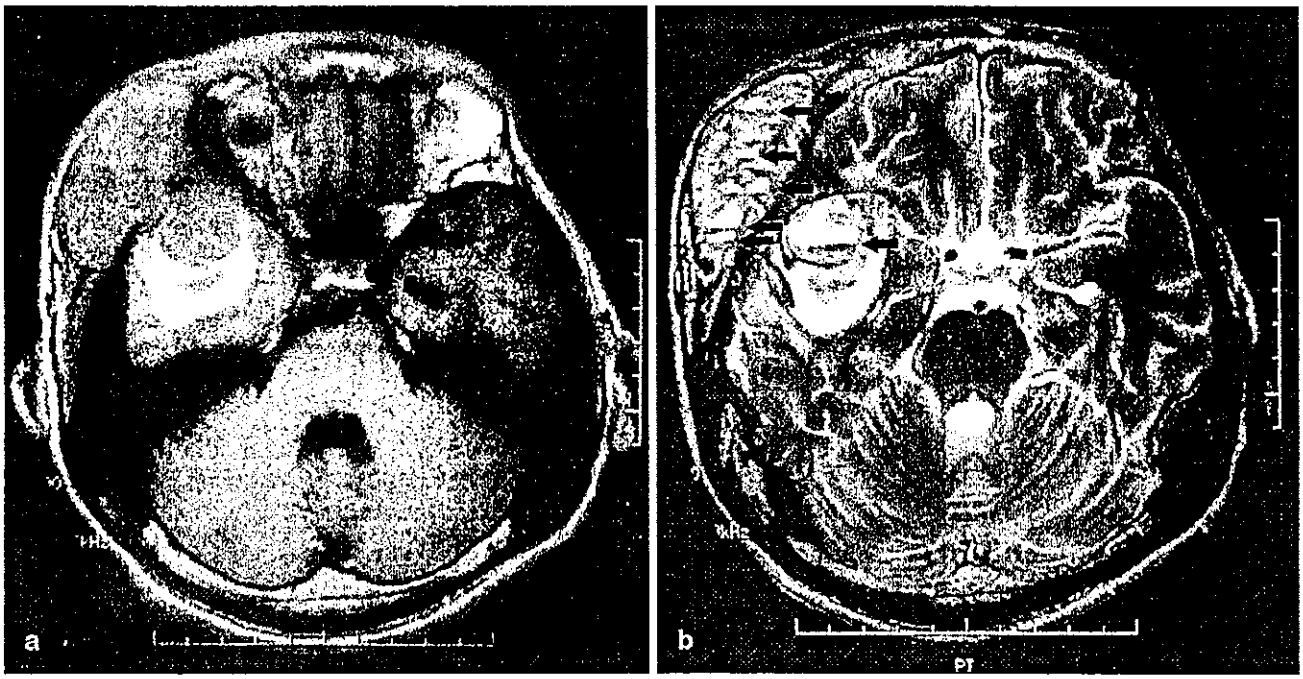

Fig. 2 **a** Axial T1-weighted magnetic resonance (MR) image (TR/TE: 440/7.3 ms) showed a mass of iso signal intensity relative to white matter. Focal subarachnoid hemorrhage corresponded to area of high signal intensity surrounding the tumor. **b** Axial T2-weighted MR image (TR/TE: 4000/118 ms) showed a mass of heterogeneous and high signal intensity relative to white matter. Also noted was the presence of multiple fluid levels within the tumor (*arrows*)

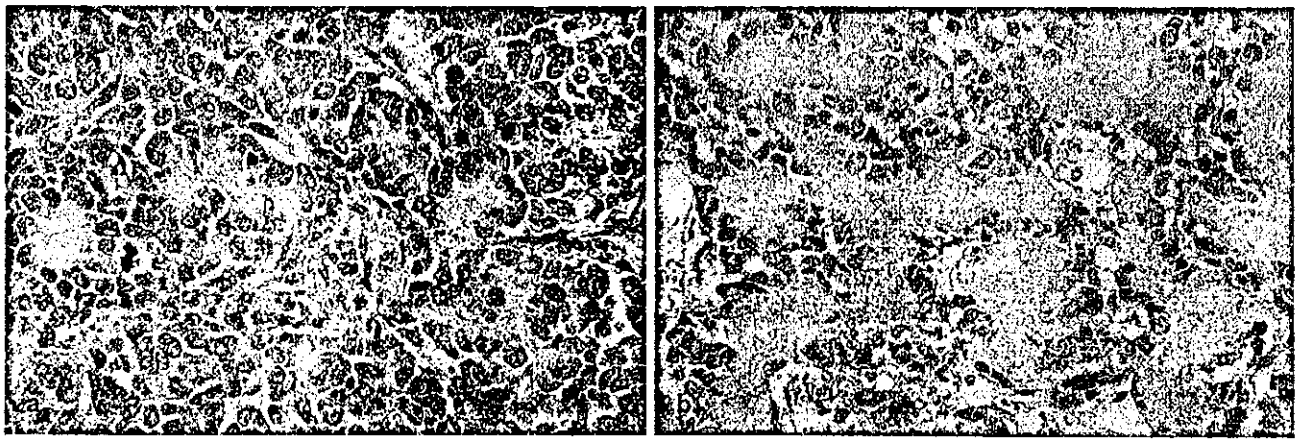

Fig. 3 a Microphotograph of the biopsy specimen showed a proliferation of tumor cells with prominent rosette-like structures. The tumor cells were generally small and round with abundant mitoses. Eosinophilic material was seen in the center of the rosette (×400). b Microphotograph showed conventional lace-like osteoid formation with partial mineralization (×400)

Results

Light microscopic findings

The biopsy showed a small multi-nodular proliferation of the tumor cells. These tumor cells formed a rosette-like structure, and eosinophilic material was seen in the center of the rosette. Focal mineralization was apparent in the eosinophilic material. Between the rosette-like structures, many small vessels proliferated, showing a hemangiopericytoma-like pattern. Under high power, tumor cells were generally small and round with abundant mitoses. In several areas, conventional lace-like osteoid was seen. The rosette-like structures were also observed around the conventional osteoid area. There was no typical Flexner-Wintersteiner rosette in the biopsied specimen (Fig. 3A, B).

Immunostainings

In immunohistochemical features, CD56 was 2+ positive, CD 99 and EMA were 1+ positive (Fig. 4), but cytokeratin was negative.

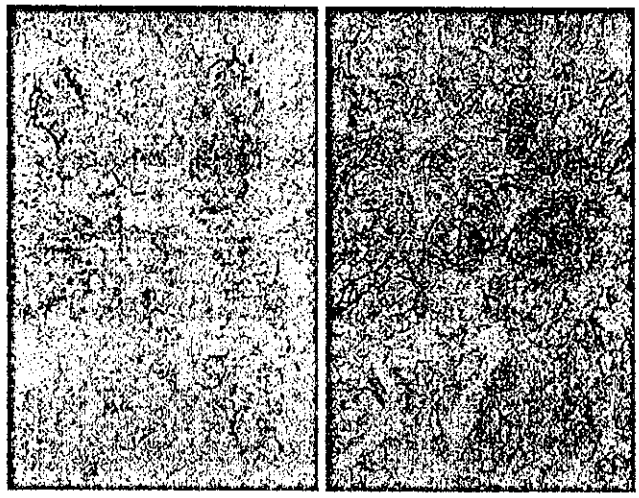

Fig. 4 Immunostaining of the tumor cells with EMA and CD56. The tumor cells showed a characteristic membrane-positive appearance for EMA (left, ×200) and for CD56 (right, ×200)

Discussion

Osteosarcoma is the most common type of second malignancy associated with hereditary retinoblastoma [11, 14]. Hawkins et al. reported that the incidence of osteosarcoma after heritable retinoblastoma is 300 times greater than the risk in the general population [8]. More than 100 cases of second primary osteosarcoma in the irradiated fields in patients with retinoblastoma have been reported in the literature [2, 3, 7, 14]. However, to our knowledge, there has not been any report describing second primary osteosarcoma having rosette-like structures. The rosette-like feature of osteosarcoma was first described in the textbook by Unni in 1996 [23]. In 2002, we reported that several primary osteosarcomas in the extremities showed a rosette-like structure with production of osteoid in the center. This special type of osteosarcoma usually showed a positive immunoreaction for EMA, CD56, and CD99 [15]. The histological and immunohistochemical features described in the literature were identical to those of the current case.

Second primary sarcomas often demonstrate both high-grade and undifferentiated features, making them difficult to distinguish from small, undifferentiated round cell tumors [19]. In addition, the rosette-like structure raises the possibility of recurrent retinoblastoma. However, the long interval (24 years) in the current case between the primary retinoblastoma and development of the second tumor suggests that a recurrence of retinoblastoma is less likely [22]. Furthermore, a definite tumor osteoid

in the center of a rosette-like structure and scattered typical lace-like osteoid, indicating osteosarcoma, and the absence of a typical Flexner-Wintersteiner rosette should be emphasized in the differential diagnosis between osteosarcoma with rosette-like features and recurrent retinoblastoma.

Careful immunohistochemical examinations were useful for distinguishing the current tumor from recurrent retinoblastoma or other small round cell tumors such as rhabdomyosarcoma, Ewing sarcoma/Primitive neuroectodermal tumor (ES/PNET), and metastatic cancer [9]. Positive immunoreactivity for EMA, CD56, CD99, but negativity for cytokeratin in the current case were consistent with those of osteosarcoma with rosette-like features [15]. Positive immunoreaction for EMA would suggest metastatic cancer, but the patient had no cancer history or visceral lesion. Furthermore, negative reaction for cytokeratin indicates that metastatic cancer would be an unlikely diagnosis. It is well known that CD 56 (N-CAM) and CD99 are expressed not only in neural neoplasms or ES/PNET but also in other several kinds of tumors [13, 18, 20]. Definite osteoid formation by tumor cells strongly favored the diagnosis of osteosarcoma.

Little is known in the literature regarding the relationship between treatment of the second primary osteosarcoma and prognosis [2, 4, 10, 16, 19]. Recently, encouraging results have been achieved with adjuvant chemotherapy and/or aggressive surgery [2, 21]. The current patient has remained alive with disease after effective treatment by systemic chemotherapy without any regrowth of the tumor or distant metastasis for 43 months. Similarly, in the review of the other four cases registered in the National Cancer Center in Tokyo (data not shown), three died of disease, but one patient was alive with disease for 99 months after systemic chemotherapy. However, our previous study showed that rosette-like feature in primary osteosarcoma is an adverse prognostic factor [15]. Further study is required with regard to prognosis of osteosarcoma with this specific background and histology.

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CASE REPORT

Extramedullary myeloid tumour (EMMT) of the gallbladder

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J Clin Pathol 2005;58:211–213. doi: 10.1136/jcp.2004.019729

This report describes a rare case of an extramedullary myeloid tumour (EMMT) of the gallbladder in a patient without leukaemia. A 33 year old man visited a local hospital because of jaundice. Abdominal computed tomography revealed a tumorous mass measuring 6.0 × 4.5 cm and involving the entire gallbladder. A percutaneous needle biopsy was attempted, but because adenocarcinoma could not be completely ruled out, the use of undue force was considered dangerous. Under a preoperative diagnosis of gallbladder carcinoma, a hepatopancreatoduodenectomy was performed. The tumour cells exhibited various amounts of eosinophilic cytoplasm, had medium sized round nuclei with indentation and grooving, and were strongly immunoreactive for myeloperoxidase, CD43, and c-kit protein (CD117). After surgery, the patient underwent combination chemotherapy as prescribed for cases of acute myeloblastic leukaemia. The patient did not develop acute leukaemia during a follow up period of four years. In conclusion, a correct diagnosis of EMMT can be made using appropriate immunohistochemical staining.

Extramedullary myeloid tumour (EMMT), otherwise termed granulocytic sarcoma or chloroma, is a rare extramedullary tumour composed of immature cells of the myelomonocytic series. Males and females are equally affected, with a mean age of 48 years (range, 2–81).[1] About 70% of reported cases are in patients with acute myelogenous leukaemia, chronic myelogenous leukaemia, or other myeloproliferative diseases, but in the remaining 30% no known underlying disease has been noted at the time of diagnosis.[1] The most common sites of involvement are the skin, lymph nodes, and bone, although other organs have been implicated. A large proportion (75–86%) of EMMTs in non-leukaemic patients are initially misdiagnosed.[2] An EMMT developing in the gallbladder of a patient without leukaemia is extremely rare. Geddy and Wedgwood reported a case of myelofibrosis of the gallbladder,[3] but unlike our case, lesions outside the gallbladder were recognised in the bone marrow. Here, we report a rare case of EMMT of the gallbladder, detailing the clinicopathological and immunohistochemical features of this entity, which was accurately diagnosed and has been followed up for a long period.

"A large proportion (75–86%) of extramedullary myeloid tumours in non-leukaemic patients are initially misdiagnosed"

CASE REPORT

A 33 year old man visited a local hospital because of jaundice. He was diagnosed as having gallbladder carcinoma based on a radiographic examination, and was referred to the National Cancer Centre, Tokyo, Japan. Laboratory data indicated that carcinoembryonic antigen, CA19-9, and elastase concentrations were within normal limits, and that T-bilirubin (51 mg/litre), D-bilirubin (37 mg/litre), alkaline phosphatase (688 IU/litre), glutamic oxaloacetic transaminase (84 U/litre), glutamic pyruvate transaminase (317 U/litre), lactate dehydrogenase (431U/litre), and the white blood cell count (9.6×10^9/litre) were slightly raised.

Abdominal computed tomography imaging showed partial infiltration of the tumour into the gallbladder wall. We tried to perform a percutaneous needle biopsy, but because adenocarcinoma could not be completely ruled out the use of undue force was considered dangerous. We performed a cytological examination which was unable to provide definitive information on the lesion. The preoperative diagnosis was a malignant neoplasm that probably originated from the neck of the gallbladder, cystic duct, or common bile duct. However, it is unusual for a carcinoma to grow so large without showing signs of invasion of the liver and portal vein (fig 1A). The differential diagnosis was an extranodal malignant lymphoma, and a hepatopancreatoduodenectomy was performed.

Macroscopically, the gallbladder lumen was filled with blood and degenerative tissue, and the cut surface of the tumour had a nodular, well circumscribed, glistening appearance (fig 1B). The tumour measured 6.0 × 4.5 cm at its maximum diameter.

Microscopically, the tumour cells had various amounts of eosinophilic cytoplasm and medium sized round nuclei with indentation and grooving. They were arranged in a trabecular to sheet-like pattern within the thin fibrous septa (fig 2A), and did not show a cohesive growth pattern. The tumour cells had invaded the muscular layer of the gallbladder, but most of the gallbladder epithelium was intact (fig 2B). Tumour invasion was seen in the cystic duct, common bile duct, portal vein, part of the liver parenchyma, hepatoduodenal ligament, omentum, part of the muscular layer of the transverse colon, and duodenum. The histological differential diagnosis for such small round cell tumours included undifferentiated carcinoma, small cell carcinoma, Ewing's sarcoma, rhabdomyosarcoma, monophasic synovial sarcoma, nephroblastoma, and haemopoietic tumour.

Upon immunohistochemical examination, the tumour cells showed diffuse and strong reactivity for myeloperoxidase (MPO), CD43, and c-kit protein (CD117) (fig 2A–C), and weak reactivity for CD45 (LCA) and CD99. The cells were negative for CD20, CD79a, CD3, CD34, CD45RO (UCHL-1), CD68, CD56, terminal deoxynucleotidyl transferase, WT1, desmin, vimentin, and cytokeratins (CAM 5.2, AE 1/3, and KL1). Based on these results, we made a final diagnosis of EMMT.

After surgery, the patient underwent combination chemotherapy as prescribed for cases of acute myeloblastic leukaemia, but clinical investigations—including computerised tomography and a bone marrow trephine biopsy

Abbreviations: EMMET, extramedullary myeloid tumour; MPO, myeloperoxidase

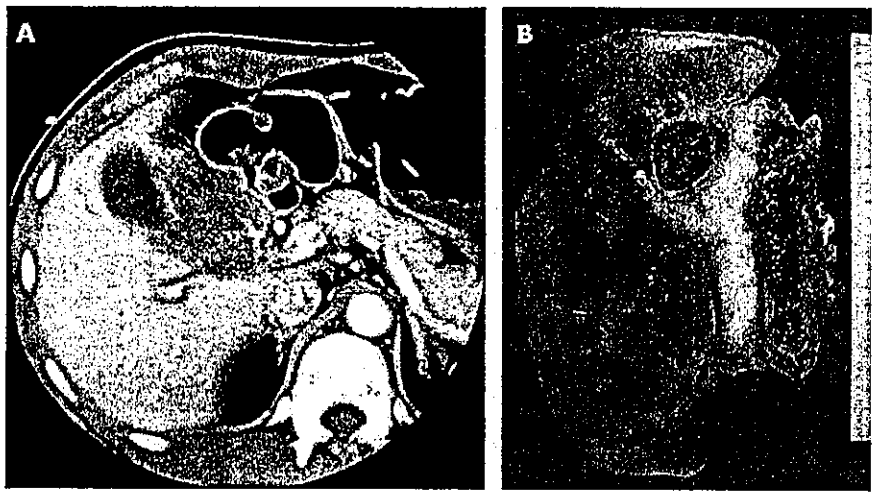

Figure 1 (A) Abdominal computerised tomography image showing partial infiltration of the tumour into the gallbladder wall but no sign of invasion of the liver or portal vein. (B) Macroscopically, the gallbladder lumen is filled with blood and degenerative tissue, and the cut surface of the tumour has a nodular, well circumscribed, glistening appearance.

specimen—did not detect more lesions. The patient did not develop acute leukaemia during a follow up period of four years.

DISCUSSION

A large proportion (75–86%) of EMMTs in non-leukaemic patients are initially misdiagnosed[3] because of their morphological and immunohistochemical similarity to other small round cell tumours. Most of them are diagnosed as malignant lymphoma, and occasionally as Ewing's sarcoma or eosinophilic granuloma.[4]

> "In cases of suspected extramedullary myeloid tumour, antibodies to myeloperoxidase and CD43 should be used, along with other B cell and T cell specific antibodies"

Some small round cell tumours, such as undifferentiated carcinoma and small cell carcinoma, usually show aggressive invasion, but in our case the lesion had not invaded the portal vein or the gallbladder epithelium, and staining for cytokeratins was negative. Other small round cell tumours, such as Ewing's sarcoma, rhabdomyosarcoma, monophasic synovial sarcoma, and nephroblastoma, were also eliminated because of negative immunostaining for desmin, cytokeratins, and WT1, respectively. Malignant lymphomas may be positive for CD45 (LCA) along with either pan B cell (CD79a, L-26) or T cell (CD3) markers, but are negative for MPO, which was positive in our case.

Immunohistochemical markers, such as CD43 and MPO, may be helpful in the diagnosis of EMMT. However, a few cases of EMMT show reactivity for pan B cell (CD79a, L-26) or T cell (CD3) markers. Furthermore, CD43 is an excellent

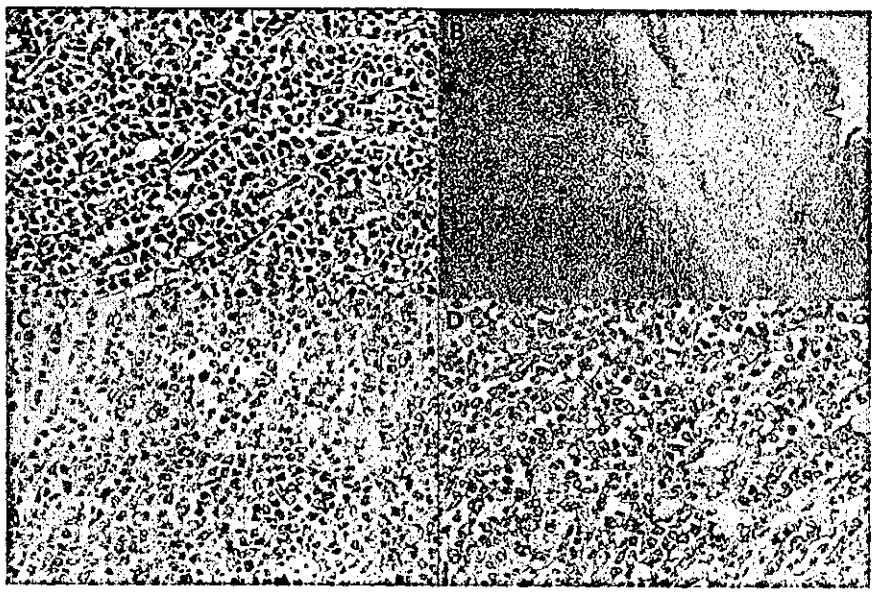

Figure 2 (A) The tumour cells have various amounts of eosinophilic cytoplasm and are arranged in a trabecular to sheet-like pattern (haematoxylin and eosin (H&E) stain; original magnification, ×400). (B) Most of the gallbladder epithelium is not involved (H&E stain; original magnification, ×40). The tumour cells show diffuse and strong reactivity for (C) myeloperoxidase and (D) CD43 (original magnification, ×400).

Take home messages

- We present a rare case of extramedullary myeloid tumour of the gallbladder in a non-leukaemic patient that was diagnosed successfully using an appropriate panel of immunohistochemical stains
- The patient underwent combination chemotherapy after diagnosis and has not developed leukaemia after four years of follow up
- It is important not to misdiagnose such cases as malignant lymphoma, because the pathological diagnosis influences the prognosis of the patient

marker for myeloid cells and is also used as a T cell marker.[3] If myeloid associated antigens are not investigated, a misdiagnosis of malignant lymphoma is likely. Therefore, in cases of suspected EMMT, antibodies to MPO and CD43 should be used, along with other B cell and T cell specific antibodies.

Recently, c-kit tyrosine kinase inhibitors (such as Glivec) have been studied as possible treatments for haemopoietic malignancies, so that c-kit detection may have important implications for treatment. Jian *et al* reported c-kit reactivity in 87% of EMMT cases,[5] and many tumours have been shown to express CD117/KIT as assessed by the anti-KIT polyclonal antibody (DakoCytomation, A4502; Glostrup, Denmark) used in our laboratory, which has low specificity. Although tumours carrying mutations of either c-kit or the platelet derived growth factor α gene (for example, gastrointestinal stromal tumours) are sensitive to tyrosine kinase inhibitors, in the absence of an accompanying mutation other KIT positive tumours show no therapeutic benefit with Glivec.[6] We were unable to isolate and sequence the c-kit gene from formalin fixed, paraffin wax embedded tissue sections of our present case.

Most non-leukaemic patients with EMMT develop acute leukaemia one to 49 months after the diagnosis (mean, 10.5).[1] If the case is correctly diagnosed as EMMT at the initial presentation, and the patient receives intensive systemic chemotherapy with or without radiotherapy, longterm survival is good.[7] Aggressive forms of treatment are necessary for patients who present with acute myeloid leukaemia after being treated with chemotherapy for non-Hodgkin lymphoma as a result of initial misdiagnosis. These patients frequently fail to attain durable remission and have a poor prognosis.[2]

In summary, we present a rare case of EMMT of the gallbladder in a non-leukaemic patient that was diagnosed successfully using an appropriate panel of immunohistochemical markers. Care should be taken not to misdiagnose such cases as malignant lymphoma, because the pathological diagnosis influences the prognosis of the patient.

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Case Reports

CD56-positive Small Round Cell Tumor: Osseous Plasmacytoma Manifested in Osteolytic Tumors of the Iliac Bone and Femora

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Monoclonal gammopathy of undetermined significance does not overexpress cluster of differentiation (CD) 56, but plasma cell myeloma frequently overexpressed it. However, plasma cell leukemia and extramedullary plasmacytoma usually down-regulate CD56 expression. Plasmacytoma, especially 'solitary plasmacytoma of bone', is difficult to diagnose as plasma cell neoplasm, because it occasionally appears similar to other bone tumors, both clinically and pathologically, and is rarely accompanied by monoclonal protein in the serum or urine. The present case was a patient with an osteolytic 'small round cell tumor' of the iliac bone, which also invaded the femora. An immunohistopathological finding of CD56 expression played a key role in making a diagnosis. The definitive diagnosis of plasmacytoma was made based on the electron microscopic findings. The plasma cells which infiltrated her sternum showed the same restriction to kappa light chain expression in their cytoplasms as that of the iliac bone tumor cells, but did not express CD56. Locally infiltrating osteolytic bone tumors should be examined for surface immunoglobulin light chains as well as CD56 expression when plasmacytoma is suspected.

Key words: CD56 – osseous plasmacytoma – osteolytic bone tumor – plasmacytoma – SBP

INTRODUCTION

'Plasmacytoma' is one of the plasma cell neoplasms locally infiltrating bones or spreading to extramedullary sites from the bones (1). The new World Health Organization (WHO) criteria defines solitary plasmacytoma of bone (SBP) as 'a localized bone tumor consisting of plasma cells identical to those seen in plasma cell myeloma, which appears as a solitary lytic lesion on radiological examination' (1). Electrophoreses of serum and urine samples in patients with SBP tend to reveal monoclonal proteins less frequently than plasma cell myeloma, and clinical diagnosis of SBP is generally difficult in such cases without monoclonal proteins (2). Previous reports showed that 65–78% of cases with plasma cell myeloma strongly expressed cluster of differentiation (CD) 56, but patients with monoclonal gammopathy of undetermined significance (MGUS) seldom showed CD56 expression (3,4). On the other hand, plasma cells obtained from patients with plasma cell leukemia (PCL) were reported to lack or barely express CD56 in either the peripheral blood (PB) or the bone marrow (BM) (5). Myeloma cells obtained from extramedullary sites revealed absence of CD56, although those of BM expressed CD56 in the same patients (6). Furthermore, CD56-negative multiple myeloma had a higher incidence of extramedullary disease, a plasmablastic morphology, and poor prognosis (7). The present case was one in which the CD56 expression played a key role in reaching a definitive diagnosis of plasma cell neoplasm.

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CASE REPORT

In February 2000, a 57-year-old female suffered from severe pain in her buttocks. She had been to a chiropractor but her pain had not been improved. In December 2000, she was referred to a hospital, where an iliac bone biopsy was performed. The histopathological diagnosis was a 'small round cell tumor'. In January, 2001, she was referred to and admitted to our hospital. Computerized tomography (CT) revealed an extensive osteolytic tumor in her right iliac bone (Fig. 1). Magnetic resonance imaging (MRI) also showed a bone

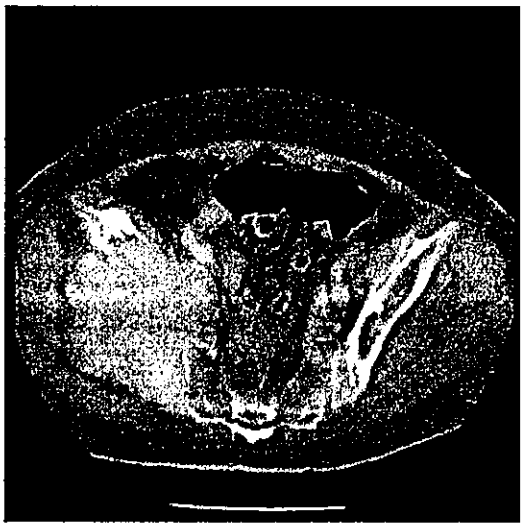

Figure 1. CT showing the osteolytic iliac bone tumor.

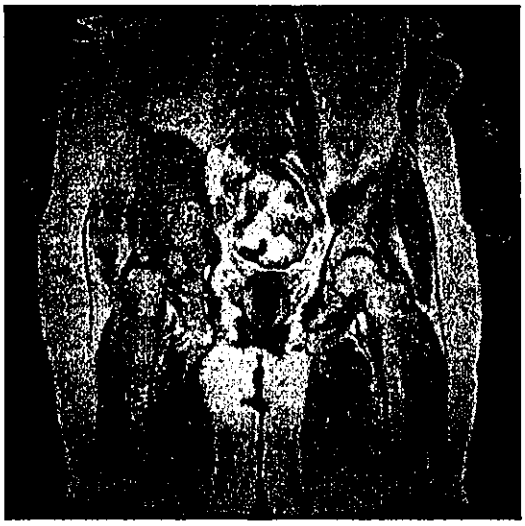

Figure 2. MRI showing the iliac bone tumor involving the femoral bones.

tumor in her right iliac bone and osteolytic lesions at the necks of her bilateral femora (Fig. 2). She had been lying in bed with such severe pain on her right hip joint that she required a lumbar subdural morphine infusion. Re-biopsy of her iliac bone tumor was performed and hematoxylin–eosin staining of the specimen showed the diffuse proliferation of condensed small round cells (Fig. 3). Although the tumor cells had scant cytoplasms, their nuclei appeared rather eccentric. Her blood tests were almost normal except for a low hemoglobin level of 10.2 g/dl and a slightly high calcium concentration of 10.9 mg/dl. As for the levels of immunoglobulins, only IgA was abnormal and slightly declined to 92 mg/dl [normal range (NR), 107–390 mg/dl]. A bone marrow aspiration of her sternum revealed 4% of plasma cells. Her findings did not meet any

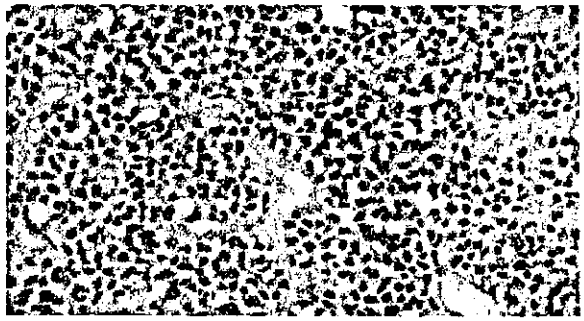

Figure 3. Hematoxylin–eosin staining of the iliac bone tumor specimen which shows a small round cell tumor (600×).

major diagnostic criteria of the Southwest Oncology Group for multiple myeloma (8). Immunohistochemical analysis of her iliac bone tumor specimen showed neither epithelial markers such as cytokeratin or epithelial membrane antigen nor the markers for neural differentiation such as neuron-specific enolase, CD57 (Leu7), synaptophysin or neurofilament. Hematopoietic cell markers such as leukocyte common antigen (CD45), CD34, terminal deoxynuceotidyl transferase, myeloperoxidase, CD3 and CD79a were not expressed, but CD56 was expressed (Fig. 4A). In February 2001, the pain worsened, because the head of her right femur was dislocated into the acetabular fossa necessitating the towing of her right femur. She had become progressively worse and it was suggested that there was a strong possibility of either malignant lymphoma or plasma cell neoplasm. Therefore, we started chemotherapy with 750 mg/m^2 cyclophosphamide, 50 mg/m^2 doxorubicin, and 1.4 mg/m^2 vincristine i.v., and 100 mg prednisolone orally for five consecutive days.

The study by Van Camp et al. (3), which concluded that strong CD56 expression was common in multiple myeloma, suggested that there was a great likelihood of plasma cell neoplasms. We therefore performed additional immunohistochemical stainings of the specimens, and plasmacytoma was confirmed by the positive immunostaining in the cytoplasms of tumor cells with an anti-kappa light chain antibody (Fig. 4B), but negative with an anti-lambda antibody (data not shown). We re-performed bone marrow aspiration and plasma cells from her sternum were clearly distinguished from other BM cells or lymphoid cells of her sternum on two-color cytogram (performed with EPICS XL-MCL flow cytometer, Beckman Coulter Inc., Miami, FL) with a fluorescein isothiocyanate (FITC)-anti-CD38 antibody staining (Immunotech, a Beckman Coulter Company, Marseille, France). The result revealed an increased ratio of the cytoplasmic kappa/lambda chains [32.2 (61.1%/1.9%)]. Furthermore, the electron microscopic examination of her iliac bone tumor cells showed abundant rough endoplasmic reticula with regular parallel arrays, which were consistent with plasma cells (Fig. 5). The immunohistochemical analysis of the plasma cells in the clot section of her sternal BM showed the same pattern of the restricted kappa chain expression as that of the tumor specimen obtained from

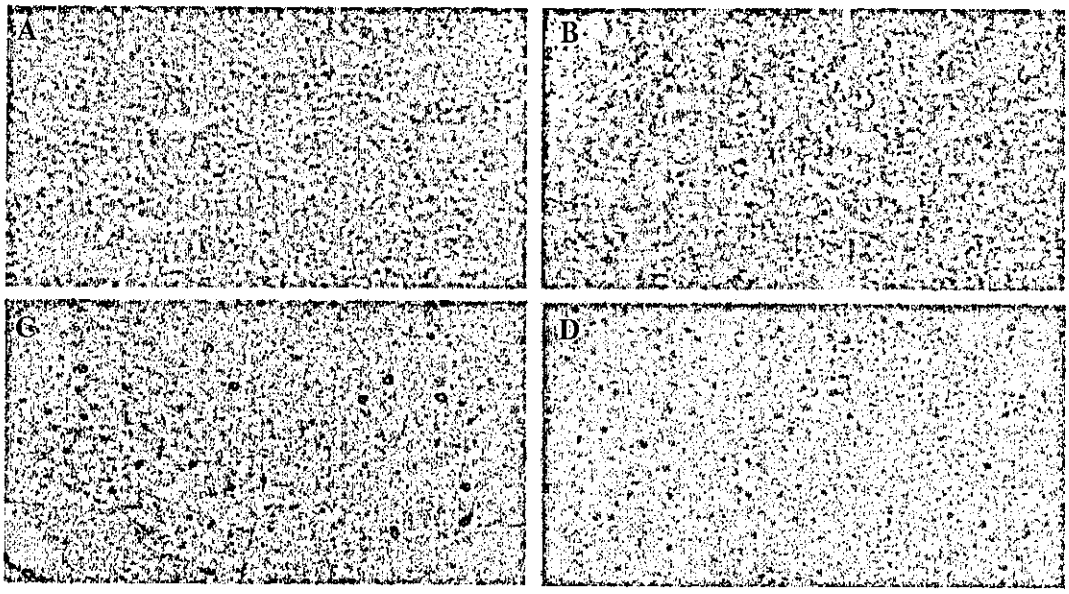

Figure 4. Immunohistochemical findings of the iliac plasmacytoma and the plasma cells in the sternal bone marrow (600×). Plasma cells in the iliac tumor showed CD56 expression (A). Plasma cells in the tumor specimen obtained from the iliac bone showed the restricted kappa chain expression (B). The clot section of the sternum also showed the same pattern of restricted kappa chain expression (C). Interestingly, plasma cells in the sternum lacked CD56 expression (D).

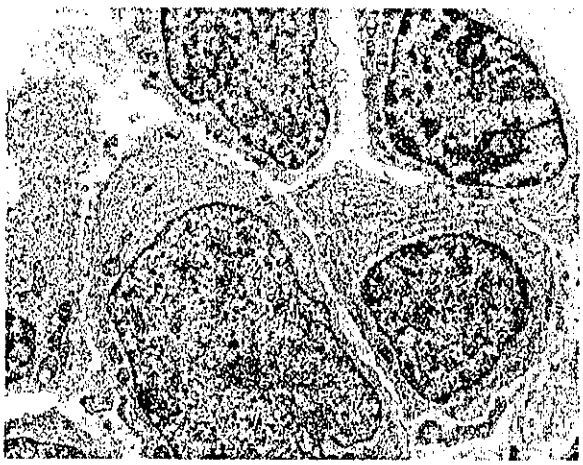

Figure 5. Electron micrograph showing the infiltration of "granular endoplasmic reticulum"-rich cells that were consistent with plasma cells.

her iliac bone (Fig. 4C). However, the plasma cells in her sternum lacked CD56 expression (Fig. 4D). Additional serum examination showed 2.58 mg/l of β2-microglobulin (NR 0.92–1.40 mg/l). Although ordinary urine examination showed no protein, immunoelectrophoresis of her concentrated urine disclosed kappa type of Bence–Jones protein (BJP).

After six courses of the described chemotherapy, partial remission was obtained. She underwent surgery for residual tumor resection in the iliac bone and artificial hip–femur joint replacement. The resected specimen revealed fibrotic and bleeding tissue without any residual tumor. The postoperative recovery was uneventful and she recovered mobility with the use of a wheelchair through rehabilitation.

DISCUSSION

When a bone tumor is encountered which is histopathologically diagnosed as 'small round cell tumor', the differential diagnosis includes Ewing sarcoma, osteosarcoma of small cell type, malignant lymphoma, plasmacytoma, metastatic small cell lung cancer and so on. To make a definitive diagnosis, immunohistochemistry is usually necessary in addition to hematoxylin–eosin staining. As for non-Hodgkin's lymphoma (NHL), some anaplastic large cell lymphomas (9) as well as NK/T-cell lymphomas have been reported to express CD56 (10). However, CD30 was negative and the sites frequently involved in NK/T-cell lymphoma were not affected in the present case. Although some tumor cells of osteosarcoma also show positive immunoreactivity for CD56 (11), the present case had CD56-positive cells in her iliac bone tumor and this was a clue to carrying the investigation forward to reach a definitive diagnosis. In plasma cell neoplasms, it has been reported that CD56 expression is common in plasma cell myeloma, but normal plasma cells of healthy volunteers and benign plasma cells of MGUS were negative for CD56 (3,4). On the other hand, expression of CD56 was down-regulated in PCL (5) and extramedullary plasmacytoma (6). CD56-negative myeloma is characterized by higher levels of β2-microglobulin, BJP, renal insufficiency, thrombocytopenia, plasmablastic morphology and high incidence of extramedullary disease (7). The level of serum β2-microglobulin in this case was not so high, and the tumor cells showed very small round shapes without blastic appearance. Renal function was intact and platelet counts were within normal limits. CD56 is a neural cell adhesion molecule (NCAM), one of the recognition molecules which operate via both homophilic (NCAM

to NCAM) and heterophilic (NCAM to heparan sulfate proteoglycan and various other collagens) binding mechanisms. During embryogenesis, this molecule is down-regulated during migrating events but re-expression usually occurs when target organs are reached (12).

Furthermore, comparing the presence of lytic bone lesions on radiography, Pellat-Deceunynck et al. (5) reported that 80% of patients with plasma cell myeloma which expressed CD56 had one or more lytic bone lesions but that they were found only in 44% of patients who lacked or weakly expressed CD56. Ely and Knowles (13) also reported that strong expression of CD56 by plasma cells correlated with the presence of lytic bone regions and strong CD56 expression by osteoblasts in BM. Although the levels of CD56 expression on plasma cells of SBP have not been reported, they were supposed to be high according to the above-mentioned reports. Although the present case with femoral invasion does not fit with the strict definition of SBP as described in the new WHO classification (1), it is suggested that such an 'osseous plasmacytoma' as an osteolytic, relatively localized plasmacyotma with CD56 expression is the opposite manifestation of plasma cell neoplasms to PCL or extramedullary myeloma. Plasma cells in the osseous plasmacytoma that overexpress CD56 might adhere to and proliferate in BM. Homophilic interactions among myeloma cells through CD56 might facilitate a mass formation of plasma cells. Moreover, the destruction of bone trabeculae can be attributable to heterophilic interactions between plasma cells and osteoblasts through CD56 (13). CD56 seemed to be up-regulated and down-regulated during development of plasma cell dyscrasia and changed its character according to the relationship of myeloma cells to BM.

CONCLUSION

"Osseous plasmacytoma" might present itself histopathologically as "small round cell tumor" and often remains undiagnosed if only hematoxylin–eosin staining is performed. In addition, low levels of the serum or urine monoclonal protein make its clinical diagnosis difficult. From previous observations and the present finding, the level of CD56 expression is suggested to be high in "osseous plasmacytoma". Therefore, locally infiltrating osteolytic bone tumors should be examined for surface immunoglobulin kappa and lamda light chains as well as CD56 expression when "osseous plasmacytoma" is suspected.

Acknowledgments

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ORIGINAL ARTICLE

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Differential diagnosis of gastrointestinal stromal tumor and other spindle cell tumors in the gastrointestinal tract based on immunohistochemical analysis

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Abstract To confirm the usefulness of an immunohistochemical panel of antibodies for KIT (c-kit/CD117), CD34, desmin, smooth-muscle actin (SMA), h-caldesmon (HCD), S-100 protein, neuron-specific enolase (NSE), and beta-catenin, 297 mesenchymal and peripheral nerve-sheath tumors of the gastrointestinal tract and intra-abdominal locations including 211 gastrointestinal stromal tumors (GISTs), 12 leiomyomas, 18 leiomyosarcomas, 17 solitary fibrous tumors (SFTs), 14 schwannomas, and 25 desmoid-type fibromatoses (DTFs) were analyzed immunohistochemically. Consistent (100%) immunoreactivity for KIT, CD34, desmin and S-100, and nuclear accumulation of beta-catenin were detected in GISTs, SFTs, smooth-muscle tumors, schwannomas, and DTFs, respectively. Immunoreactivity for SMA, HCD, and NSE was observed in a wide range of these tumors. In addition, 418 bone and soft tissue tumors were enrolled in this study for KIT immunostaining. As a result, a limited number of these tumors were KIT positive, including synovial sarcoma that showed morphological similarity to GISTs. These findings suggest that KIT, CD34, desmin, S-100, and beta-catenin are key markers for clinical diagnosis of GISTs and other spindle cell tumors that may involve the gastrointestinal tract, whereas SMA, HCD, and NSE have only limited value.

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Keywords Gastrointestinal stromal tumor (GIST) · Spindle cell tumor · KIT · Beta-catenin · S-100 · Soft tissue sarcoma

Introduction

The primary mesenchymal and peripheral nerve sheath tumors arising in the gastrointestinal tract of adults form a large, diverse group that differ in their histological features and clinical behavior, but sometimes pose diagnostic problems. Gastrointestinal stromal tumors (GISTs) comprise the majority of this tumor group. GIST is an entity that was defined recently on the basis of its ultrastructural and immunohistochemical similarity to the interstitial cells of Cajal [22, 27, 46]. Immunohistochemically, most GISTs are KIT (c-kit proto-oncogene protein) positive, and c-kit tyrosine kinase is rendered constitutively active by mutations [17, 23, 25, 26, 31, 44, 47]. Application of c-kit tyrosine kinase inhibitor STI-571 (Glivec, imatinib) to the treatment of GISTs has proved to be promising for clinical management [21, 56]. Accordingly, it has become more important for pathologists to diagnose GISTs accurately.

Although numerous studies have evaluated various pathological and immunohistochemical parameters in an attempt to identify GISTs specifically [10, 11, 35, 36, 37, 40, 41, 43, 58, 59], there have been no systematic studies that attempt to distinguish GISTs from other spindle cell tumors arising in the gastrointestinal tract and intra-abdominal locations. The present study was conducted to confirm the usefulness of an immunohistochemical panel of antibodies for KIT, CD34, desmin, smooth-muscle actin (SMA), h-caldesmon (HCD), S-100 protein, neuron-specific enolase (NSE), and beta-catenin, which might be used to clinically diagnose GISTs and other spindle cell tumors that can arise in the gastrointestinal tract. In addition, as relatively little is known about expression of KIT in other mesenchymal tumors, we studied its ex-