

Utility of the immunohistochemical detection of FLI-1 expression in round cell and vascular neoplasm using a monoclonal antibody

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FLI-1 nuclear transcription factor has been proposed as a useful tool in the differential diagnosis of small round cell sarcomas. Recently, FLI-1 has been reported as the first nuclear marker of endothelial differentiation. However, its clinical use has been hampered by major interpretation problems, due to the presence of background staining as well as staining variation between different lots of the same antiserum. In this study, a novel monoclonal antibody raised against the carboxyl terminal of the FLI-1 protein (clone GI146-222, BD Pharmingen) was tested in a series of small round cell and vascular neoplasms. Furthermore, in order to assess FLI-1 specificity, we analyzed its expression in a series of common epithelial and nonepithelial malignancies. In total, 15 Ewing's sarcomas, 10 rhabdomyosarcomas, 5 desmoplastic small round cell tumors, 10 synovial sarcomas, 10 high-grade pleomorphic sarcomas, 10 malignant melanomas, 5 Merkel's carcinomas, 10 colonic adenocarcinomas, 10 breast carcinomas, 10 lung adenocarcinomas, 20 angiosarcomas, 5 epithelioid hemangioendotheliomas, 10 Kaposi's sarcomas and 10 benign hemangiomas, were stained. A strong FLI-1 immunoreactivity was detected in all Ewing's sarcomas and vascular neoplasms, highlighting the high sensitivity of FLI-1 monoclonal antibody. However, 2/5 Merkel's carcinomas and 1/10 malignant melanomas showed a strong nuclear immunostaining, suggesting that FLI-1 may not be so helpful in the differential diagnosis of cutaneous Ewing's sarcoma. In addition, a weak immunoreactivity was found in 3/5 Merkel cell carcinomas, 3/10 synovial sarcomas, 5/10 malignant melanomas, 6/10 lung adenocarcinomas and in 1/10 breast carcinomas. In contrast, all the rhabdomyosarcomas, desmoplastic small round cell tumors, high-grade pleomorphic sarcomas and colonic adenocarcinomas tested were negative. Importantly, in contrast with previous studies, no background staining was observed. Our results indicate that FLI-1 monoclonal antibody can be reliably applied to the differential diagnosis of small round cell neoplasms of soft tissue, and confirm its important role as nuclear marker of endothelial differentiation, mainly helpful in those cases in which technical artifacts are seen by using the traditional membranous and cytoplasmic endothelial markers.

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FLI-1 is a member of ETS genes, which encode for a family of transcription factors, defined by a highly conserved DNA-binding domain.¹ Its clinical role is most apparent in the human Ewing's sarcoma/primitive neuroectodermal tumor (ES/PNET) category, wherein FLI-1 is the target of a characteristic

balanced chromosomal translocation t(11;22)(q24;q12) which results in the production of the EWS/FLI-1 fusion gene. The resultant EWS/FLI-1 chimeric protein includes the N-terminal transactivation domain of EWS and the C-terminal DNA-binding domain of FLI-1.² The EWS/FLI-1 chimeric protein binds DNA with the same affinity as FLI-1, but EWS/FLI-1 transcriptional activation of the target genes is much more efficient.³

ES/PNET belongs to a heterogeneous group of mesenchymal neoplasms featuring round cell morphology and therefore collectively known as small round cell sarcomas. Alveolar rhabdomyosarcoma (RMS), desmoplastic small round cell tumor (DSRCT), mesenchymal chondrosarcoma and poorly differentiated synovial sarcoma (SS) also belong to

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this group. Immunohistochemistry plays a key role in the differential diagnosis of round cell tumors and CD99 is a very sensitive marker of ES/PNET. However, its expression is not limited to these tumors. CD99 immunoreactivity has been observed in RMS,⁴ SS,⁵⁻⁷ lymphoblastic lymphoma,⁸ mesenchymal chondrosarcoma,^{9,10} Merkel cell carcinoma (MCC)¹¹ and has been also reported in rare cases of Wilms' tumor,¹² small cell osteosarcoma¹³ and DSRCT.^{14,15} Thus, FLI-1 has been recently proposed as an additional immunohistochemical marker of ES/PNET, along side the traditional CD99.¹⁶⁻¹⁸

Moreover, as FLI-1 protein expression is consistently found in endothelial cells as well as neoplasm thereof,¹⁸ FLI-1 has been also proposed as a nuclear marker of endothelial differentiation.¹⁹

In the previous series, the use of a polyclonal antiserum has led to major interpretation problems, due to the presence of background staining as well as staining variation between different lots of the same antiserum.^{18,19} As a consequence, FLI-1 immunohistochemistry has never become popular. In the present study, a novel monoclonal antibody raised against the carboxyl terminal of the FLI-1 protein (clone GI146-222, BD Pharmingen) was tested. FLI-1 expression was investigated in a series of both small round cell neoplasms and vascular neoplasms. In addition, in order to assess FLI-1 specificity, its expression was analyzed in a series of common epithelial and non-epithelial malignancies.

Materials and methods

Formalin-fixed, paraffin-embedded archival tissue from 15 ES/PNET, 10 RMS, five DSRCT, 10 SS, 10 high-grade pleomorphic sarcomas (PS), 10 malignant melanomas (MM), five MCC, 10 colonic adenocarcinomas (CA), 10 breast carcinomas (BC), 10 lung adenocarcinomas (LA), 20 angiosarcomas (AS), five epithelioid hemangioendotheliomas (EHE), 10 Kaposi's sarcomas (KS) and 10 benign hemangiomas (BH), were immunostained with a monoclonal antibody raised against the carboxyl terminal of the FLI-1 protein (clone G146-222, BD Pharmingen, dilution 1:50). Immunostains were performed using a sensitive polymer based revelation system (Envision Plus, DakoCytomation, Glostrup Denmark). Heat-induced antigen retrieval was performed by incubating the slides for 40' at 98°C in a waterbath using an 8.5 pH EDTA buffer. All immunostains were performed on an automated immunostainer (Dako Autostainer, DakoCytomation). Positivity was scored using a four-tiered scale (1+ = <25% positive cells; 2+ = 26-50%; 3+ = 51-75%; 4+ = 76-100%). Normal endothelium served as built-in positive control in all cases. Only nuclear immunoreactivity was evaluated.

Results

All ES/PNET (Figures 1a and b) as well as all vascular tumors (Figures 1c and d), exhibited a 4+ ,

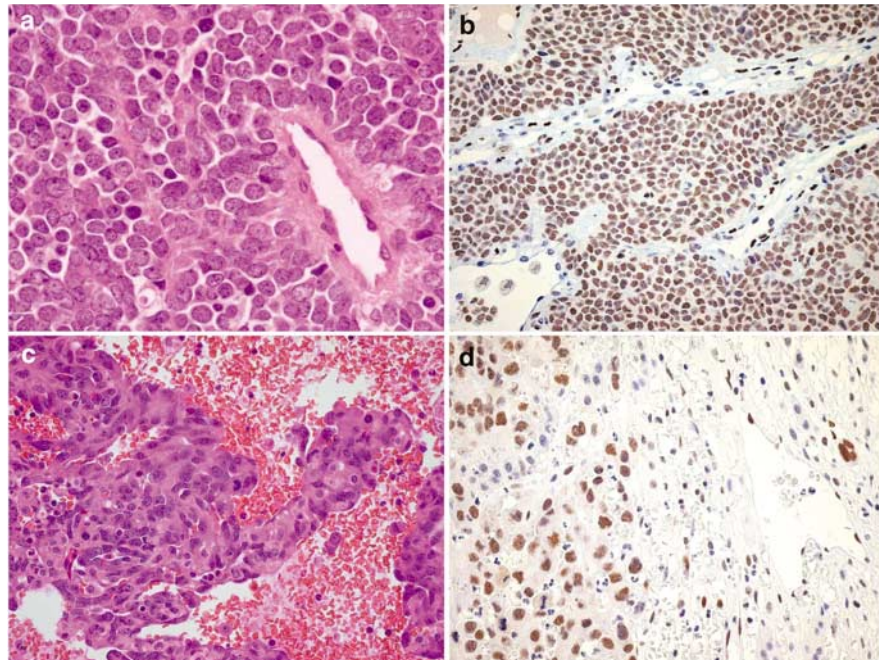


Figure 1 (a) Ewing's sarcoma/PNET represents the prototype of small round cell mesenchymal malignancies ($\times 400$); (b) FLI-1 monoclonal antibody decorates all Ewing's sarcoma cell nuclei ($\times 200$); (c) epithelioid angiosarcoma may exhibit a solid growth pattern that mimics carcinoma ($\times 400$); (d) all cases of epithelioid angiosarcoma exhibited strong FLI-1 immunoreactivity ($\times 400$); Importantly, FLI-1 immunopositivity is detectable in normal endothelium as well as in benign and malignant vascular neoplasm.

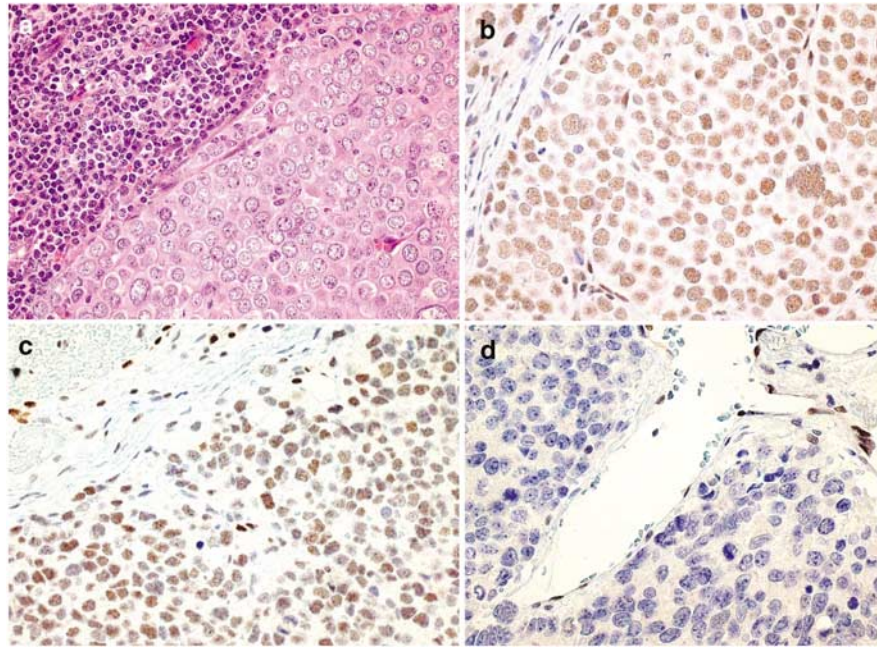


Figure 2 (a) Merkel cell carcinoma may represent an important diagnostic challenge when dealing with round cell neoplasm arising in adults ($\times 200$); (b) FLI-1 immunopositivity was observed in all Merkel cell carcinomas tested ($\times 400$); (c) exceptionally, malignant melanoma exhibited strong FLI-1 immunoreactivity; (d) infiltrating ductal breast carcinoma showing no FLI-1 staining. Endothelial cells serve as built-in positive control ($\times 400$).

Table 1 Immunohistochemical results

Tumor	FLI-1 Immunopositivity	Score
ES	15/15 (100%)	4+
RMS	0/10 (0%)	—
DSRCT	0/5 (0%)	—
SS	3/10 (30%)	1–2+
PS	0/10 (0%)	—
MM	6/10 (60%)	2+
MCC	5/5 (100%)	3+
LA	6/10 (60%)	1–2+
BC	1/10 (10%)	2+
CA	0/10 (0%)	—
EHE	5/5 (100%)	4+
KS	10/10 (100%)	4+
BH	10/10 (100%)	4+
AS	20/20 (100%)	4+

ES, Ewing's sarcoma; RMS, rhabdomyosarcoma; DSRCT, desmoplastic small round cell tumor; SS, synovial sarcoma; PS, high-grade pleomorphic sarcoma; MM, malignant melanoma; MCC, Merkel cell carcinoma; CA, colonic adenocarcinoma; BC, breast carcinoma; LA, lung adenocarcinoma; AS, angiosarcoma; EHE, epithelioid hemangioendothelioma; KS, Kaposi's sarcoma; BH, benign hemangiomas.

strong nuclear immunoreactivity. Strong immunoreactivity was also found in 2/5 MCC (Figures 2a and b) and in 1/10 MM (Figure 2c). Weak nuclear immunoreactivity, ranging between 1+ and 2+ was observed in 3/5 MCC, 5/10 MM, 3/10 SS, 6/10 LA, 1/10 BC (Figure 2d). All RMS, DSRCT, PS and CA tested were negative, as shown in Table 1. No cytoplasmic background staining was observed.

Discussion

FLI-1 gene was first identified in Friend virus-induced erythroleukemia at the site of retroviral integration.²⁰ Subsequent analyses in different models of virus-induced lymphoma/leukemia have shown FLI-1 gene rearrangements at the site of proviral integration.¹ FLI-1 gene belongs to the human *Ets* family of transcription factors and localizes at the *Ets* locus on human chromosome 11q23.¹ Interestingly, FLI-1 expression has been shown to be critical for hematopoiesis and early vessels development, as demonstrated in knockout mice²¹ and zebrafish.²² Moreover, FLI-1 protein expression has been found in endothelial¹⁸ and hemopoietic cells, as T-lymphocytes and megacaryocytes.¹

In most of the human ES/PNET, FLI-1 is the target of a characteristic chromosomal balanced translocation t(11;22) (q24;q12), which results in the production of EWS/FLI-1 fusion gene. In the remaining cases of ES/PNET, the EWS gene is rearranged with other partners of the *Ets* oncogene family. Interestingly, ES/PNET cases harboring the EWS/FLI-1 transcript seem to have a better prognosis than those containing other EWS/ETS transcripts.²³ Furthermore, since the copy number of 1q and 16q may identify a group of ES/PNET patients with a worse prognosis, the importance to assess the presence of additional cytogenetic aberrations has been recently highlighted.²⁴

A few mechanisms underlying EWS/FLI-1 oncogenic activity have been hypothesized. EWS/FLI-1

but not FLI-1 alone induces malignant transformation in NIH3T3 cells.^{25–27} Interestingly, this phenotype may be reversed by using a transcriptional repressor, which inhibits only the transcriptional activity of EWS/FLI-1, confirming that its oncogenic activity is related to the aberrant expression of specific EWS/FLI-1 target genes.²⁸ However, since its transforming activity is not only dependent upon the DNA-binding domain, it has been suggested that a protein–protein interaction may represent an additional FLI-1 oncogenic mechanism.²⁹ Interestingly, up-regulation of *c-myc* is observed in the cell lines expressing EWS/FLI-1.³⁰ In contrast, the TGF- β pathway and p57 protein, which are involved in the cell-cycle inhibition, have been shown to be down-regulated.³⁰

A polyclonal antiserum raised against the C-terminus of the FLI-1 protein has been previously reported as a useful tool in distinguishing ES/PNET from other round cell tumors.¹⁸ However, its clinical use has been hampered by major interpretation problems, due to the presence of background staining as well as staining variation between different lots of the same antiserum. In the current study, FLI-1 expression was investigated in 15 cases of ES/PNET and a strong nuclear immunoreactivity was detected in all cases. Since in the previous series FLI-1 was expressed in 71%¹⁸ and 92%¹⁷ of ES/PNET tested, the higher sensitivity of this FLI-1 monoclonal antibody is apparent. It may be correctly argued that some of the cases in Folpe's study¹⁸ might be characterized by other EWS/ETS translocations. However, it should be noted that in Llombart-Bosch's series,¹⁷ RT-PCR analysis demonstrated the presence of EWS/FLI-1 translocation in all the cases included.

No FLI-1 expression was found in RMS and DSRCT, suggesting that FLI-1 may be used in the differential diagnosis of round small cell tumors. Importantly, lower levels of FLI-1 immunopositivity were observed in a significant proportion of SS, MM as well as common epithelial malignancies. Even if, with the exceptions of one case of MM and two cases of MCC, the intensity of the nuclear staining ranged from weak to moderate, this underlines the importance of evaluating immunohistochemical results in context with morphology and in panel with other immunoreagents.

The detection of FLI-1 expression in MCC, limits its role in the differential diagnosis of cutaneous ES^{31–33} and expands further the morphologic overlap between MCC and those rare examples of ES arising primarily in the skin.

It has also to be emphasized that the immunohistochemical detection of proteins involved in the formation of fusion transcripts may represent a faster as well as cheaper mean to predict the presence of the underlying chromosome abnormalities. This has been proved true for alk-1 immunostaining in both anaplastic large cell lymphomas^{34–36} and inflammatory myofibroblastic tumors.^{37,38}

Immunohistochemical detection of WT1 in desmoplastic small round cell tumor^{39,40} and of TFE3 in alveolar soft part sarcoma^{41,42} represent additional examples of this approach.

FLI-1 clinical use is not limited to the differential diagnosis of small round cell tumors. Since FLI-1 protein is present in normal endothelial cells,¹⁸ its expression has been recently investigated in a series of endothelial neoplasms.¹⁹ In Folpe's study, the sensitivity of the FLI-1 polyclonal antibody was about 90% in AS and EHE and 100% in BH and KS. In our series, strong immunoreactivity was detected in all the endothelial neoplasms, including AS and EHE, suggesting that, even in this setting, FLI-1 monoclonal antibody appears to exhibit a higher sensitivity than the polyclonal antiserum. Our results confirm the important role of FLI-1 immunohistochemistry as a very reliable nuclear marker of endothelial differentiation, mainly helpful in those cases in which the evaluation of the traditional membranous and cytoplasmic endothelial markers such as CD31, CD34 and FVIII-RA is hampered by technical artifacts. Importantly, FLI-1 is totally useless in distinguishing benign from malignant vascular proliferations. In fact, strong immunoreactivity is present in normal endothelial as well as in any neoplasm thereof.

In contrast with a previous series,¹⁸ we found a weak/moderate FLI-1 expression in 60% of LA and 10% of BC. Interestingly, our results are consistent with the previous observation that FLI-1 is expressed in normal epithelia, as breast epithelium and cutaneous eccrine glands.¹⁹

In conclusion, our results indicate that FLI-1 monoclonal antibody, when evaluated in context with morphology, can be reliably applied to the differential diagnosis of small round cell neoplasms of soft tissue. In addition, FLI-1 currently emerges as the most sensitive marker of endothelial differentiation.

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