HHS Public Access

Author manuscript

Am J Surg Pathol. Author manuscript; available in PMC 2016 October 01.

Published in final edited form as:

Am J Surg Pathol. 2015 October ; 39(10): 1305–1312. doi:10.1097/PAS.0000000000000462.

NUCLEAR BRACHYURY EXPRESSION IS CONSISTENT IN CHORDOMA, COMMON IN GERM CELL TUMORS AND SMALL CELL CARCINOMAS AND RARE IN OTHER CARCINOMAS AND SARCOMAS. AN IMMUNOHISTOCHEMICAL STUDY OF 5229 CASES

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Abstract

Brachyury is a transcription factor of the T-box family typically expressed in notochord and chordoma. Some studies report brachyury as highly specific for chordoma, whereas others have concluded that brachyury is expressed in many types of common carcinomas by RT-PCR and immunohistochemistry and could be involved in the epithelial-mesenchymal transition and metastatic process. In this study, we immunohistochemically evaluated 5229 different tumors for nuclear brachyury expression using a new rabbit monoclonal antibody and automated immunostaining (Leica Bond Max). Only nuclear labeling was scored, and antibody dilution of 1:2000 was used. In normal tissues, only rare cells in seminiferous tubules were labeled; all other organs were negative. All chordomas (75/76), except a sarcomatous one, were positive, whereas chondrosarcomas were negative. Among epithelial tumors, positivity was often detected in embryonal carcinoma (74%) and seminoma (45%). Pulmonary small cell carcinoma was often positive (41%), whereas pulmonary and pancreatic adenocarcinomas only rarely showed nuclear brachyury-positivity (3–4%). Common carcinomas such as ductal carcinomas of breast, or adenocarcinomas of the prostate only exceptionally showed nuclear positivity (< 1%). No colorectal, hepatocellular, renal cell, squamous cell, thyroid or urothelial carcinoma, or mesothelioma showed nuclear brachyury-positivity. Among mesenchymal and neuroectodermal tumors, only isolated cases of melanoma, malignant peripheral nerve sheath tumor, rhabdomyosarcoma, synovial sarcoma, and follicular lymphoma showed nuclear expression. However, as shown previously with lung carcinoma, experiments with lower antibody dilutions (1:200–1:500) showed weak cytoplasmic and nuclear labeling in breast cancers. In addition to chordoma, we show here for the first time that nuclear brachyury expression is prevalent in embryonal carcinoma, seminoma, and small cell carcinoma of the lung but very rare in common carcinomas, sarcomas, and melanoma. With these reservations, we have demonstrated the

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The remaining authors declare no conflict of interest.

presence of nuclear brachyury immunoreactivity to be a sensitive and fairly specific marker for chordoma.

Keywords

Brachyury; chordoma; seminoma; germ cell tumor; small cell carcinoma

INTRODUCTION

The brachyury gene ("short tail" from Greek, also designated as "T gene") encodes a transcription factor in the T-box group of morphogenetic genes and has homologous forms in mice and humans.[1,2] The encoded brachyury protein is important in the development of the posterior and caudal body elements including the notochord of mice. While initially expressed in subsets of primitive epithelial and mesenchymal cells, in adult tissues brachyury is almost exclusively expressed in the notochord. [3–6] In animals, loss-of-function mutations cause defects in the tail development;[2] in humans, germline gene duplications have been associated with familial chordoma and similar somatic changes have been associated with sporadic chordoma.[7,8]

Nuclear expression of brachyury protein has been immunohistochemically detected in notochord and their neoplastic counterpart, chordomas. [9] In one study, chordomas showed strong nuclear brachyury expression, while brachyury was absent in other types of tumors, including carcinomas, sarcomas, and gliomas.[9] A small number of soft tissue tumors considered extra-axial chordomas were also shown to be brachyury-positive.[10,11] Another study found no immunohistochemically detectable brachyury expression in potential chordoma mimics, such as different types of germ cell tumors and renal cell carcinomas, in contrast to chordoma.[12] These studies used a variety of polyclonal and monoclonal antibodies.

The transcription factor brachyury has been also identified as a driver in the epithelial-mesenchymal transition (EMT) for several types of carcinomas and is being explored as a target antigen in tumor vaccine studies.[13] Brachyury expression has been detected, among others, in mammary[14], pulmonary[15,16], prostatic[17] and colorectal carcinomas[18] and is often associated with aggressive disease and poor prognosis. In those studies, brachyury has been detected by RT-PCR and immunohistochemistry, finding both nuclear and cytoplasmic expression. Human T-cells generated against brachyury peptide were shown to lyse a range of carcinomas including those of breast, lung, and colon.[13] In addition, immunohistochemical studies demonstrated nuclear brachyury expression in oral squamous cell carcinoma, finding it associated with the development of lymph node metastases.[19] In cancer biology, brachyury has also been linked with EMT leading into a more invasive phenotype of tumors.[20]

Previous immunohistochemical studies have reported high specificity of brachyury for chordoma vs. carcinomas[9] while several other studies reported widespread expression of brachyury in various carcinomas;[14–19] these studies seem to contradict each other but may be explained by differences in technique, antibody, or definition of expression. In this study,

we utilized a newly developed rabbit monoclonal antibody (Mab) to examine nuclear brachyury expression in over 5,000 human tumors, including chordoma and its mimics, and a wide variety of carcinomas, sarcomas, and melanomas. Our aim was to examine the specificity of this antibody to chordoma and reveal its other diagnostic potential employing exclusive scoring of nuclear labeling.

MATERIALS AND METHODS

Large numbers of chordomas, chondrosarcomas, and other tumors relevant in the differential diagnosis of chordoma, and other epithelial, mesenchymal, neuroectodermal, and lymphohematopoietic tumors were organized in multitumor blocks containing 30–70 different cases each. The size of each sample exceeded the size of a 0.6 mm^2 microarray core by a factor of at least 5–15. Normal adult and fetal human tissues were also studied. All tissues were derived from anonymized surgical specimens.

The previously described primary rabbit monoclonal antibody (MAb 54-1, IgG1 isotype) was raised to brachyury protein and was predicted to recognize a carboxyterminal epitope.[21] This antibody reacted with a 49 kD protein by immunoblotting, and immunoblotting reactivity was absent in cells in which the brachyury gene was silenced by RNA-interference. The antibody did not react with other T-box proteins during in vitro testing.[21]

Immunohistochemical staining was performed in a Leica Bond Max automated system (Leica, Bannockburn, IL) using the Leica-Refine detection kit. Immunostaining was preceded by Leica high-pH epitope retrieval for 25 min. The primary antibody was diluted 1:2000, corresponding to antibody concentration of 0.6 ug/ml. The primary antibody was incubated for 30 min and was followed by the Leica polymer and post-polymer (each 15 min), DAB for 10 min, and a hematoxylin counterstain for 5 min. A chordoma or brachyury-positive seminoma was used as a positive control.

Cytoplasmic staining was generally rare with the antibody dilution used and was not scored. However, if a lower dilution of primary antibody was used (1:200 to 1:500), a variably weak cytoplasmic and nuclear labeling was obtained in a multitumor block containing ductal carcinomas of the breast.

RESULTS

All results presented here were obtained with a 1:2000 dilution of the primary antibody, following the methods described above.

Normal fetal and adult tissues

Fetal tissues (10 week) did not show nuclear brachyury immunoreactivity in the skin, paraspinal and limb soft tissues, liver, intestines, or kidney. However, notochord elements were not available for observation. None of the normal adult tissues examined, including skin, gastrointestinal tract, liver, pancreas, lung, endometrium, prostate, thyroid, and lymphoid tissue of tonsil, showed any nuclear immunoreactivity. However, scattered cells in seminiferous tubules variably showed brachyury-positive nuclei.

Chordoma, chondrosarcoma, and mixed tumors/myoepitheliomas

The results on epithelial tumors have been summarized in Table 1. All chordomas except one case with spindle cell sarcomatous transformation showed nuclear brachyury expression (Fig. 1A). In addition, variable cytoplasmic staining was observed. In contrast, none of the chondrosarcomas of bone were positive (Table 2). However, these tumors showed variable cytoplasmic labeling (Fig. 1B). All extraskeletal myxoid chondrosarcomas were also negative. Although none of the 131 mixed tumors/myoepitheliomas of the salivary gland showed nuclear positivity, two cutaneous or soft tissue examples showed nuclear positivity (Fig. 1C, D). One of these tumors contained p63-positive cells as expected of tumors with myoepithelial differentiation. All renal cell carcinomas were negative.

Other epithelial neoplasms

In general, nuclear brachyury expression was rare in other epithelial neoplasms but occurred often in germ cell tumors, small cell carcinomas, and very occasionally in common carcinomas (Table 1).

Among germ cell tumors, 46% of seminomas showed typically uniform nuclear labeling (Fig. 2A), whereas the other cases were negative. Embryonal carcinomas (74%) often showed focal positivity in a minority of tumor cells, typically less than 10–20% (Fig. 2B, C). Endodermal sinus tumors also showed focal positivity (Fig. 2D). Choriocarcinomas or components with trophoblastic differentiation were negative.

Small cell carcinoma of the lung was the most frequently positive non germ-cell tumor (41%). In these cases, 10–30% of tumor cells showed nuclear labeling (Fig. 3A). Other pulmonary carcinomas were only rarely positive. These included 9/212 (4%) of pulmonary adenocarcinomas, which usually showed very focal labeling (range: 1–15%, median 3%). Some usual acinar adenocarcinomas also showed focal positivity (Fig. 3B, C). Multifocal strong nuclear brachyury was seen in one so-called embryonal carcinoma of the lung, a tumor showing glands resembling secretory endometrium (Fig. 3D).

When using a 1:2000 dilution of the MAb, only 1/427 ductal carcinomas of the breast (<1%) showed nuclear brachyury expression, which was focal and seen in 10% of tumor cells (Fig. 4A). This carcinoma was ER/PR+, Her2 2+, without basal cell phenotype (negative for CK5/6 and Trim29). However, if a lower dilution of primary antibody was used (1:200 to 1:500), a variably weak cytoplasmic and nuclear labeling was obtained in a multitumor block containing ductal carcinomas of the breast. Only one prostatic adenocarcinoma (0.5%) had nuclear brachyury labeling, which was focal. This case had Gleason score 5+4 features and contained nuclear brachyury labeling in 30% of tumor cells (Fig. 4B). Three pancreatic adenocarcinomas (3%) showed focal positivity (<10% of tumor cells). The sole endometrial carcinoma (1/236) with nuclear brachyury was a high-grade carcinoma with a solid non-glandular pattern (Fig. 4C) showing low estrogen receptor expression (10% of tumor cells), MSH2/MSH6 mismatch repair deficiency status, and focal nuclear beta-catenin expression. One endometrioid carcinoma of the ovary was also positive (Fig. 4D).

All adrenocortical, colorectal, hepatocellular, renal, thyroid, and ovarian serous, and clear cell carcinomas were negative for nuclear brachyury labeling. None of the squamous carcinomas of skin, head and neck, or lung showed nuclear brachyury-positivity.

Mesenchymal, neuroectodermal and lymphoid neoplasms

In malignant mesenchymal and neuroectodermal tumors, nuclear brachyury expression was distinctly rare and seen in only one each of metastatic melanoma (Fig. 5A), high-grade malignant peripheral nerve sheath tumor (Fig. 5B), Ewing sarcoma (Fig. 5C), monophasic synovial sarcoma (Fig. 5D), and embryonal rhabdomyosarcoma. In these cases, 5–10% of tumor cells were positive. Lymphomas (45 diffuse large B-cell, 24 small lymphocytic, 26 follicular, 20 T-cell, miscellaneous) were negative, except one follicular lymphoma that showed brachyury-positivity in approximately 10% of nuclei.

DISCUSSION

In this study we examined immunohistochemically detectable nuclear brachyury expression in chordomas and a wide variety of epithelial, mesenchymal, neuroectodermal, and lymphohematopoietic neoplasms, to further explore the distribution of brachyury in human tumors, and the potential specificity of brachyury for chordoma. To our knowledge, we are the first to observe frequent nuclear brachyury-positivity in germ cell tumors and small cell carcinomas of the lung.

Our findings confirm the observations that nuclear brachyury is consistently expressed in chordoma.[9–12] The only exceptions seem to be inconsistent or absent expression in chordomas that have undergone sarcomatoid transformation. Furthermore, there is a strong contrast between chordoma and chondrosarcoma, including extraskeletal myxoid chondrosarcoma, as these tumors lack nuclear brachyury expression. Rare examples of soft tissue mixed tumor/myoepitheliomas expressed brachyury. Some of these tumors may represent peripheral (extra-axial) counterparts of chordomas, as previously described and found to be brachyury-positive.[10–11] However, in these cases, it is advisable to rule out the possibility of chordoma metastatic into soft tissues.

Unlike chordomas, only a few types of epithelial neoplasms demonstrated significant nuclear brachyury expression with the specific dilution of rabbit anti-brachyury MAb (1:2000) and technique reported here, including germ cell tumors and pulmonary small cell carcinomas. Among germ cell tumors, testicular seminomas commonly show extensive brachyury expression. We could not determine how the positive and negative cases differ. In embryonal carcinoma, brachyury expression is typically restricted to focal areas. A previous study with a different antibody found that all germ cell tumors were negative. However, studies on embryonal carcinoma cell lines have detected brachyury expression.[22,23] Therefore, brachyury expression in germ cell tumors may reflect phenotypic similarity to early embryonic tissues. Additional studies are warranted to examine the possible clinical significance of brachyury expression.

Small cell carcinomas also showed a high frequency of positivity for brachyury nuclear expression. However, common carcinomas of breast, prostate, lung, and endometrium only

exceptionally showed nuclear positivity, further indicating that nuclear brachyury-positivity is fairly specific for chordoma when facing the differential diagnosis of chordoma vs. common carcinomas. However, numerous studies have found brachyury expression in carcinomas and many have indicated that brachyury is associated with poor prognosis. Those studies[14–19] have variably detected brachyury transcripts by RT-PCR, but also by immunohistochemistry, and nuclear positivity along with cytoplasmic positivity have been reported. However, all these studies used other antibodies or a lower dilution of the rabbit MAb.[21] The uniqueness and high degree of specificity of the anti-brachyury rabbit MAb employed here, in comparison to other commercially available antibodies, has been previously shown in ELISA and Western blot analyses.[21] It therefore seems that the rabbit MAb we used is exceptionally specific for chordoma vs. carcinoma, when using a high antibody dilution (1:2000). Lower antibody dilutions result in variably weak cytoplasmic and nuclear labeling in other tumors, such as breast and lung carcinomas.[21]

In conclusion, nuclear brachyury expression, as tested with a new rabbit MAb, is relatively specific for chordoma and is not expressed in chondrosarcoma and other sarcomas, with few exceptions. Occasional peripheral mixed tumors can express brachyury and may represent peripheral counterparts of chordoma. Among epithelial neoplasms other than chordomas, embryonal carcinomas and seminomas, and small cell carcinomas frequently express nuclear brachyury, whereas the common carcinomas very rarely do, when tested with the conditions reported here. With these reservations, we have demonstrated the presence of nuclear brachyury immunoreactivity to be a sensitive and fairly specific marker for chordoma. Potential brachyury expression in non-chordomas has to be considered in diagnostic practice.

Acknowledgments

CP and JS have submitted, as part of their official duties at NIH, an employee invention report concerning the monoclonal anti-brachyury antibody used in this study. This work was supported by the Intramural Research Program of the Center for Cancer Research, National Cancer Institute, NIH.

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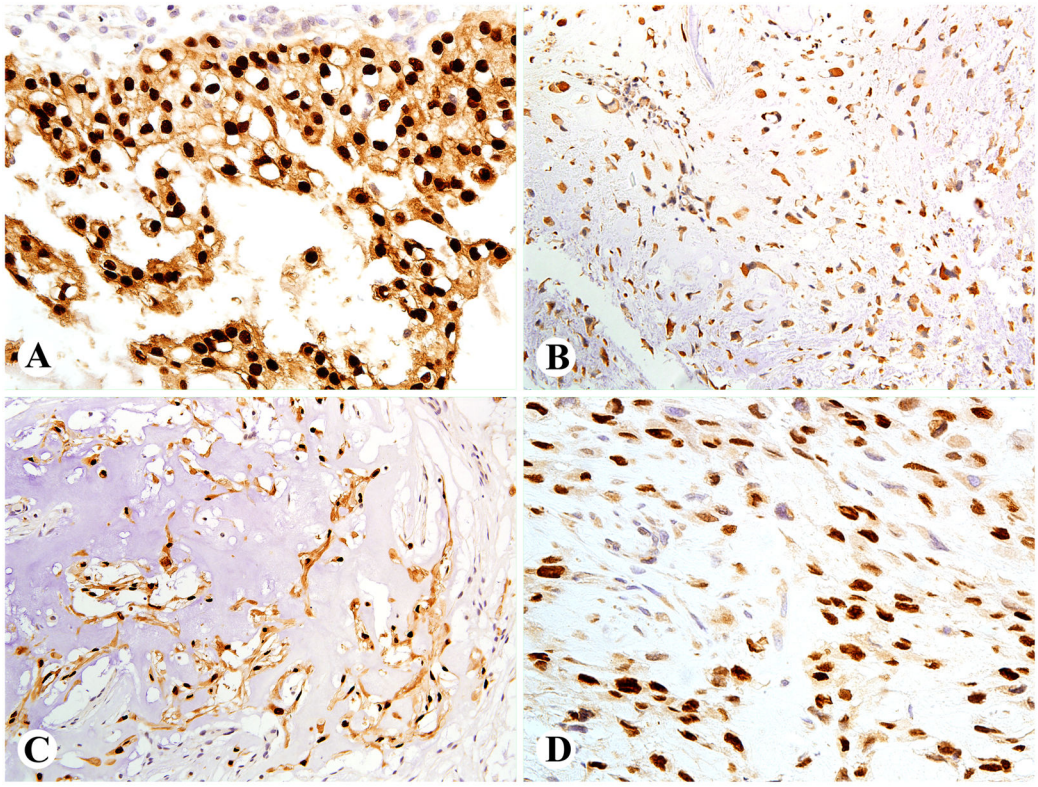

Fig. 1.

Nuclear brachyury expression in chordoma and its histologic mimics. A. Chordoma cells show strong nuclear and moderate cytoplasmic expression. B. Chondrosarcoma with myxoid stroma shows cytoplasmic but no nuclear brachyury-positivity. C. A chordoma-like extra-axial tumor shows both nuclear and cytoplasmic positivity. D. A mixed tumor of soft tissue shows nuclear positivity.

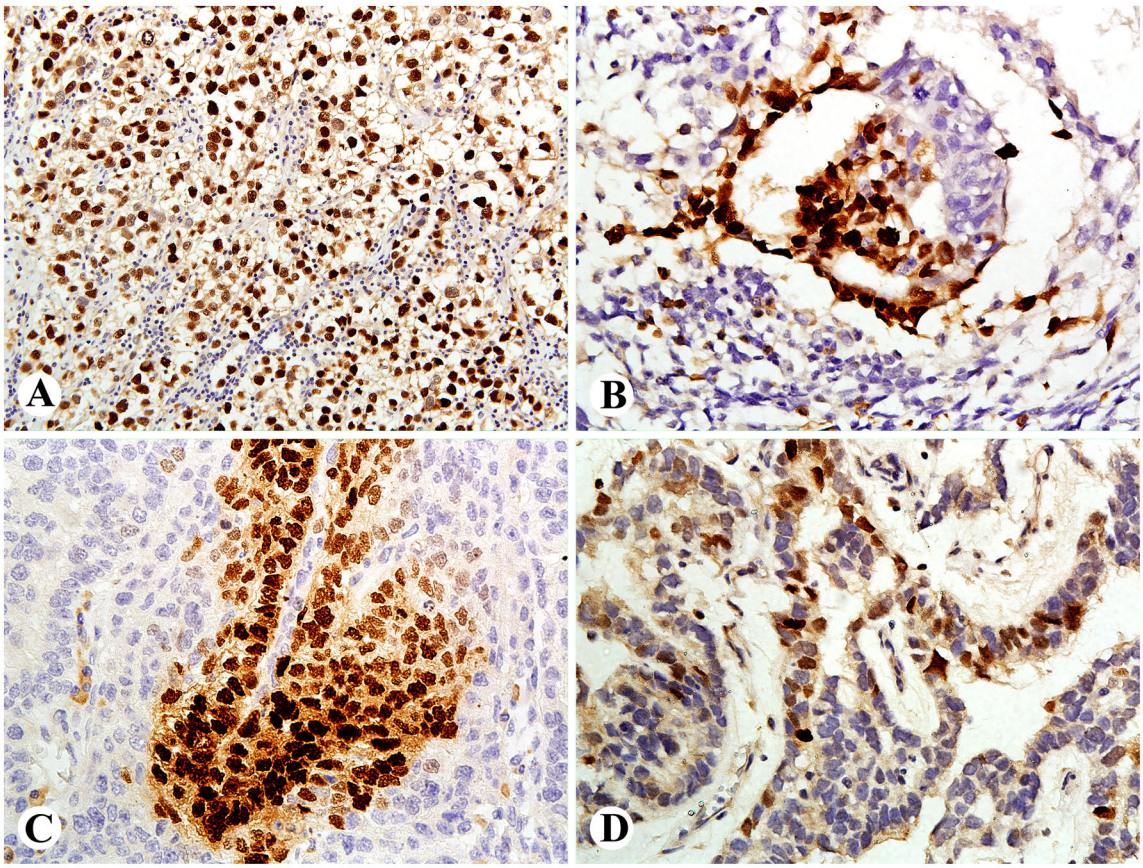

Fig. 2.

Nuclear brachyury expression in germ cell tumors. A. Seminoma commonly showing nuclear positivity. B. Embryoid body in embryonal carcinoma and rare cells outside it are positive. C. Embryonal carcinoma with a strongly positive area showing brachyury expression. D. Yolk sac tumor with focal positivity.

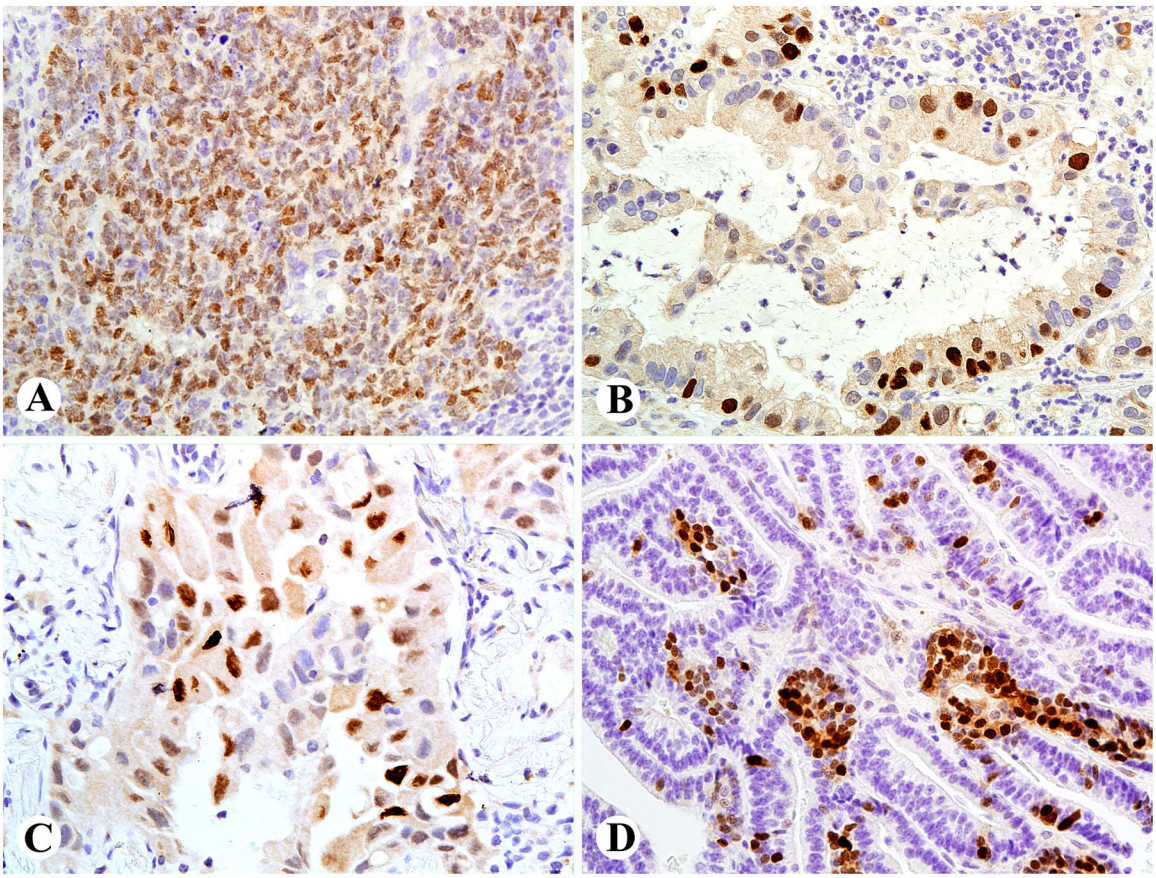

Fig. 3.

Lung cancers with nuclear brachyury expression. A. Small cell carcinoma was the most common carcinoma showing positivity. B–C. Two examples of acinar adenocarcinomas with focal brachyury expression. D. An embryonal type adenocarcinoma showing brachyury expression.

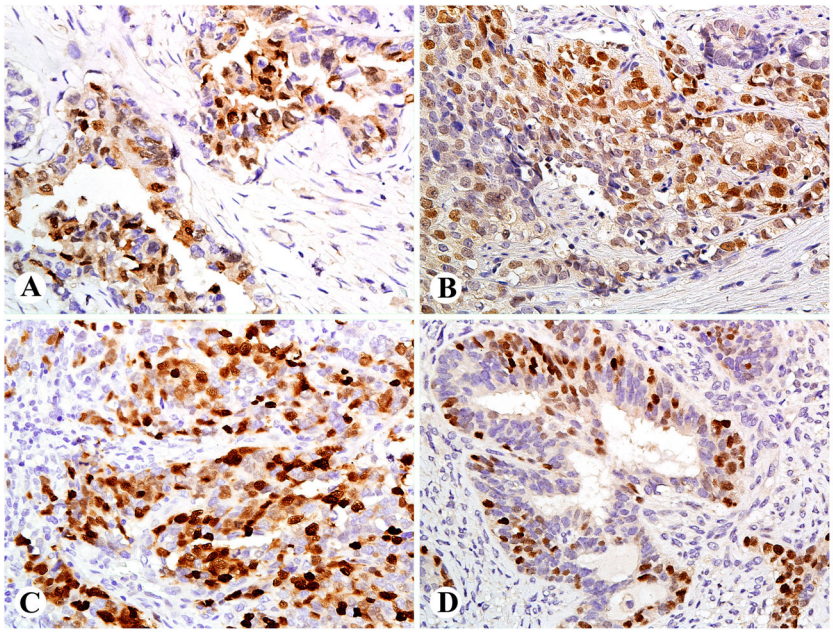

Fig. 4.

Other carcinomas with nuclear brachyury expression. A. Only one breast cancer, a high-grade ductal carcinoma was focally positive. B. A poorly differentiated prostatic adenocarcinoma was positive. C. Endometrial adenocarcinoma and D. ovarian endometrioid carcinomas with brachyury expression.

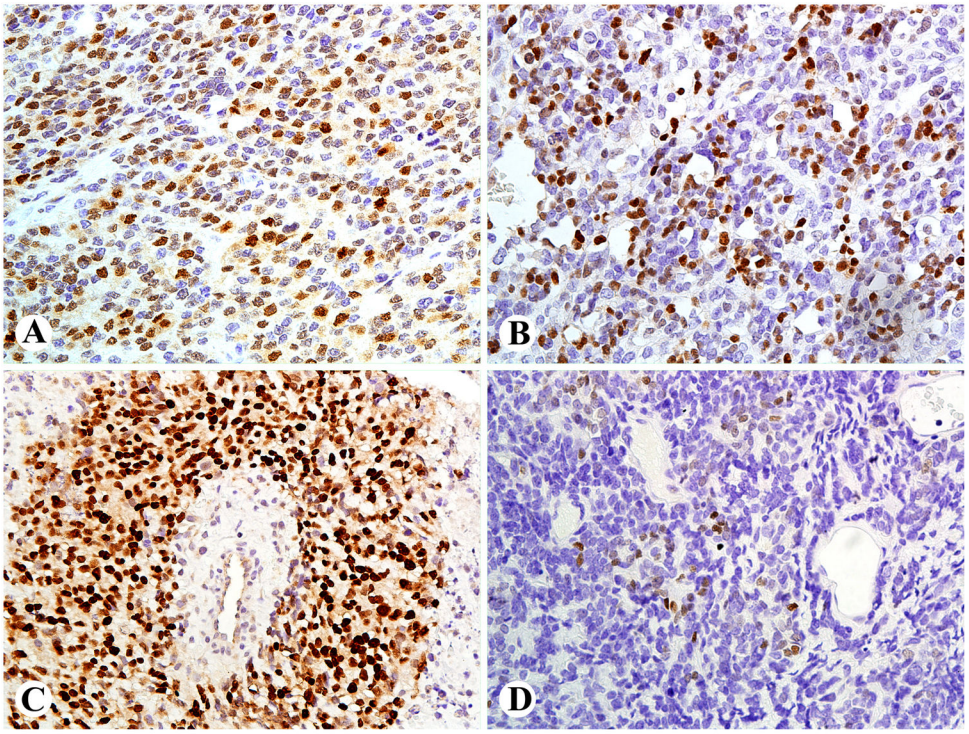

Fig. 5.

Brachyury expression in melanoma and sarcomas. A. A minority of melanoma cells showed nuclear positivity. B. Only occasional malignant peripheral nerve tumors showed nuclear positivity. C. A Ewing sarcoma with strong nuclear immunoreactivity. D. Isolated cells were positive in a synovial sarcoma.

Table 1

Nuclear brachyury expression in chordomas and 4119 carcinomas and some other epithelial neoplasms detected with a novel rabbit monoclonal antibody at 1:2000 dilution.

Tumor type	Positive/all	% positive
Adrenocortical carcinoma	0/31	
Basal cell carcinoma of skin	0/45	
Breast, ductal carcinoma	1/427	<1
Breast, lobular carcinoma	0/76	
Cholangiocarcinoma	0/52	
Colon, adenocarcinoma	0/213	
Chordoma	75/76	99
Endometrial adenocarcinoma	1/236	1
Germ cell tumor, choriocarcinoma	0/7	
Germ cell tumor, embryonal carcinoma	25/34	74
Germ cell tumor, seminoma	33/71	46
Germ cell tumor, yolk sac tumor	1/6	16
Hepatocellular carcinoma	0/112	
Kidney, carcinomas of various types*	0/393	
Lung, adenocarcinoma	9/212	4
Malignant mesothelioma	0/96	
Ovary, clear cell carcinoma	0/14	
Mixed tumor/myoepithelioma, salivary gland	0/105	
Mixed tumor/meyoepithelioma, skin/soft tissue	2/32	6
Ovary, endometrioid carcinoma	2/61	3
Ovary, serous carcinoma	0/190	
Ovary, granulosa cell tumor	0/22	
Pancreas, adenocarcinoma	3/115	3
Pancreas, neuroendocrine tumor	0/42	
Prostate, adenocarcinoma	1/263	0.5
Rectum, adenocarcinoma	0/119	
Small cell carcinoma, lung	12/29	41
Stomach, adenocarcinoma	3/165	2
Squamous cell carcinoma, lung	0/80	
Squamous cell carcinoma, oral/tongue	0/67	
Squamous cell carcinoma, tonsil	0/41	
Squamous cell carcinoma, skin	0/37	
Thymoma	0/58	
Thyroid carcinoma (21 follicular, 86 papillary)	0/107	
Thyroid, other (10 anaplastic, 6 medullary)	0/16	

Tumor type	Positive/all	% positive
Urothelial carcinoma	0/167	

* Kidney carcinomas included 148 low-grade clear cell carcinomas, 134 high-grade clear cell carcinomas, 56 papillary type 1, 10 papillary type 2, and 45 chromophobe carcinomas.

Table 2

Nuclear brachyury expression in 1,110 mesenchymal, neuroectodermal and lymphoid neoplasms detected with a novel rabbit monoclonal antibody at 1:2000 dilution.

Tumor type	Positive/all	% positive
Angiosarcoma	0/10	
Chondrosarcoma, bone	0/54	
Chondrosarcoma, extraskeletal myxoid	0/18	
Chondrosarcoma, dedifferentiated	0/9	
Clear cell sarcoma	0/12	
Dermatofibrosarcoma protuberans	0/39	
Desmoplastic small cell tumor	0/13	
Epithelioid hemangioendothelioma	0/13	
Epithelioid sarcoma	0/20	
Ewing sarcoma	0/34	
Fibroma/fibrothecoma, ovary	0/21	
Gastrointestinal stromal tumor	0/46	
Granulosa cell tumor, ovary	0/22	
Leiomyoma, uterus	0/57	
Leiomyosarcoma, non-visceral	0/32	
Liposarcoma, well-differentiated	0/43	
Liposarcoma, dedifferentiated	0/33	
Lymphoma, small B-cell/CLL	0/27	
Lymphoma, follicular	1/25	4
Lymphoma, diffuse large B-cell	0/72	
Lymphoma, T-cell, miscellaneous	0/30	
Malignant peripheral nerve sheath tumor	1/40	3
Melanoma, metastatic	1/105	
Neuroblastoma	0/26	
Paraganglioma	0/51	
Pleomorphic undifferentiated sarcoma	0/60	
Rhabdoid tumor	0/2	
Rhabdomyosarcoma, embryonal	1/41	2
Rhabdomyosarcoma, alveolar	0/28	
Schwannoma	0/57	
Solitary fibrous tumor	0/24	
Synovial sarcoma	1/57	2
Wilms tumor	0/19	
Total	3/1110	