Original Article

Special Expression of Thy-1 in Different Malignant Tumors

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ABSTRACT

Objective: To investigate the expression status of Thy-1 in malignant cancerous tissues and to evaluate whether Thy-1 could be a potential tumor marker.

Methods: Immunohistochemistry method was used to examine Thy-1 expressions in different malignant tumor tissues. We used a monoclonal antibody specific for Thy-1 and the indirect Catalysis Signal Amplification method. With the help of tissue microarray (TMA), we examined expression the status of Thy-1 in 1451 different types of malignant tumor tissues, 144 normal tissues and benign lesions.

Results: All the malignant tumor cells were grouped as one group (malignant cells group) and normal or benign tumor tissue cells as another group (normal cells group). Among the entire 1451 malignant cases group, positive Thy-1 expression with diffuse and strong staining was observed in 734 (734/1451; 50.59%) cases. In the benign cases only 6 chronic cervicitis cases showed weak staining for Thy-1. All the normal tissue showed negative staining. One-Way ANVOA analysis showed F value between these two groups was 147.229 (P<0.0001).

Conclusion: A significantly stronger expression of Thy-1 in malignant tumors was observed. Special overexpression of Thy-1 in malignant tumors suggests that Thy-1 might be a potential novel tumor marker with respect to cancer progression. Chronic cervicitis has some relationship with cervix carcinoma. When it develops into atypical hyperplasia, it will be a precancerous lesion for cervix carcinoma. In this research we found weak staining for Thy-1 in chronic cervicitis lesion, so Thy-1 may play a crucial role in carcinogenesis for cervix carcinoma. The research about the relationship between Thy-1 expressions in cancer cells and in precancerous lesion will provide some clues to understand the mechanism for carcinogenesis process.

Key words: Thy-1/CD90; Malignant tumor; Tumor marker

INTRODUCTION

Thy-1 is a glycoprotein with a high homology to the immunoglobulin V domain and belongs to the immunoglobulin-like superfamily of cell adhesion molecules[1]. Thy-1 gene locates at chromosome 11q23−24 and its protein was firstly described as a marker for thymocyte differentiation in mice[2]. As an antigen and cell differentiation marker, which was initially defined using liver-absorbed rabbit antiserum to human brain[3], Thy-1 was expressed in large amount on cell membrane of different types of human tissues, such as lymphoid cells, neural cells, fibroblasts, endothelial cells, and smooth muscle cells[4]. Activation of Thy-1 can promote T cell activation. Thy-1 also affects numerous nonimmunologic biological processes, including neurite outgrowth, tumor growth and migration. Besides，Thy-1 is a regulator of cell-cell and cell matrix interaction in axon regeneration, apoptosis, and fibrosis[5−7].

Immunostaining research of fresh cells and frozen tissues identified that Thy-1 locates on cell membrane, in cytoplasm or expresses secretory

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forms. So far, no immunostaining investigations of Thy-1 in paraffin embedded human tissues has been reported. Tissue microarray (TMA) technology allows rapid visualization of molecular targets in thousands of tissue specimens at a time, either at the DNA, RNA or protein level. This technique facilitates rapid translation from basic research to clinical applications. By revealing the cellular localization, prevalence and clinical significance of candidate molecules, TMAs are ideally suitable for genomics-based diagnostic. In previous study[9], we have examined Thy-1 expression in lung cancer and found its overexpression in tumor cells and weak staining in the adjacent normal tissue cells suggesting that Thy-1 might be a novel tumor marker. In this study TMA combined with Immunohistochemical (IHC) was applied to detect the aberrant expression pattern of Thy-1 in different types of malignant tumors in order to identify the Thy-1 expression status and its clinical implications.

MATERIALS AND METHODS

Materials and Tumor Samples

TMA was purchased from Cybrdi Corporation (China). The different malignant tumors included colon carcinoma, cervix carcinoma, ovary carcinoma, rectal cancer, gastric carcinoma, brain glioma, esophageal carcinoma, lung cancer, hepatocarcinoma, mammary carcinoma, corpus uteri carcinoma, soft tissue sarcoma, prostate carcinoma and kidney carcinoma. The number of validated malignant cases was 1451. There were also 144 benign lesions including chronic inflammation and normal tissues. The primary mouse monoclonal anti-Thy-1 antibody (clone F15-42-1) was purchased from Research Diagnostic, Inc., NJ, USA. The Signal Amplification Kit protocol was purchased from DAKO, CA. Other kits and regents used in the process of immunohistochemistry method were purchased from DAKO, CA.

Immunohistochemical Staining for Thy-1 Protein in Different Malignant Tumors

Antigen retrieval was performed by heating the samples without boiling in 200 ml of DAKO Target Retrieval Solution (pH 6.10) in a microwave oven for 40 min. IHC staining was performed according to the Signal Amplification Kit protocol. The primary mouse monoclonal anti-Thy-1 antibody was used at 1:20 dilution. Bound primary antibody was visualized by the avidin-biotin-peroxidase technique (Signal Amplification Kit, DAKO, CA) according to the instruction. The final staining was done in DAB solution for 5 min. The slides were counterstained with Mayers Hematoxylin Blue in 0.3% ammonia. Fatty tissue was selected as positive control and the adjacent normal tissue around malignant tumor served as a negative internal control. Immunohistochemical staining for Thy-1 was considered positive when there was greater than 10% reactivity. We grouped Thy-1 expression status, using immunostaining method, as positive, strong staining in the cells, and negative, weak staining in the cells.

Statistical Analysis

For statistical analysis, the cases were divided into two groups, positive and negative, based on Thy-1 expression status. One-Way ANVOA analysis was used to determine the difference of Thy-1 expression levels observed between the malignant tumor cells and the normal cells. Differences were considered statistically significant at $P<0.05$. All statistical analyses were performed using the software SPSS 12.0.0 (SPSS Inc., Chicago, IL).

RESULTS

Expression Characteristic of Thy-1 Protein in Different Malignant Tumors

The monoclonal antibody has been shown to recognize Thy-1 specifically[10]. Table 1 summarized the results of immunohistochemistry for all 1610 cases, which included efficiency 1451 malignant points (HE staining confirmed the pathological diagnosis), 144 normal tissue points and 15 inefficiency points (HE staining confirmed the points had no malignant tumor cells). A total of 734 (734/1451; 50.59%) cases of malignant tumors presented Thy-1 positive expression. The positive rate was high in colon, cervix, ovary, rectal, gastric carcinomas and low in soft tissue sarcoma, prostate and kidney carcinomas. Figure 1 to Figure 5 showed strong Thy-1 staining in different types of tumors, while in the adjacent normal tissue cells Thy-1 was low and mainly existed in the cytoplasm. All the normal tissue cells showed weak or negative staining of Thy-1. Almost all benign lesions except 6 chronic cervicitis cases were observed (data not shown). Our investigation

indicated that Thy-1 expression was up-regulated in malignant tumors.

Table 1. Thy-1 immunohistochemistry results of all types of tumors

Cat #	Pathology	Case number	Efficiency point of malignant	Normal tissue	Inefficiency point	Positive cases for malignant	Positive rate
CC05-01-001 CC04-01-006	Colon carcinoma	147	133	13	1	80	60.15%
CC10-02-001	Cervix carcinoma	63	22	39	2	13	59.09%
CC11-01-002	Ovary carcinoma	72	69	3	0	40	57.97%
CC01-08-001	Rectal cancer	84	77	6	1	42	54.55%
CC01-01-004 CC01-08-001	Gastric carcinoma	436	387	45	4	211	54.52%
CS17-01-005	Brain glioma	63	59	4	0	31	52.54%
CC02-01-003	Esophageal carcinoma	72	70	1	1	37	52.86%
CC04-01-002 CC04-01-006	Lung cancer	304	286	16	2	146	51.05%
CC03-01-001	Hepatocarcinoma	63	57	5	1	29	50.88%
CC08-01-003	Mammary carcinoma	63	59	3	1	26	44.07%
CC09-01-002	Corpus uteri carcinoma	63	59	3	1	25	42.37%
CC24-01-001	Soft tissue sarcoma	63	63	0	0	23	36.51%
CC19-01-003	Prostate carcinoma	63	59	3	1	18	30.51%
CC07-01-002	Kidney carcinoma	54	51	3	0	13	25.49%
Total		1610	1451	144	15	734	50.59%

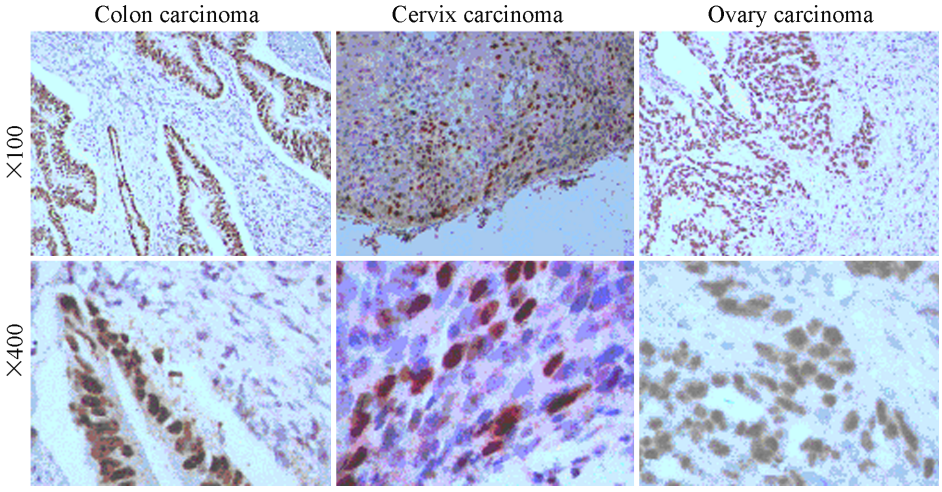

Figure 1. Thy-1 expression status immunostaining in colon cancer, cervix cancer and ovary cancer samples. Strong expression of Thy-1 was detected in cancer cells and the adjacent normal cells showed weak staining, (×100 and ×400 magnifications for upper and lower panel, respectively).

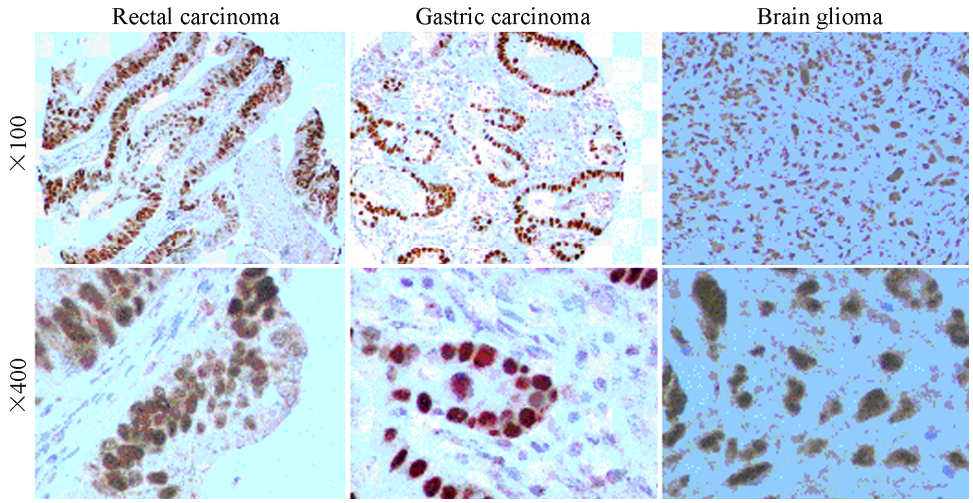

Figure 2. Representative Thy-1 immunostaining in rectal cancer, gastric cancer and brain glioma samples. Strong expression of Thy-1 was detected in cancer cells and weak staining was detected in the adjacent normal cells, (×100 and ×400 magnifications for upper and lower panel, respectively).

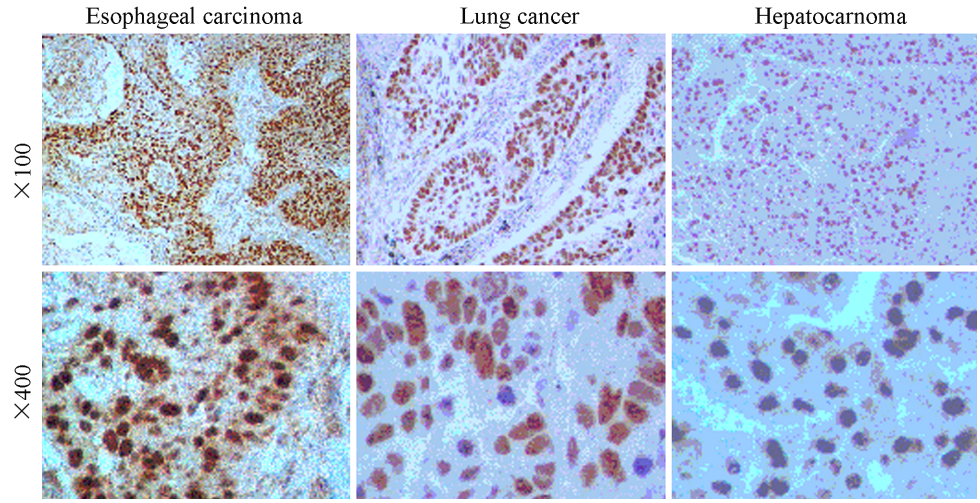

Figure 3. Representative Thy-1 immunostaining in esophageal cancer, lung cancer and hepatocarcinoma samples. Strong expression of Thy-1 was detected in cancer cells and the adjacent normal cells showed weak staining, (×100 and ×400 magnifications for upper and lower panel, respectively).

Thy-1 Protein Expressed in Malignant Tumor Cells Specially

We grouped all the malignant tumor cells as one group (malignant cells group) and normal tissue cells as another group (normal cells group). We tested Thy-1 expression status difference between these two groups using One-Way ANVOA analysis method. As shown in Table 2 there were 734 samples as positive expression of Thy-1 and 717 samples as negative expression of Thy-1 in malignant cells group. There were 144 samples as negative Thy-1 expression and no positive expression in normal cells group. The F value between the two groups was 147.229 and the P value between the two groups was below 0.0001. So Thy-1 expression status of malignant tumor group was significantly different from normal cells group.

DISCUSSION

A common concern is whether the small core

samples used in TMA analysis give reliable information on large tumor specimens. However, we should keep in mind that the basic principle of TMA analysis is fundamentally different from conventional histological analyses. This technology is a population-level research tool but not intended for making clinical diagnoses of individual cases.

Table 2. Statistic analysis of Thy-1 expression status

	Positive	Negative	F	Sig.
Malignant cells	734	717	147.229	P<0.0001
Normal cells	0	144		

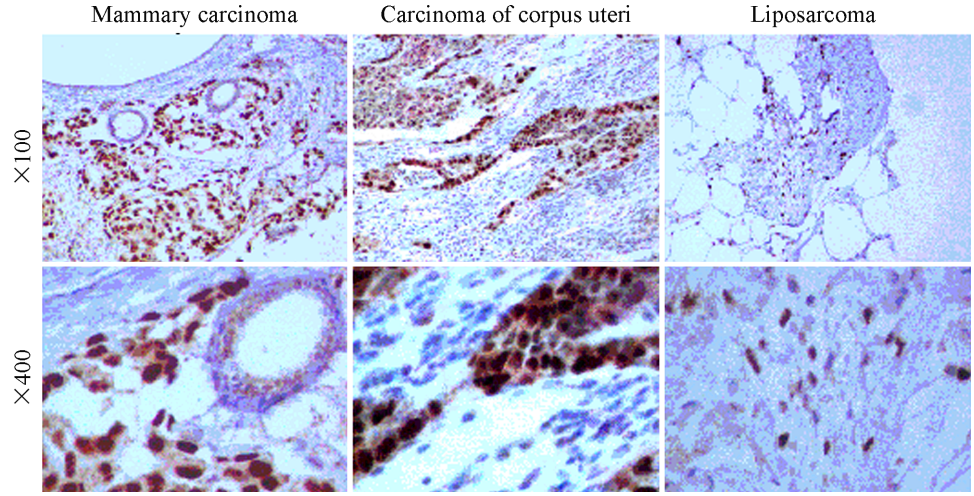

Figure 4. Representative Thy-1 immunostaining in mammary cancer, corpus uteri cancer and liposarcoma. Strong expression of Thy-1 was detected in tumors and the adjacent normal cells showed weak staining, (×100 and ×400 magnification for upper and lower panel, respectively).

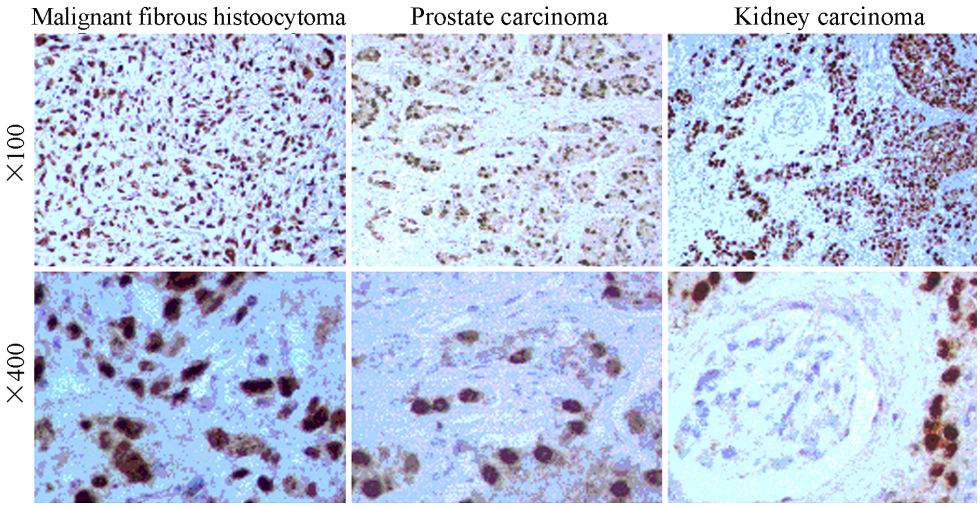

Figure 5. Representative Thy-1 immunostaining in malignant fibrous histiocytoma, prostate cancer and kidney cancer samples. Strong expression of Thy-1 was detected in tumors cells and the adjacent normal cells showed weak staining, (×100 and ×400 magnifications for upper and lower panel, respectively).

Thy-1 is also called CDw90. The human Thy-1 gene is located on chromosome 11.22. The Thy-1 protein is a glycosyl-phosphatidylinositol-anchored member of the immunoglobin superfamily (IgSF). It was originally described as a cell differentiation marker, which expressed predominantly in mouse brain and thymus[2, 11]. It was clear that Thy-1 is expressed in many different tissues, such as brain and kidney[12]. Mature human Thy-1 protein consists of a polypeptide core of 111 amino acids derived from a 161-amino acid precursor. In brain Thy-1 is expressed exclusively in neurons[13], forming 2%–7% of the total surface proteins and representing one of the most abundant cell-surface glycoproteins in nervous systems. It has been shown to function as an adhesion molecule. Thy-1 plays a role in heterophilic cell adhesion, mediating Ca^{2+}-independent cell contact between mouse thymocytes and thymic epithelial cells[14]. Thy-1 is able to trigger mouse thymocyte death in vitro through a bcl-2-resistant mechanism[15]. Mason et al.[16] found that TNF can induce Thy-1 expression, so it is possible that human endothelial Thy-1 may interact with leucocytes during their adhesion and migration into tissues during inflammation. Thy-1 may have many functions in different physical process in human cells. Thy-1 has been demonstrated to affect adhesion, signal transduction and motility in different cell types, such as the adhesion of thymocytes to thymic epithelium, the activation of lymphocytes and neurite outgrowth on astrocytes[17, 18]. Several groups indicated that Thy-1 could trigger many cellular functions, such as proliferation, lymphokine release, differentiation, and apoptosis.

Recently, several studies have shown that Thy-1 is a putative tumor suppressor gene in ovarian and nasopharyngeal carcinoma[19–21]. Abeysinghe et al. reported that fibronectin and thrombospondin, which are involved in cellular differentiation and the regulation of tumor angiogenesis, were found to be up-regulated upon Thy-1 induction. In contrast, the gene SPARC was found to be independent of Thy-1 expression. Their study suggested that Thy-1 plays a critical role in regulating downstream genes associated with the regulation of ovarian tumor growth and cellular differentiation[15]. Thus, Thy-1 may have an important role in other tumor process and we focused on Thy-1 expression status in different malignant tumors.

The primary objective of this study was to determine the potential value of Thy-1 expression characteristics in different malignant tumors. Our previous study has demonstrated the different Thy-1 expression status between non-small cell lung cancer cells and the adjacent normal cells. Nearly all the Thy-1 positive staining cells were cancer cells. In univariate and multivariate analysis for 91 NSCLC patients we found TNM staging, lymph node status and Thy-1 overexpression in nuclei were independent factors to affect the prognosis of NSCLC patients[9]. In this study, we examined Thy-1 expression in 1451 cases of different type of malignant tumors and 144 normal or benign lesion tissues, and almost all the positive staining was observed in malignant tumor cells except the chronic cervicitis. These results suggest that Thy-1 could be a potential tumor marker in surgical pathology diagnosis, and it may have some important role in tumor development and progression, which may be in clinical diagnosis in the future. HPV inflammation has some relationship with cervix carcinoma. When chronic cervicitis develops into atypical hyperplasia, it will be a precancerous lesion for cervix carcinoma. In this research we found that Thy-1 had low expression in cervicitis, which indicates that Thy-1 might have some role in the process from precancerous to carcinoma. Several reports demonstrated that Thy-1 was an activation marker in human dermal microvascular endothelial cells for adult but not embryonic angiogenesis, which implies that Thy-1 should be involved in the progression of tumor metastasis[22]. The research on the relationship between Thy-1 expression and the precancerous lesion will help to understand the molecular mechanisms in carcinogenesis process.

In summary, there is a specific overexpression of Thy-1 in malignant tumor cells compared to normal or benign tumor tissues. Thy-1 may be a potential tumor marker for pathological diagnosis in malignant tumors and may play an important regulatory role in carcinogenesis and tumor progression. Additional studies will be required to demonstrate these initial observations.

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