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Cancer Testis Antigen (CTA) T21, as a Potential Diagnostic, Prognostic, and Immune-Therapeutic Targets for Malignant Tumours.

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ABSTRACT

CTAs, are a family of tumour-specific shared antigens that represent promising targets for cancer immunotherapy, as well as, diagnostic and prognostic markers for tumour development. T21 is a novel antigen with little information known about its protein expression in malignant cells. This study was aimed at evaluating the expression of T21 antigen as a potential diagnostic and prognostic marker for diverse malignancies. Material and methods: IHC staining was applied using a mono-specific polyclonal antibody against T21 to diverse paraffin-embedded malignant tissue microarrays. The specificity of the staining was confirmed by the negativity of the isotype controls. The slides were imaged to visualise the positive T21 staining using an inverted light microscope (x 10) with digital net camera. IBM-SPSS statistic software, version 22, was used for descriptive and statistical data analysis. Results: T21 was expressed in a large percentage of the examined primary (84.9%) and metastatic (65%) tumour sections. T21 expression was evident in ccRCC, adenocarcinoma of small intestine, testicular tumours, adenocarcinoma of rectum, squamous cell carcinoma of oesophagus and astrocytoma. For metastatic tumours, the expression was remarkable in metastatic squamous cell carcinoma of unknown origin, metastatic breast carcinoma and metastatic thyroid carcinoma. There was no correlation the between T21 expression and tumour staging. Conclusion: T21 represents a potential target for cancer immunotherapy and vaccination. Overexpression of T21 antigen represents a useful diagnostic biomarker for cancer progression, but caution should be used when considering its potential for use as a prognostic marker. Further inclusive work focusing on the correlation of T21 expression and tumour staging is needed.

مستضد السرطان T21 (CTA) كهدف محتمل للتشخيص والتنبؤ والعلاج المناعي للأورام الخبيثة

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الكلمات المفتاحية:
المستضدات الخاصة بالأورام
المستضد T21
التنبؤ وتشخيص السرطان

الملخص

عائلة (CTA) تعتبر من المستضدات الخاصة بالأورام وتمثل حاليا هدفا واعدا في العلاجات المناعية للأورام. يعتبر المستضد T21 من المستضدات الجديدة مع القليل من المعلومات المتوفرة عن مستوي تعبيره البروتيني في الخلايا السرطانية. هدفت هذه الدراسة على تقييم تواجد المستضد T21 في الانسجة السرطانية المختلفة وإمكانية استعماله كعلامات تشخيصية وتنبؤيه محتملة لمختلف الأورام. أظهرت الدراسة تواجد هذا المستضد بنسب متفاوتة في بعض الأورام مثل اورام الأمعاء، البروستاتا، القولون، والمرئي، خصوصا في الأورام الأولية منها وبنسبة تصل الي 84.9%. كما أوضحت الدراسة عدم وجود ارتباط بين درجة تعبير المستضد T21 ومرحلة الورم. الزيادة في تواجد المستضد T21 في الانسجة السرطانية يدل إمكانية استخدامه كعلامة بيولوجية لوجود

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السرطان، الا انه يجب النظر في إمكانية استخدامه كعلامة تنبؤيه لتطور مراحل المرض. المزيد من الدراسات التي تركز على الارتباط بين نسبة تواجد وتعبير المستضد T21 مع مراحل المرض حاليا مطلوبة.

1 Introduction

1.1 Tumour antigens.

Tumour antigens are immunogenic proteins that are either expressed on the surface of tumour cells or could be secreted into the blood stream (1). Tumour antigens can also be an intracellular molecule or gene; its synthesised peptides can become the targets of a tumour-specific T-cell response (1; 2). In consequence, this can lead to cytotoxic immune responses against the tumour cells, which is crucial for cancer immunotherapy and cancer vaccination approaches (3).

Tumour antigens are classified into various categories with their diverse expression profiles. Onco-foetal antigens (i.e. alpha-fetoprotein and carcino-embryonic antigen (CEA)), extracellular antigens, are typically expressed in early foetal tissues and tumour cells, but not in normal adult somatic tissues (4). These antigens are frequently considered in clinic as tumour markers of several tumours such as liver, colon, breast, and pancreatic cancer (4). Tissue-specific differentiation antigens (i.e. tyrosinase, TRP-1, and GP100) are also tumour markers that are expressed by malignant cells and their normal counterpart in a linage specific manner, whereas, their expression in other normal adult tissues are undetectable (5; 4). Other categories of tumour antigens are known as tumour-specific shared antigens, or cancer-testis antigens (CTAs). Their expression is up-regulated in germ-line cells and diverse tumour cells, but not in their normal counterpart (6; 2). Actually, because the unique expression of CTAs, they signify as potential targets for cancer diagnosis, prognosis, and immunotherapy approaches.

1.2 Definition of Cancer Testis Antigens

CTA are heterogeneous multigene families, predominantly expressed in gametogenic tissues and cancers (6). About 44 different CTA gene families have been identified up to date using T-lymphocyte epitope cloning or SEREX approach (7). Most of CTAs are expressed at mRNA levels, with a lesser extent to protein levels (7; 6). Roughly, 19 of the CTA families encode immunogenic proteins that provoke immune-reactivity in humans (6; 2). The majority of CTAs genes direct protein transcription during cell growth (8). For example, HAGE gene, a member of the CTA family, encoding a DEAD-box protein, which is integrated in RNA metabolism, embryogenesis, and spermatogenesis (9). Also, CTAs have been allied to stem cell biology, and are associated with early embryonic development. NY-ESO, MAGE, and SSX cancer testis antigens have up-regulated expression in mesenchymal stem cells in the bone marrow (10).

1.3 Expression of CTAs during cancer development

To date, there is an argument as to whether up-regulation of CTAs is linked to cancer development and progression, or if they are randomly expressed in malignant tissues. In 2006, a study conducted by Sharma *et al* has indicated a strong correlation between CTAs expression and progression of urothelial carcinomas of the urinary bladder. The study showed that at least one CTA was expressed in 77% of the examined samples and 61% of these tumours expressed more than one CTA (11). Another recent systematic review and Meta-analysis of 7428 patients and 44 studies have reported an overexpression of CTAs members, with MAGE-A subfamily linked to poor prognosis in multiple cancers (12). Thus, it could serve as a potential prognostic marker of poor prognosis in cancers. Furthermore, up-regulation of the CTAs HAGE, MAGE-A3 and MAGE-A6, has been concomitant to blast crisis in patients with chronic myeloid leukaemia (13), suggesting that expression of CTAs is likely associated with tumour development and progression.

Cancer immunotherapy and vaccination is signified as a safe and promising approach for cancer therapy (6; 10). It is based on stimulation and enhancement of the cellular and the humoral immunity of the host against cancer cells (6). CTAs represent a suitable target because of their ability to enhance both cellular and humoral immuno-reactivity via stimulation of CD8+ and CD4+ T lymphocytes (10). Spontaneous immuno-response against MAGE-A, NY-ESO-1, and SSX has also been recognised in melanoma and leukemia patients (12; 13). Furthermore, humoral and cellular immune responses against NY-ESO-1 have been detected, and the specific epitopes have been recognized as the recognition sites for CD8+ cytotoxic T lymphocytes (1; 12; 14). In clinical trials, NY-ESO-1–restricted immunogenic peptides exhibit potential antitumor effects (15; 14). Moreover, certain constructed peptides from the CTA subfamily have been considered a key tool for the protective and effective immunotherapy of some tumours. For example, the cancer peptide vaccine S-288310, which is a constructed peptide vaccine from the DEPDC1-CTA subfamily, and has shown to be well tolerated with an effective outcome among patients with advanced urethral carcinoma in phase I/II clinical trial (16; 17). This peptide is composed of two HLA-A*24:02-restricted peptides that are derived from M-phase phosphoprotein 1 (MPHOSPH1), and DEP domain-containing 1 (DEPDC1) onco-antigens (17). At this point in time, personalized treatment for cancer has become a trend, and accumulating evidence suggesting that CTAs are highly suitable targets for cancer vaccination and therapy because of their tissue-restricted expression and pre-defined immunogenicity, particularly in solid tumours (16) is emerging.

1.4 Expression of Testis clone 21 (T21) in malignancies.

T21 gene is one of the new CTAs identified by using a SEREX approach to screen a normal testicular cDNA library with sera from prostate cancer patients (18). The gene is positioned at chromosome 12q21.23 and fully sequenced to 534 amino-acids with 19 exons (18). However, its biological function is still not fully understood, but presence of bZIP sites at position 60-67, 130-165, and 424-453 of the gene suggesting that T21 gene product might act as transcription factor (18). Lately , T21 gene has been acknowledged as being homologous to CEP290 gene with the exception of a unique T21 region derived from a CEP290 intron (19). CEP290 protein was found to play a role in centromeres, cilia, and basal bodies function (20). Up-regulation of T21 gene was found in germ cells of the testis and diverse malignant tissues, however generally not in other normal tissue counterparts (18).

T21 was firstly identified as a promising prostate-associated tumour antigen as a significant up regulation in T21 expression was found in malignant prostatic glands relative to benign prostatic glandular tissues and stroma, and in this particular type of malignancy it is associated with tumour stage (18; 21). Silencing of T21 in a prostate cancer cell line significantly inhibits cancer cell proliferation, suggesting a crucial role of T21 in prostatic cancer cell survival (21). However, RNA expression levels of T21 does not always correlate to protein expression level of the gene. T21 also represents a potential and promising target for cancer immunotherapy and vaccination of other malignancies such as breast, kidney, ovary, melanoma, colon and stomach cancer. Hence, this study is aimed to evaluate cancer testis antigens T21, as tumour biomarker and prospective prognostic indicators for a variety of tumours using an immunohistochemistry staining technique.

2 Materials and methods

2.1 Patients and tissue samples

Three matched histological slides of formalin-fixed paraffin-embedded tissue samples from US Biomax (USA) were used for T21

staining. Two slides were stained for T21 expression, and the other for isotype control. Each slide contained tumour microarray cores of various tumours at different stages. Each core measured 1.5mm in diameter and 5µm in thickness. Cores of normal tissue samples that were obtained 1.5 cm away from tumour site, were also included in the microarray tissue samples.

2.2 Antibodies

Mono-specific polyclonal rabbit antibody (PACIFIC immunology, USA) against T21 antigen was used as a primary antibody. For negative controls anti-rabbit polyclonal antibody (Affinity Bio-Reagents (ABR), USA) was used. Biotinylated goat anti-rabbit polyclonal immunoglobulin (Dako, USA) was used as secondary antibodies to visualise immune-reactivity of primary antibodies. All of these antibodies were reconstituted and stored according to their manufacturer's instructions.

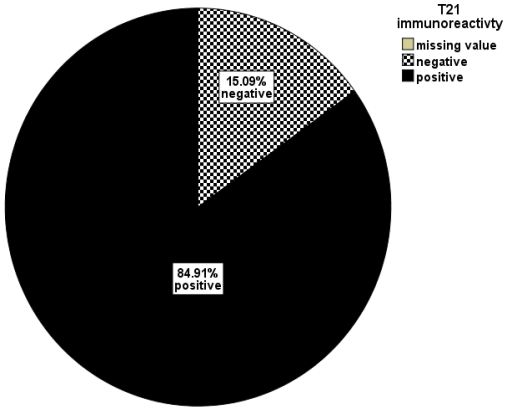
2.3 Immunohistochemically (IHC) Staining Techniques

In the current study, IHC staining of paraffin embedded sections (TMA) was performed to look at T21 expression in diverse malignancies. The sections were de-paraffinized and rehydrated by incubation of the examined slides in an oven at 60 ^{0}C for 2 hours. Then, a series of xylene concentrations (xylene 1 & 2) and ethanol concentrations (100%, and 70%) (Fisher scientific, UK) were applied in a fume hood for 5 and 3 minutes, respectively, at room temperature. For antigen retrieval, the slides were incubated with boiled citrate phosphate buffer (pH 6.0, for 10 minutes). The slides then were rinsed with running tap water for 5 minutes, and then allowed to stand in the water bath for 15 minutes. To block endogenous peroxidase activity, 0.3% hydrogen peroxide for 5 minutes in a black box was applied. Non-specific binding sites were blocked with 10% normal goat serum (Sigma, UK) for 20 minutes. To target T21 antigen, primary antibodies against T21 were diluted using 5% goat serum, and applied on the section overnight at 4 ^{0}C. To visualise the immune-reactivity, the slides were incubated with diluted biotinylated goat anti-rabbit polyclonal secondary antibody for 30 minutes at room temperature. Then, the immunoreactivity of the antibodies was visualised using the Avidin-Biotin-Complex system (ABC) (Vector Laboratories Ltd, UK) for 30 minutes, followed by incubation of the slides with DAB reagent (3, 3 diaminobenzidine tetra-hydrochloride, Vector Laboratories Ltd, UK) until appearance of a specific brown colouration. Following colour development, counterstaining and dehydration of the slides was performed. Gills haematoxylin stain (Sigma, UK) was applied to the slide for 10 seconds for counterstaining. Following that, a series of ethanol concentrations (70% for one minute, 100% ethanol (1) for one minute, 100% ethanol (2) for 2 minutes), and xylene concentrations (xylene 1 and 2 for one minute each) were applied to dehydrate the sections. After covering and mounting of the slides using DPX mounting medium (Fluka BioChemika, Switzerland), the slides were examined for T21 positivity under an inverted light microscope (x 10) with digital net camera (Nikon, Japan). The images were campared against isotype control staining, which was used to determine any potential background staining. SPSS software, version 22, was utilised for the statistical analysis.

3 Results

3.1 T21 is highly expressed in various primary tumours

To evaluate T21 expression at the protein level in primary malignancies, 161 tissue cores of diverse tumours were stained for T21 antigen using an IHC staining technique. T21 staining was categorised to positive or negative by appearance of a brown colour at the stained sections (positive staining). The specificity of the staining was confirmed by negativity of the isotype controls. Our results showed overexpression of T21 antigen in nearly 84.9% of the total examined cases of primary malignancies of diverse organs (Figure 1). The expression was not noticeable in normal adjacent tissues or isotope controls. The expression was evident in ccRCC and adenocarcinoma of small intestine (i.e. the T21 positivity was 100% of the all examined samples) (Table1). T21 antigen was also overexpressed in other tumours including: testicular seminoma (92% positive), adenocarcinoma of rectum (91.6% positive), squamous cell carcinoma of oesophagus (95% positive), adenocarcinoma of colon (83% positive), adenocarcinoma of the stomach (75% positive), astrocytoma (90% positive) (Table 1). The expression of T21 in lung squamous cell carcinoma was lower than other tissues examined as it was detected in only 50% of the examined samples (Table 1).



T21 immunoreactivty

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	135	83.9	84.9	84.9
	negative	24	14.9	15.1	100.0
	Total	159	98.8	100.0	
Missing	System	2	1.2		
Total		161	100.0		

Figure 1: T21 immunoreactivity in various primary tumours of different organs. T21 staining was positive in nearly 84.9% of the examined cases, and only 15% of the cases were negative for T21 expression.

Table 1: T21 Immunoreactivity in primary tumours. T21 expression is noticeably high in tissue cores of primary tumours. The expression was evident mainly in ccRCC (nearly 100% positive), and adenocarcinoma of small intestine (100% positive), but was not so highly expressed in squamous cell carcinoma of lung (only 50% positive).

	T21 immunoreactivty		
	positive	negative	missing value
	Count	Count	Count
Seminoma	12	1	0
Non-Hodgkin's Lymphoma of Tests	4	2	0
adenocarcinoma of recrum	11	1	0
Clear cell carcinoma	20	0	0
Squamous cell carcinoma of lung	9	9	0
Astrocytoma	18	2	0
Squamous cell carcinoma of esophegus	19	1	0
adenocarcinoma of stomach	15	5	0
adenocarcinoma of small intestine	12	0	0
adenocarcinoma of colon	15	3	0

In colon cancer, T21 staining was not detected in mucinous adenocarcinoma subtype, suggesting a specific expression pattern of T21 antigen at certain tumour subtype of colon cancers. Low expression intensity of T21 was restricted to the epithelial cells of the normal intestinal glands of colon, but not in stroma. However, high levels of T21 expression were found in both malignant (A) and corresponding normal (B) tissue of the stomach. The expression was restricted to gastric glandular tissues rather than stroma (Figure 2).

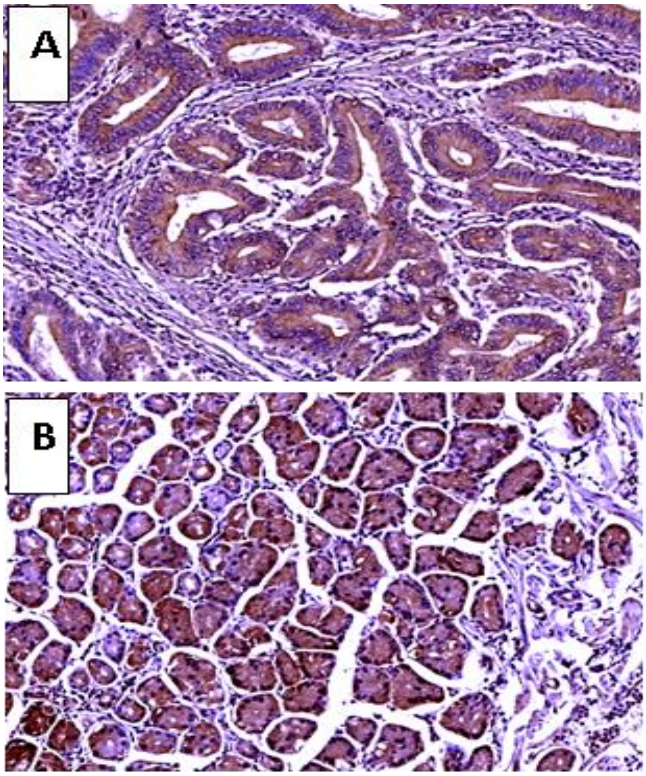
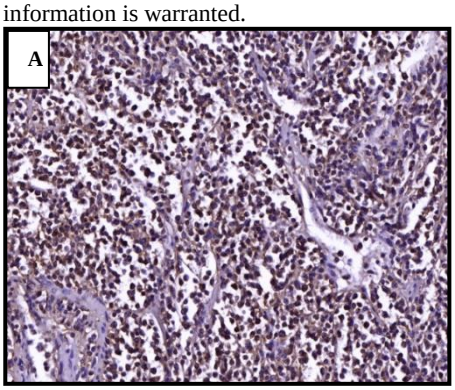


Figure 2: IHC staining for T21 antigen at malignant and normal glandular stomach tissues. T21 staining in stomach adenocarcinoma grade I (A): positive T21 staining at glandular tissues, mainly at the endothelial layer but not at the stromal tissues of the normal stomach tissue sections (B)

Since T21 expression was initially considered as a potential target for prostatic cancer, here we assessed expression of T21 as diagnostic biomarker for testicular tumours. Our results showed overexpression of T21 antigen in testicular tumours generally. The positivity of T21 staining was as follows: 92% positive at testicular seminoma, 66% at testicular non-Hodgkin's lymphoma cases (Table 1). T21 staining in normal cases were restricted to the germ cells "spermatocytes" in seminiferous tubules of the testis without noticeable staining in the surrounding normal interstitial tissues (Figure 3 & 4), suggesting T21 antigen could be a promising biomarker for testicular tumours, particularly, testicular seminoma.

To outline, expression of T21 was noticeable at malignant tissues compared to negligible levels at normal counterparts and stromal tissues except for normal stomach tissue. The expression was evident at testicular seminoma, ccRCC, oesophageal squamous cell carcinoma, and adenocarcinomas. T21 is highly suggested to be a diagnostic biomarker for diverse primary malignancies. Although, a more comprehensive study for T21 expression with a larger sample information is warranted.



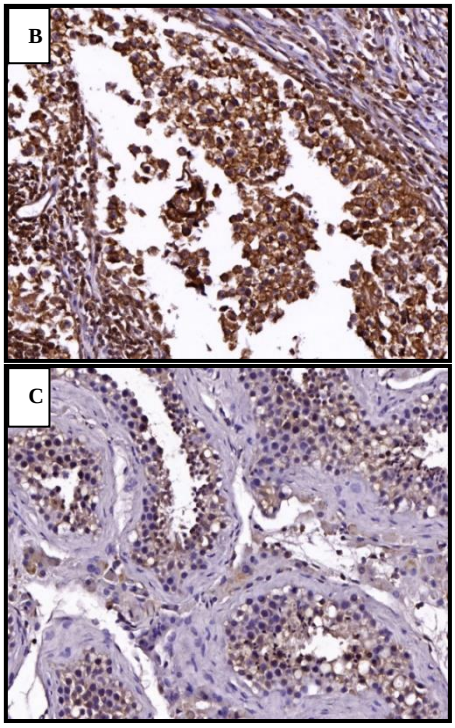


Figure 3: T21 staining at different testicular tumours: positive staining of T21 in testicular seminoma (A); in testicular non-Hodgkin's lymphoma (B); in germ cell lines in normal testicular tissues rather than the surrounding glandular tissues (C).

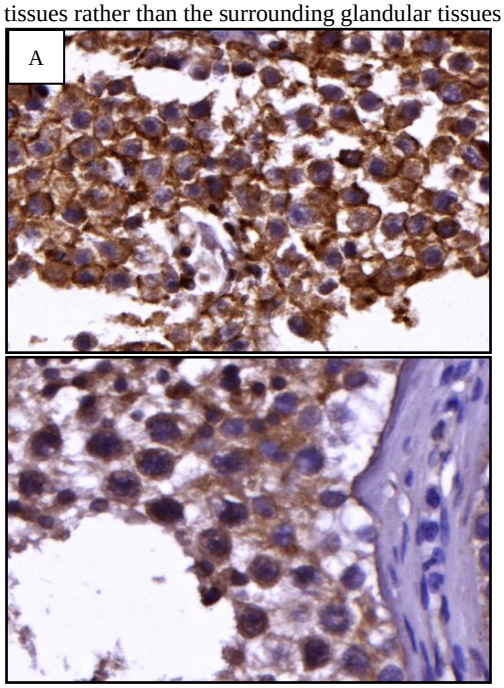
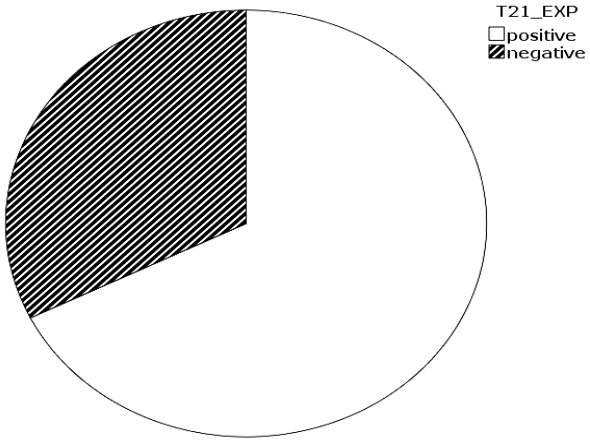


Figure 4: high intracellular staining for T21 antigen in testicular tumour cells (A) relative to testicular germline cells (B).

3.2 High expression of T21 in metastatic tumours

To evaluate if T21 expression correlates with tumour progression, we assessed T21 expression in different metastatic tumour types and in different metastatic sites. Our results showed that T21 antigen was overexpressed in nearly 67.7% of metastatic malignant tissues at different metastatic sites, and the negative expression of T21 was observed in nearly 32.3% of tissues (Figure 5). Expression of T21 antigen was observed at metastatic squamous cell carcinoma of unknown origin, metastatic breast carcinoma and metastatic thyroid carcinoma by nearly 83%, 75%, and 70% of all the examined cases, respectively (Table 3 & Figure 6). With an inferior percentage, T21 antigen was also expressed in other metastatic carcinoma subtypes including lung carcinoma, gastric adenocarcinoma, rectal carcinoma,

squamous cell carcinoma of penis, and pancreatic adenocarcinoma (Table 3 & Figure 6). Though, the expression was negligible in metastatic nasopharyngeal carcinoma, metastatic ovarian cystadenocarcinoma, and metastatic colon carcinoma (Table 3 & Figure 6).



T21_EXP

		Frequency	Percent	Valid Percent	Cumulative Percent
Valid	positive	65	67.7	67.7	67.7
	negative	31	32.3	32.3	100.0
	Total	96	100.0	100.0	

Figure 5: T21 immunostaining at metastatic tumour cells. T21 was positively staining in nearly 67.7% of the examined tissue cores. About 32% of the examined tissues were negative for T21 staining.

M_location * T21_EXP Crosstabulation

Count

		T21_EXP		Total
		positive	negative	
M_location	ovary	3	1	4
	great omen	5	11	16
	neck	9	5	14
	lymph node	23	7	30
	liver	12	0	12
	spleen	3	3	6
	mesentry	5	1	6
	abdominal wall	1	3	4
	lung	2	0	2
	abdomen	2	0	2
Total		65	31	96

Table 2: T21 expression in metastatic cells at different locations. T21 was highly expressed at metastatic tumour cells of lymph nodes (nearly in 78% of the cases) and liver (in nearly 100% of the cases).

When observing the expression in metastatic sites, the highest expression of T21 antigen was observed in metastatic tumour cells from the liver (i.e. at all the examined cases), mesentery (by 83%), lymph nodes (by78%), and ovaries (by 75%) (Table 2 & figure 7). Conversely, T21 expression was not substantial in other metastatic tumour tissues in certain locations; namely, lung, abdomen, neck, spleen, great omentum and abdominal wall (Table 2). Although, the limited numbers of the examined samples should be considered. The current data also showed no evident correlation between frequency of T21 antigen expression and tumour progression. The intensity of T21 staining was not increased with tumour grading. a further study with a larger examined samples and complete clinical data would be advised.

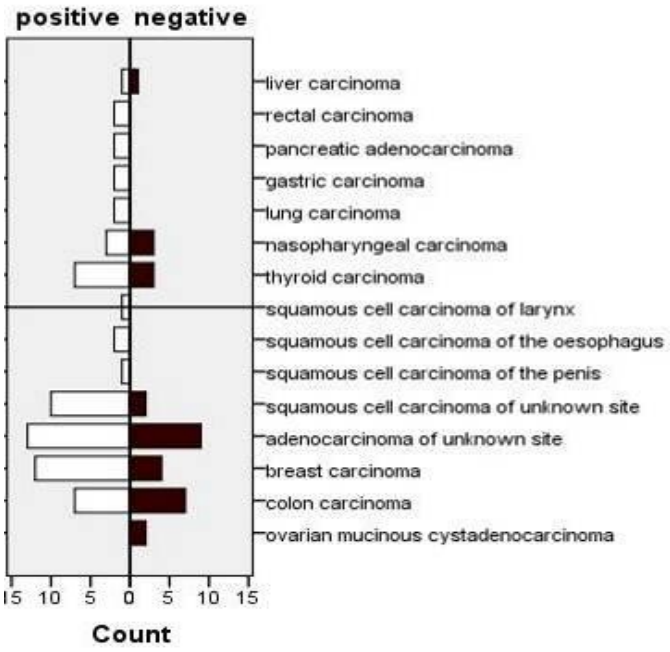


Figure 6: T21 expression in metastatic tumour tissues of different tumour types. T21 antigen was noticeably expressed at metastatic breast carcinoma (75%), metastatic squamous cell carcinoma of unknown origin (83%), and metastatic thyroid carcinoma (70%).

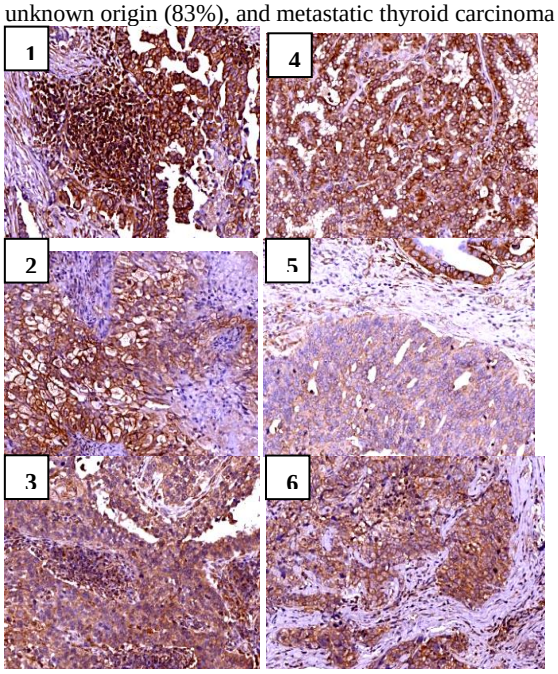


Figure 7: T21 staining in diverse metastatic tumours. T21 expression was noticeable in metastatic breast adenocarcinoma in lymph node (1); metastatic lung carcinoma in lymph node (2), metastatic squamous cell carcinoma of unknown site in the neck (3), metastatic adenocarcinoma of unknown origin in lymph node (4); metastatic thyroid adenocarcinoma in lymph node (5); and metastatic nasopharyngeal carcinoma in the neck (6).

Count

		T21_EXP		
		positive	negative	Total
T_type	ovarian mucinous cystadenocarcinoma	0	2	2
	colon carcinoma	7	7	14
	breast carcinoma	12	4	16
	adenocarcinoma of unknown site	13	9	22
	squamous cell carcinoma of unknown site	10	2	12
	squamous cell carcinoma of the penis	1	0	1
	squamous cell carcinoma of the oesophagus	2	0	2
	squamous cell carcinoma of larynx	1	0	1
	thyroid carcinoma	7	3	10
	liver carcinoma	1	1	2
	nasopharyngeal carcinoma	3	3	6
	lung carcinoma	2	0	2
	gastric carcinoma	2	0	2
	pancreatic adenocarcinoma	2	0	2
	rectal carcinoma	2	0	2
Total		65	31	96

Table 3: T21 expression in different metastatic tumour cells types. T21 staining was noticeably positive in metastatic breast carcinoma (nearly 75%), and metastatic squamous cells carcinoma of unknown origin (nearly 83%).

4 Discussion

Cancer immunotherapy is a hopeful approach for cancer suffering patients, especially with the advanced knowledge about cancer biology and immunology. Cancer immunotherapy is dependent on the stimulation of the host immune response against an appropriate targeted tumour- associated antigen, and ultimately to overcome the host immune tolerance (4; 10). The ideal tumour antigen to target should have a natural immunogenicity to stimulate immune responses selectively against tumour cells. It should also be expressed at high levels and frequency in malignant tissues rather than the normal counterparts, thus potential severe autoimmune reaction can be avoided, particularly if the selected antigen is a self-protein (22). In addition, the selected antigen should not be rare, and expressed in a considerable percentage of tumours from patients with cancer (22). Moreover, the chosen antigen should be vital for tumour cell survival (22; 4).

Recently, CTAs represent an attractive target for cancer treatment and immunotherapy due to their unique expression among diverse malignant tissues. CTAs are highly expressed in several malignant tissues compared to negligible or limited expression in corresponding normal counterparts, except in germ line cells such as "spermatocytes" in testis (8; 2). These antigens also have the ability to induce both humoral and cellular immune responses against cancer cells in the host (6; 2). The majority of CTA families serve as transcription factors, and their expression could be essential for cancer cell survival (7). Some studies have shown up-regulation of CTAs is directly correlated to progression of various tumours and tumour staging (21; 12; 2). Overall, CTAs could be useful diagnostic and prognostic biomarkers for cancer progression, and could be potential targets for cancer vaccination and immunotherapy.

T21 is a new CTA antigen, heterogeneously overexpressed in diverse malignancies with a negligible level in their normal counterparts (18; 21). The current study also demonstrated an overexpression of T21 antigen at the protein level in several malignancies, namely; ccRCC, small intestinal carcinoma, colon carcinoma, oesophageal carcinoma, testicular seminoma and stomach carcinoma. Its expression was restricted to malignant tissues rather than normal counterparts and stroma, with exception for colon and gastric epithelial cells and germ cells "spermatocytes" in seminiferous tubules of the testis. Our findings further support others studies (18; 21) and suggest that T21 antigen could be a promising target for cancer vaccination and immunotherapy. However, as a newly discovered cancer-associated antigen, the biological function and roles of T21 in cancer development are still not fully understood and need to be investigated further before this antigen can proceed forward. A future study investigation the key roles of T21 expression during carcinogenesis would be very interesting.

Indeed, studying the correlation between expression of the targeted antigens with tumour progression and staging is essential for an effective cancer immunotherapy regime, and estimation of cancer prognosis. Studies have shown that the effectiveness of cancer immunotherapy approaches is highly reliant on tumour size and stage, as well as the presence of other combination therapies for cancer (1; 22; 23).

It has also been noticed that expression of certain CTAs positively correlates with tumour progression (24). For instance, expression of BAP31, a newly defined CTA, is linked to progression and metastasis of several malignancies, in particular cervical cancer (25). Also, over-expression of BAP31 has been highly correlated with a poor clinic outcome in cervical cancer (25). In addition, MAGE-C1, a cancer testes antigen, has been shown to have both diagnostic and prognostic values in myeloma cancer (26); its expression could signify an important indicator of early caner relapse and reduced survival in myeloma patients (26). Likewise, overexpression of T21 in correlation to prostatic tumour development has been reported (21). It has been shown that expression of T21 is associated with Gleason grading of prostatic cancer (21). However, data from this IHC study did not indicate a strong correlation between intensity of T21 expression and tumour grading despite its up-regulation in all examined metastatic tumours. This could be due to the limited number of examined cases in each malignancy cohort in this study and increasing the study size is advised. Absence of the clinical information for each examined tumour core also restricted our data analysis. Thus, currently the prognostic efficacy of T21 expression for cancer development should be taken with some caution. Besides, a more inclusive study evaluating the prognostic value of T21 expression during cancer development is the ultimate aim.

5 Conclusion and future direction

T21 antigen, a CTA, is heterogeneously overexpressed in several malignancies, with a negligible level in normal tissue counterparts. T21 expression is noticeable mainly in testicular seminoma, non-Hodgkin's lymphoma of the testis, ccRCC, oesophageal squamous cell carcinoma, and adenocarcinoma. Overexpression of T21 antigen might be useful as a diagnostic biomarker for carcinogenesis, but not as prognostic marker yet. Further validation of our results with a future study involving a larger number of examined malignant cases is desirable. Also, to make a correlation between T21 expression and tumour prognosis, a clinical follow-up study for the examined patients would be advantageous.

6 References

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