World Journal of Gastrointestinal Oncology

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DOI: 10.4251/wjgo.v7.i12.492

World J Gastrointest Oncol 2015 December 15; 7(12): 492-502
ISSN 1948-5204 (online)

Targeting cancer testis antigens for biomarkers and immunotherapy in colorectal cancer: Current status and challenges

Anil Suri, Nirmala Jagadish, Shikha Saini, Namita Gupta

Anil Suri, Nirmala Jagadish, Shikha Saini, Namita Gupta, Cancer Microarray, Genes and Proteins Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India

Author contributions: All the authors solely contributed to this Review.

Supported by Indo-UK Cancer Research Program, No. BT/IN/UK/NII/2006; Centre for Molecular Medicine, No. BT/PR/14549/MED/14/1291; and NII-core funding, Department of Biotechnology, Government of India.

Conflict-of-interest statement: The authors declare that they have no conflict of interests.

Correspondence to: Dr. Anil Suri, FNASc, FAMS, Cancer Microarray, Genes and Proteins Laboratory, National Institute of Immunology, Aruna Asaf Ali Marg, New Delhi 110067, India. anil@nii.res.in
Telephone: +91-11-26703700
Fax: +91-11-26742125

Received: June 15, 2015
Peer-review started: June 17, 2015
First decision: July 27, 2015
Revised: September 21, 2015
Accepted: October 20, 2015
Article in press: October 27, 2015
Published online: December 15, 2015

Abstract

Colorectal cancer ranks third among the estimated cancer cases and cancer related mortalities in United States in 2014. Early detection and efficient therapy remains a significant clinical challenge for this disease. Therefore, there is a need to identify novel tumor associated molecules to target for biomarker development and immunotherapy. In this regard, cancer testis antigens have emerged as a potential targets for developing novel clinical biomarkers and immunotherapy for various malignancies. These germ cell specific proteins exhibit aberrant expression in cancer cells and contribute in tumorigenesis. Owing to their unique expression profile and immunogenicity in cancer patients, cancer testis antigens are clinically referred as the most promising tumor associated antigens. Several cancer testis antigens have been studied in colorectal cancer but none of them could be used in clinical practice. This review is an attempt to address the promising cancer testis antigens in colorectal cancer and their possible clinical implications as biomarkers and immunotherapeutic targets with particular focus on challenges and future interventions.

Key words: Cancer testis antigens; Colorectal cancer; Testis specific genes; Biomarkers; Immunotherapy

Core tip: Despite of the availability of enormous tumor antigens, there is a dearth of targets for biomarkers and immunotherapy for clinical cancer management. Cost-effectiveness and invasiveness associated with colonoscopy hinders its implications in less developed and developing countries. Colorectal cancer treatment including surgery and radiation has significant side effects on normal tissues. Recently a new category of antigens has been discovered which are expressed in tumor cells but not in normal tissues except the immuno-privileged testis. Targeting such antigens would be specific to the cancer cells with no deleterious

effects on normal cells. Scope of these magic bullets in colorectal cancer is discussed in this review.

Suri A, Jagadish N, Saini S, Gupta N. Targeting cancer testis antigens for biomarkers and immunotherapy in colorectal cancer: Current status and challenges. *World J Gastrointest Oncol* 2015; 7(12): 492-502 Available from: URL: http://www.wjgnet.com/1948-5204/full/v7/i12/492.htm DOI: http://dx.doi.org/10.4251/wjgo.v7.i12.492

INTRODUCTION

Colorectal cancer (CRC) is the third most common cancer in both men and women causing the global incidence of more than 1.2 million cases and 600000 deaths every year[1]. Histologically, Adenocarcinoma represents the most common type of CRC (about 95%) and other histotypes include neuroendocrine neoplasms, gastrointestinal stromal tumors, primary colorectal lymphoma, leiomyosarcoma, melanoma and squamous cell carcinoma. Clinically CRC can be classified as genetic/hereditary and non-hereditary/sporadic[2,3]. Hereditary CRCs can be further categorized as hereditary non-polyposis colorectal cancer and multiple polyps CRC which can be further sub-divided in to several subgroups depending upon the genetic basis[4]. Considering the slow development of CRC in comparison to other cancers, early detection of precancerous lesions may significantly improve the efficacy of therapeutic modalities and consequently, reducing the CRC-related deaths. Detection of CRC and its precursors (polyps) mainly relies upon colonoscopy but due to its invasive nature and the cost involved, it has limited applications in developing countries like India[5]. Less invasive detection test such as fecal occult blood test and stool analysis have low sensitivities which highlights the need to explore novel, sensitive, non-invasive biomarkers that can facilitate early detection, staging, disease progression and prediction of therapeutic outcome to determine optimized treatment for CRC. CRC treatment encompasses surgical or endoscopic resection, followed by second line of therapeutic interventions including chemotherapy, radiation and targeted therapy which often causes systemic toxicity and side effects. The toxicity incurred results in compromised quality of life of CRC patients and emphasizes the need to explore other therapeutic modalities such as immunotherapy. Immunotherapy is not commonly used as a treatment option but recent advances in tumor immunology and identification of tumor specific antigens reignited the interest in immunotherapy. Molecular identification of tumor antigens for immunotherapy may pave the way for novel therapeutics and their integration with conventional therapies can have substantial impact towards improving the outcomes of patients with CRC. In this context, cancer testis (CT) antigens are regarded as the promising targets for biomarker development and immunotherapy. The aberrant expression of CT antigens in cancer cells but not in other somatic tissues except testis forms the basis for their clinical implications as biomarkers and immunotherapy[6-8]. Over the past two decades, there is a huge influx of promising clinical studies which revealed significant future prospects to study these Cancer testis antigens for clinical translation.

DISCOVERY OF CANCER TESTIS ANTIGENS

The search for novel tumor associated antigens (TAA) for biomarker development and immunotherapeutic targets led to the identification of distinct categories of TAAs. Broadly, TAAs can be divided as tumor shared antigens [antigens present in both differentiated and cancer cells such as overexpressed antigens (MUC1)] and tumor specific antigens [antigens expressed specifically in cancer cells such as mutated antigens (p53, Ras)]. However, both tumor shared antigens and tumor specific antigens have their respective limitations which hamper their clinical implications. Tumor shared antigens cannot serve as targets for biomarker and targeted therapy because of their non-specific expression in other somatic tissues whereas tumor specific antigens are not abundantly expressed in cancers. Interestingly, in the early 1990s, a unique class of TAAs designated as CT antigens was identified which are primarily expressed in germinal cells of immuno-privileged testis and placenta and yet exhibits aberrant expression in multiple malignancies. The term cancer testis antigen (CTA) was coined by Old *et al*[9]. Melanoma associated antigen-1 (MAGE-1) was the first identified CT antigen which exhibited autologous T cell response in melanoma patients[10]. Later, it was shown to be expressed in several other malignancies as well[11,12]. The method (T cell epitope cloning) used for the identification of MAGE-1 was based on *in vitro* stimulation of peripheral blood cells with autologous tumor cells and subsequent gene identification by re-stimulation with cells transfected with cDNA libraries of tumor cells. Employing the same strategy, some other members of MAGE family (MAGE-A2, MAGE-A3), BAGE and GAGE-1 were identified[13-16]. Later, serological analysis of cDNA expression libraries (SEREX) was developed and led to the discovery of several CT antigens including synovial sarcoma/X breakpoint 2 (SSX-2) and New York oesophageal squamous cell carcinoma 1 (NY-ESO-1)[17-19]. Shortly after this, differential gene expression libraries were employed to compare total mRNA of normal tissues *vs* testis and resulted in the discovery of Sperm associated antigen 9 (SPAG9) and A-kinase anchor protein 4 (AKAP4)[20,21]. To compile the growing list of CT antigens, a database was developed by Ludwing Institute for Cancer Research (http://www.cta.Incc.br/)[22]. So far, more

than 180 members of CT antigens have been identified. Because of their exceptionally restricted expression in cancer cells, CT antigens are considered as excellent targets for diagnostic and prognostic biomarkers and immunotherapy. Theoretically, targeting these CT antigens will not cause any deleterious side-effects on normal cells[23]. However, there are many challenges to be addressed before translating their implications from benchside to bedside.

CANCER TESTIS ANTIGENS IN COLORECTAL CANCER

There is growing line of evidences indicating the expression of several cancer testis (CT) antigens in CRC. However, only few of the CT antigens exhibited high frequency of expression that could provide clinical applications. Chronologically, the first CT antigen characterized in CRC was MAGE family[10]. MAGE family of genes comprises of over 65 genes that are encoded from X chromosome[24]. Their function in germ cells of testis is poorly defined but they are highly immunogenic in cancer patients, generating both humoral as well as cytotoxic T cell responses. That's why there are several ongoing clinical studies to analyze the antitumor immunotherapeutic potential of MAGE antigens and/or their epitopes. It has been demonstrated that CRC tissues expressed some of MAGE antigen with low frequency, particularly, MAGE-A1 and MAGE-A3. Importantly, there are few contradictory reports regarding other members of MAGE family such as MAGE-A12, MAGE-B1 and MAGE-B2[24,25]. These antigens were demonstrated to have no expression in 34 CRC specimens tested by employing RT-PCR[24]. In an independent study by Burgdorf *et al*[26], 47% liver biopsy specimens with metastatic CRC were shown to express six distinct members of MAGE family (MAGE A-1, A-3, A-4, A-6, A-10 and A-12). Furthermore, MAGE-A12 expression was also shown in disseminated tumor cells (found in blood) of CRC patients[27]. These findings are corroborated with the earlier studies of MAGE antigen in melanoma where, MAGE-A1 expression was demonstrated in 48% of metastatic melanoma *vs* 16% of primary melanoma indicating the possible correlation of MAGE expression in late metastatic cancers[28]. Such inter-tumor variations in the expression of CT antigens are common. Yet, another study by Chen *et al*[29] in 250 CRC tissue specimens revealed that 36% of CRC specimens expressed at least one member of MAGE-A family. So far, MAGE-8 is the member of MAGE family that displayed highest frequency of expression (44%) in CRC tumor specimens[30]. Apart from MAGE family, in a cohort of 121 CRC patients, it was demonstrated that several CT-X antigens are expressed in CRC tissue specimens in contrast to matched adjacent non-cancerous tissues including *SCP-1* (1.7%), *SSX-2* (2.5%), *SSX-4* (2.5%), *SSX-1* (5.0%), *CT10* (6.6%), *NY-ESO-1* (9.9%), *MAGE-1*, (11.6%) *LAGE-1* (15.7%), *MAGE-4* (22.3%) and *MAGE-3* (27.3%)[31]. While most of the CT antigens are testis-restricted, some of them also show weak expression in normal tissues and are termed as testis specific genes. Some examples of testis specific CT antigens expressed in CRC includes *HSP105*[32], *GPA34*[33], *RAP80/UIMC1*[34], *TRAG-3*[35], *cTAGE* variants[36], *NY-CO-58/KNSL6*[37], *NW-BR-3*[38], *RBP1L1*[39], *KU-MEL-1*[40], *HSP60*[41], *RNF43*[42], *KIF18A* (*SW#108*)[43] and *TOMM34*[44]. Some of the CT antigens such as ADAM-1, FTHL17, GAGE-1 to 8, MORC, MMA-1A, MMA-1B, PAGE-1, RAGE-4, SCAGE-ac, SGY-1, SPO11, TAF2Q, TDRD, TEX15 and TPX-1are reported to be not expressed in CRC tissue sections[24,45-47]. However, these studies were conducted with small sample size hence confirmation in a large cohort is required to validate the results.

FUNCTIONS OF CT ANTIGENS

A lot of clinical research and trials have been conducted to explore the clinical potential of Cancer testis antigens but their role in carcinogenesis is still poorly-understood. CT antigens are proposed to be activated due to global demethylation associated with carcinogenesis[48,49]. In a different speculative proposal, CT antigen expression is considered as a part of gametogenesis gene activation program that imparts the oncogenic potential and malignant properties to a neoplastic cell[50,51]. At the same time, these CT antigens being highly immunogenic also render the cancer cells prone to the immuno-surveillance thereby raising a concern about their positive role in cancer progression. To understand the role of CT antigens in metastasis, Alves *et al*[52] compared the expression of CT antigens in primary and metastatic lesions and found no significant difference between the two sets indicating no correlation of CT antigen expression with metastasis. However, there are some CT antigens including prostate-associated gene 4, SCP-1, and SPANX, expression of which is directly correlated with liver metastasis of CRC[29]. Likewise, a well-characterized CT antigen, SPAG9 was also shown to be associated with early stages of CRC suggestive of its potential implications as an early diagnostic biomarker. In addition, the role of SPAG9 is also proposed in cellular migration and invasion as depicted by reduced migratory and immiratory potential of CRC cells post-siRNA mediated downregulation of SPAG9[53]. Another testis-selective cancer testis gene, *TSP50*, was demonstrated to be associated with poor prognosis in CRC[54].

Few of the CT antigens were shown to have functional relevance as well. For example, MAGE family members are proposed to be involved in modulation of p53[55]. Outside the *MAGE* gene families, antiapoptotic properties of GAGE-7 have been reported, as GAGE-7C was shown to render a human tumor-derived cell line resistant to apoptosis induced by interferon-γ (INF-γ) or Fas and also prevented killing induced by taxol

and ionizing radiation[56]. Yet another CT-X antigen, AKAP4 interacts with cyclic adenosine monophosphate dependent protein kinase A and is involved in sperm motility[57]. In contrast to our very limited knowledge of CT-X function, most of the non-X CT antigens have well-defined roles in spermatogenesis and fertilization. For instance, SCP-1, is a part of the synaptonemal complex and is involved in chromosome pairing during meiosis[58], OY-TES-1 acts in acrosin packaging in the acrosome of sperm heads[59], SPO11 is a meiosis-specific endonuclease[60] and the brother of the regulator of imprinted sites is a recently described paralog of the epigenetic modulatory protein CCCTC-binding factor (CTCF), and is involved in the epigenetic reprogramming occurring during spermatogenesis[61]. SPAG9 is a sperm-associated JNK-binding protein that has a role in spermatozoa-egg interaction[62]. Although, some of the CT antigens such as MAGE family members and NY-ESO-1 have been well characterized even in clinical studies and trials, we still have limited knowledge about how these CT antigens contribute in cancer cell development and evolution. In this context, there are reports suggesting that CT antigens are intrinsically disordered proteins which play important roles in transcriptional regulation and signaling *via* regulatory protein networks in cancer cells[63]. Considering the wide ranged expression of more than 170 CT antigens in tumors of different histological origins at different stages of disease progression, it can be speculated that these testis specific genes are activated as a part of dedifferentiation program during carcinogenesis and are crucial for tumor development. During tumorigenesis, neoplastic cells undergo tremendous metabolic stress and bypass several anti-tumor processes including apoptotic signals and immune attack[64]. Under such stressful conditions, it would be quite inappropriate for a cancer cell to channelize its energy towards gene activation and formation of proteins which are irrelevant to the cancer cell. In fact, cancer cells would selectively use the part of the cellular energy to activate a set of gene expression program involved in gametogenesis and embryogenesis, in order to counteract stress signals and attain the malignant characteristics such as motility and invasion which eventually helps the cancer cells to thrive under stressful conditions. As for now, the functional relevance of CT antigens might not be well characterized but their role in carcinogenesis seems to be very vital.

CURRENT CHALLENGES

Although CT antigens are undoubtedly the sure-shot promising targets for various clinical interventions based on their unique expression patterns, there is a marked variation in the expression frequencies observed by different studies. *CT* genes are classified into three major groups based on their expression pattern which include testis restricted, testis/brain restricted and testis selective. This classification is based on genome wide analysis of gene expression data that showed out of 153 *CT* genes, 39 genes are present only in adult testis and placenta classified as testis-restricted, 14 genes are expressed in brain termed as testis/brain-restricted, and 85 genes, ranked in testis selective based on the ratio of testis/placenta expression relative to normal adult tissue[65]. An example of such discrepancy is clearly represented by CAGE-1. Shi *et al*[66] reported the expression of *CAGE* mRNA in 30.8% colorectal tumors whereas, an independent study revealed *CAGE* expression in 90% tissue specimens by RT-PCR. Importantly, both groups found weak expression of CAGE in a portion of normal matched tissue specimens. Such discrepancies might be due to the differences in the experimental procedures, epidemiological variations, and inter and intra-tumor heterogeneity.

There are few CT antigens which are reported in CRC cell lines but not in tissue specimens. Some studies have reported the expression of *CTA* genes in CRC cell lines but not within CRC tissue. Such examples and their expression frequency in CRC cell lines are *MCAK* (5/6), *TAG-1* (4/4), *TAG-2A* (2/4), *TAG-2B* (1/4), *TAG-2C* (2/4)[67,68]. Some of these genes are quite promising however, further studies in tissue specimens are needed to establish their clinical utility. To validate the expression of above mentioned CT antigens, recently, MCAK expression was examined in paired colorectal tumor tissue samples and the corresponding normal tissues of 120 patients. Results showed the expression of MCAK in normal tissues and significant increased expression in CRC tissue specimens which correlated with poor prognosis and lymph node metastasis[69]. The expression of MCAK in normal tissues puts a significant challenge for its clinical implications as a diagnostic biomarker. Yet, another CT antigen POTE was shown to be differentially expressed in 6 of 6 prostate, 12 of 13 breast, 5 of 5 colon, 5 of 6 lung, and 4 of 5 ovarian cancers[70]. However, the expression of *POTE* gene was also confirmed by *in situ* RNA hybridization in normal tissues including prostate, ovary, testis, and placenta[71]. Thus, an important control that should be taken in to account while determining the expression of CT antigens is matched adjacent non-cancerous tissues (ANCT) especially for clinically relevant data. It is also noteworthy to point out the fact that ANCT may not be considered as "normal" because these tissues might have underlying, undiagnosed disease. In this regard, Chen *et al*[72] reported variations in the expression of CTA genes in sets of disease free normal tissues suggesting that CT antigens might be expressed before clinical manifestation or histopathopathological changes in the tissues. Also, depending on the sampling method, ANCT can be a section of the tumor with no morphological signs of hyperplasia but may have underlying genomic lesions that cause CTA expression. This inherent heterogeneity in a clinical challenge while exploring the clinical potential of CT antigens.

In the earlier years of CT antigens identification by SEREX, most of the studies in CRC were focused at gene expression analysis which restricted their clinical

translation because of the variability observed in gene and protein expression of certain CT antigens. In this context, the gene expression of *NY-ESO-1* in CRC was established by several studies ranging from 6% (34/567[49] to 9.9% (12/121)[31] with few exceptions. In particular, Chen *et al*[19] reported no expression of *NY-ESO-1* in CRC (0/16) by employing RT-PCR. In terms of protein expression also, NY-ESO-1 expression was detected in 8.3% (1/12) by immunohistochemical analysis[31] which is again contradicted by another study showing no NY-ESO-1 protein expression[73]. MAGE is another CT antigen which is well-studied in CRC. Immnohistochemical analysis of MAGE family members revealed by Jungbluth *et al*[74] revealed no expression of MAGE family in 15 CRC tissue specimens tested. Later, serological analysis of CRC patients demonstrated anti-MAGE-A3 antibodies in 8% of CRC patients indicating the MAGE antigen expression at least, in a fraction of CRC patients[37]. Such variations in the analysis of expression of CT antigens might also stem from the demographic variations but it is quite important to validate the protein expression in clinical samples to minimize the genomic instability driven discordances. Confirmed antigen expression also paves the way for future immunotherapeutic studies towards designing the better vaccines to improve the mounted immune response.

SPAG9: A CT ANTIGEN THAT STANDS OUT AS A BIOMARKER

Over the past two decades, there is an emergence of innumerable biomarkers and therapeutic targets for various malignancies but it is rare to find a tumor antigen that is expressed in almost all cancers. Interestingly, there is only one CT antigen that appears to be most promising biomarker and therapeutic target among all other antigens. This testis specific gene called as Sperm associated antigen 9 was first identified by Shankar *et al*[20], in 1998 as a testis specific gene having unique palindromic sequences and encoding a leucine zipper dimerization. It is a single copy gene encoded from chromosome 17q21. Further characterization of SPAG9 revealed it as a c-Jun N-terminal kinase-interacting protein involved in MAPK pathway[62,75]. The first report showing the expression of SPAG9 in cancer cells demonstrated its mRNA and protein expression in 90% epithelial ovarian cancer (EOC) tissue specimens but not in matched ANCT specimens. In addition 67% EOC patients exhibited circulating antibodies against SPAG9 suggesting its implications as an immunotherapeutic target[76]. Later, same group demonstrated SPAG9 expression (both mRNA and protein) in renal cell carcinoma[77], cervical cancer[78,79], breast cancer[80,81], thyroid cancer[82], chronic myeloid leukemia[83], colorectal cancer[53] and bladder transitional cell carcinoma[84] establishing its clinical utility as a biomarker. In CRC *per se*, SPAG9 expression was detected in 74% of CRC tissue specimens with no discrepancy in gene and protein expression[53]. Further, humoral response was generated in 70% CRC patients. In addition, depletion of SPAG9 in colorectal cancer cells resulted in inhibition of cellular proliferation, migration and invasion *in vitro*[53]. Recently, SPAG9 serum levels were determined in endometrial cancer patients and the cut off levels of 15 ng/mL could provide the sensitivity of 74% and specificity of 83% to detect endometrial cancers[85,86]. SPAG9 expression was also found in brain cancer/astrocytoma[87,88], prostate cancer[89,90], hepatocellular carcinoma[91], lung cancer[92], vulva cancer and non skin melanoma[93]. Till date, SPAG9 is the most versatile and promising CT antigen that can be clinically translated for biomarker development and immunotherapeutic use. To the best of our knowledge, none of the other CT antigens studied so far have showed such a consistency in clinical data among different studies.

Mechanistically, SPAG9 is involved in cellular proliferation, probably by regulating cyclin proteins as reported in hepatocellular carcinoma and prostate cancer[89-91]. In prostate cancer, the role of SPAG9 is not only restricted to cellular growth/proliferation but also in angiogenesis[90]. In astrocytoma and prostate cancer, SPAG9 is associated with cellular migration and invasion by modulating MMPs[88,90]. Table 1 summarizes the gene and protein expression of SPAG9, serological analyses of SPAG9 antigen levels and antibody responses in different cancers studied so far. There is a growing line of evidences that SPAG9 is indeed important for oncogenic properties of cancer cells and contributes towards tumor progression. Hence, future studies to establish its clinical implications in a large cohort of patients are warranted.

IMMUNOTHERAPEUTIC IMPLICATIONS OF CT ANTIGENS IN COLORECTAL CANCER

The search for tumor-specific and tumor-abundant antigens is still going on to facilitate the rational design of cancer immunotherapy strategies. In CRC, while conventional therapy such as chemotherapy and radiation are useful for the majority of patients, it is not good enough for patients with relapsed cancer and for those of advanced CRC stages. Chemoresistance is another problem that develops with increased exposure of conventional chemotherapy. At that time, immunotherapy can be a good choice to integrate with the conventional interventions to kill the residual tumor cells, strengthen the immune system and further improve the survival rate. There have been limited studies exploring the relevance of CT antigens for immunotherapeutic purposes in CRC patients. MAGE

Table 1 Expression and humoral response of SPAG9 in various cancers demonstrating its clinical relevance as a biomarker and mmunotherapeutic target

Cancer	SPAG9 mRNA expression *n* (%)	SPAG9 Protein expression *n* (%)	SPAG9 expression in matched adjacent non-cancerous tissues	Serological detection of SPAG9 antibodies *n* (%)	Expression in cell lines	Clinical relevance and concluding remarks	Ref.
Epithelial ovarian cancer	18 (90)	18 (90)	No	20 (67)	A-10, SKOV-6, Caov-2	No correlation between SPAG9 expression and tumor stages	[76]
Cervical cancer	54 (82)	54 (82)	No	53 (80)	SiHa, HeLa, CaSki, C-33A	SPAG9 expression in cervical tissue specimens was associated with early stages of cervical cancer Ablation of SPAG9 in cervical cancer cells resulted in inhibition of cellular proliferation, migration an invasion *in vitro* and *in vivo*	[78,79]
Breast cancer	88 (88)	88 (88)	No	80 (80)	MCF-7, BT-474, SK-BR-3, MDA-MB-231	SPAG9 expression was not correlated with tumor stages but showed significant association with early grades. In addition, High SPAG9 immunoreactivity score correlated with lymphovascular invasion and high risk of recurrence SPAG9 ablation in triple negative breast cancer cells resulted in inhibited cellular proliferation, colony formation , migration and invasion and reduced tumor growth *in vivo*	[80,81]
Renal cell carcinoma	46 (88)	46 (88)	No	40 (77)	A704, ACHN, Caki-1, Caki-2 NII-AKS395 NII-AKS413 NII-AKS414	SPAG9 expression was significantly associated with lymph node invasion and metastasis in clinical specimens siRNA mediated SPAG9 downregulation inhibited cellular proliferation, migration and invasion *in vitro* and *in vivo*	[77]
Thyroid cancer	108 (78)	108 (78)	No (not in multinodal goitres and follicular adenoma samples tested)	92 (78)	WRO, FTC-133, BC-PAP, 8305C	Both SPAG9 expression and humoral response were associated with early stages of thyroid cancer Depletion of SPAG9 resulted in inhibition of cellular growth and colony forming ability of thyroid cancer cells	[82]
Fine needle aspirates of PTC	6 (38) PTC	-	8 (40) benign nodules	-	-	No clinical relevance	[94]
Endometrial cancer	-	Serum SPAG9 antigen (with cut off 17 ng/mL) was used to determine endometrial malignancy (sensitivity = 74%, specificity = 83%)	No SPAG9 levels found in women benign diseases	36 (72)		No significant association of serum SPAG9 antigen levels with histological type, FIGO stage, tumor grade, size, myometrial invasion, lymphovascular space invasion, cervical involvement, adnexal involvement, peritoneal cytology or lymph node status of endometrial tumors Serum SPAG9 levels were found to be negatively correlated with tumor grades	[85,86]
Colorectal cancer	58 (74)	58 (74)	No	38 (70)	COLO 205, HCT 116	SPAG9 expression was correlated with early stages but not with grades, lymph nodes positivity or metastasis SPAG9 expression depletion resulted in decreased tumor growth *in vivo* and reduced migration and invasion *in vitro*	[53]
Bladder transitional cell carcinoma	101 (81)	101 (81)	No	96 (77)	HTB-2, HTB-9, HTB-1, UM-UC-3	High SPAG9 expression (> 60% SPAG9 positive cells) was found to be significantly associated with superficial non-muscle invasive stage and low grade tumors *In vitro* downregulation of SPAG9 caused G_0-G_1 arrest, inhibition of cellular proliferation, migration and invasion	[84]

Chronic myeloid leukemia	106 (88)	106 (88)	No	106 (88)	K562, KCL-22	No correlation with stages	[83]
Prostate cancer	-	54 (36.5)	No	-	REPW-1, PC-3, DU-145	SPAG9 expression in clinical specimens is associated with advanced tumor stages and gleason score SPAG9 could supercharge prostate cancer proliferation with cyclin D1 and cyclin E upregulation SPAG9 depletion caused reduction in angiogenesis and migration	[89,90]
Brain cancer (Astrocytoma)	-	63 (60)	No	-	SW1783, SF295, TG905, U251 and U87 (SPAG9 not expressed in A172)	SPAG9 expression was found to positively correlated with tumor grades. SPAG9 depletion was accompanied by downregulation of MMP9 suggesting the possible role of SPAG9 in cellular invasion. PODXL is a critical mediator of the promoting effect of SPAG9 on astrocytoma cell invasion, possibly through upregulation of MMP9 expression	[89,90]
Hepatocellul-ar carcinoma	-	47 (48.5)	No	-		High SPAG9 expression is strongly correlated with multiple tumors, advanced TNM stage, tumor size, serum AFP levels and tumor relapse SPAG9 modulates cell proliferation through cyclin regulation	[91]
Non small cell Lung cancer		63 (52.5)	No	-	A549, H1299	Overexpression of SPAG9 correlated with poor tumor differentiation, advanced p-TNM stage, nodal metastasis and poor overall survival SPAG9 might act as an important promoter in lung cancer progression and invasion *via* MMP9 regulation and JNK activation	[92]
Non melanoma Skin cancer	-	18 (90) basal cell carcinoma and 18 (82) squamous cell carcinoma	weak SPAG9 expression in 25% normal skin cases	-	-	Significant negative correlation between SPAG9 expression and tumor grade and significantly higher H score values in grade I SCC cases	[93]

EOC: Epithelial ovarian cancer; SPAG9: Sperm associated antigen 9; FIGO staging: Federation of Gynecology and Obstetrics staging; G_0: G_0 phase of cell cycle; G_1: G_1 phase of cell cycle; REPW-1: Human normal prostate epithelial cells; cyclin D1: Cyclin D1protein; cyclin E: Cyclin E protein; SCC: Squamous cell carcinoma; PTC: Papillary thyroid cancer.

antigens have also been tested as immunotherapy targets in phase II clinical trials in metastatic CRC patients and results were promising with low toxicity. This vaccine was artificially synthesized by using helper/killer-hybrid epitope long peptide of MAGE-A4 cancer antigen and was used in combination with OK432 and Montanide ISA-51[95]. In CRC, HSP105 also showed promising results in mouse model system in preclinical investigation[32]. In addition, in a recent phase I clinical trial for advanced CRC using combination of chemotherapy and immunotherapy illustrated some limited positive responses[96]. This limited success rate of immunotherapy might be attributed to the low frequency of CT antigen expression in CRC tissues. However, we have several other new CT antigens which are not yet characterized in CRC patients. Recently emerged promising CT antigens such as SPAG9 antigens can be targeted in combination with other CT antigen to improve the efficacy of immunotherapeutic vaccines. Conceptually, these testis specific genes might provide new clinical tools as we move even closer to an era of more personalized therapeutics.

CONCLUSION

Our current understanding of CT antigen expression and immune response in CRC is still in early stages of translational clinical research. Compiling together, there is a scope for improvement despite the low frequency of expression of several CT antigens in CRC. It might be related to the fact that only a sub-population of CRC patients can derive the benefit from CT antigens based therapies or multi-biomarker approach is the answer to improve the clinical management through detection, prediction and prognosis. The combination of CT antigens that can be employed for this purpose needs to be explored. With the advent of personalized therapy, CT antigens can provide an option to the clinicians to design the targeted and tailored medicine for the patients to obtain maximum benefit from their therapeutics. Analysis of antigen specific humoral and cellular response will shed more light towards designing the optimal therapeutic regimen for the patients. In conclusion, CT antigens are promising targets and might provide a new avenue for improved biomarkers

and therapeutics.

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P- Reviewer: Balatti V, Guilbert MC **S- Editor**: Tian YL
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