**Name of Journal: *World Journal of Gastrointestinal Oncology***

**ESPS Manuscript NO: 20671**

**Manuscript Type: MINIREVIEWS**

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

Suri A *et al*. Targeting cancer testis antigen in colorectal cancer

**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.

**Open-Access:** This article is an open-access article which was selected by an in-house editor and fully peer-reviewed by external reviewers. It is distributed in accordance with the Creative Commons Attribution Non Commercial (CC BY-NC 4.0) license, which permits others to distribute, remix, adapt, build upon this work non-commercially, and license their derivative works on different terms, provided the original work is properly cited and the use is non-commercial. See: <http://creativecommons.org/licenses/by-nc/4.0/>

**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:**

**Published online:**

**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

**© The Author(s) 2015.** Published by Baishideng Publishing Group Inc. All rights reserved.

**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; In press

**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 (HNPCC) 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 (CT) 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 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 (MAGEA-2, 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 versus 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 (CT) 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 melanoma associated antigen (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 versus 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 genes 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 PAGE4, 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 cAMP 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 (BORIS) 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 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*[24,66] 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 *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 of15ng/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 while 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 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 ever 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.

**REFERENCES**

1 **Mäkinen MJ.** Serrated polyps and colorectal cancer risk. *Colorectal Cancer* 2014; **3**: 77-91[DOI: 10.2217/crc.13.84]

2 **Legolvan MP**, Taliano RJ, Resnick MB. Application of molecular techniques in the diagnosis, prognosis and management of patients with colorectal cancer: a practical approach. *Hum Pathol* 2012; **43**: 1157-1168 [PMID: 22658275 DOI: 10.1016/j.humpath.2012.03.003]

3 **Strate LL**, Syngal S. Hereditary colorectal cancer syndromes. *Cancer Causes Control* 2005; **16**: 201-213 [PMID: 15947872 DOI: 10.1007/s10552-004-3488-4]

4 **Singh H**, Nugent Z, Demers AA, Kliewer EV, Mahmud SM, Bernstein CN. The reduction in colorectal cancer mortality after colonoscopy varies by site of the cancer. *Gastroenterology* 2010; **139**: 1128-1137 [PMID: 20600026 DOI: 10.1053/j.gastro.2010.06.052]

5 **Kim HJ**, Yu MH, Kim H, Byun J, Lee C. Noninvasive molecular biomarkers for the detection of colorectal cancer. *BMB Rep* 2008; **41**: 685-692 [PMID: 18959813 DOI: 10.5483/BMBRep.2008.41.10.685]

6 **Suri A**. Cancer testis antigens--their importance in immunotherapy and in the early detection of cancer. *Expert Opin Biol Ther* 2006; **6**: 379-389 [PMID: 16548764 DOI: 10.1517/14712598.6.4.379]

7 **Suri A,** Saini S, Sinha A, Agarwal S, Verma A, Parashar D, Singh S, Gupta N, Jagadish N. Cancer testis antigens: A new paradigm for cancer therapy. *Oncoimmunology* 2012; **1**: 1194-1196 [PMID: 23170277 DOI: 10.4161/onci.20686]

8 **Sammut J,** Wakeman JA, Stuart N, McFarlane RJ. Cancer/Testis Antigens and Colorectal Cancer. *J Genet Syndr Gene Ther* 2013, **4**: 5 [DOI: 10.4172/2157-7412.1000149]

9 **Old LJ**, Chen YT. New paths in human cancer serology. *J Exp Med* 1998; **187**: 1163-1167 [PMID: 9547328 DOI: 10.1084/jem.187.8.1163]

10 **van der Bruggen P**, Traversari C, Chomez P, Lurquin C, De Plaen E, Van den Eynde B, Knuth A, Boon T. A gene encoding an antigen recognized by cytolytic T lymphocytes on a human melanoma. *Science* 1991; **254**: 1643-1647 [PMID: 1840703 DOI: 10.1126/science.1840703]

11 **De Plaen E**, Arden K, Traversari C, Gaforio JJ, Szikora JP, De Smet C, Brasseur F, van der Bruggen P, Lethé B, Lurquin C. Structure, chromosomal localization, and expression of 12 genes of the MAGE family. *Immunogenetics* 1994; **40**: 360-369 [PMID: 7927540 DOI: 10.1007/BF01246677]

12 **Zendman AJ**, Ruiter DJ, Van Muijen GN. Cancer/testis-associated genes: identification, expression profile, and putative function. *J Cell Physiol* 2003; **194**: 272-288 [PMID: 12548548 DOI: 10.1002/jcp.10215]

13 **Boël P**, Wildmann C, Sensi ML, Brasseur R, Renauld JC, Coulie P, Boon T, van der Bruggen P. BAGE: a new gene encoding an antigen recognized on human melanomas by cytolytic T lymphocytes. *Immunity* 1995; **2**: 167-175 [PMID: 7895173 DOI: 10.1016/S1074-7613(95)80053-0]

14 **Chomez P**, De Backer O, Bertrand M, De Plaen E, Boon T, Lucas S. An overview of the MAGE gene family with the identification of all human members of the family. *Cancer Res* 2001; **61**: 5544-5551 [PMID: 11454705]

15 **De Backer O**, Arden KC, Boretti M, Vantomme V, De Smet C, Czekay S, Viars CS, De Plaen E, Brasseur F, Chomez P, Van den Eynde B, Boon T, van der Bruggen P. Characterization of the GAGE genes that are expressed in various human cancers and in normal testis. *Cancer Res* 1999; **59**: 3157-3165 [PMID: 10397259]

16 **Gaugler B**, Van den Eynde B, van der Bruggen P, Romero P, Gaforio JJ, De Plaen E, Lethé B, Brasseur F, Boon T. Human gene MAGE-3 codes for an antigen recognized on a melanoma by autologous cytolytic T lymphocytes. *J Exp Med* 1994; **179**: 921-930 [PMID: 8113684 DOI: 10.1084/jem.179.3.921]

17 **Sahin U**, Türeci O, Pfreundschuh M. Serological identification of human tumor antigens. *Curr Opin Immunol* 1997; **9**: 709-716 [PMID: 9368781 DOI: 10.1016/S0952-7915(97)80053-2]

18 **Türeci O**, Chen YT, Sahin U, Güre AO, Zwick C, Villena C, Tsang S, Seitz G, Old LJ, Pfreundschuh M. Expression of SSX genes in human tumors. *Int J Cancer* 1998; **77**: 19-23 [PMID: 9639388 DOI: 10.1002/(SICI)1097-0215(19980703)77: 1<19: : AID-IJC4>3.0.CO; 2-2]

19 **Chen YT**, Scanlan MJ, Sahin U, Türeci O, Gure AO, Tsang S, Williamson B, Stockert E, Pfreundschuh M, Old LJ. A testicular antigen aberrantly expressed in human cancers detected by autologous antibody screening. *Proc Natl Acad Sci U S A* 1997; **94**: 1914-1918 [PMID: 9050879 DOI: 10.1073/pnas.94.5.1914]

20 **Shankar S**, Mohapatra B, Suri A. Cloning of a novel human testis mRNA specifically expressed in testicular haploid germ cells, having unique palindromic sequences and encoding a leucine zipper dimerization motif. *Biochem Biophys Res Commun* 1998; **243**: 561-565 [PMID: 9480848 DOI: 10.1006/bbrc.1997.7943]

21 **Mohapatra B**, Verma S, Shankar S, Suri A. Molecular cloning of human testis mRNA specifically expressed in haploid germ cells, having structural homology with the A-kinase anchoring proteins. *Biochem Biophys Res Commun* 1998; **244**: 540-545 [PMID: 9514854 DOI: 10.1006/bbrc.1998.8079]

22 **Almeida LG**, Sakabe NJ, deOliveira AR, Silva MC, Mundstein AS, Cohen T, Chen YT, Chua R, Gurung S, Gnjatic S, Jungbluth AA, Caballero OL, Bairoch A, Kiesler E, White SL, Simpson AJ, Old LJ, Camargo AA, Vasconcelos AT. CTdatabase: a knowledge-base of high-throughput and curated data on cancer-testis antigens. *Nucleic Acids Res* 2009; **37**: D816-D819 [PMID: 18838390 DOI: 10.1093/nar/gkn673]

23 **Scudellari M**. A ballsy search for cancer targets. *Nat Med* 2011; **17**: 916-918 [PMID: 21818078 DOI: 10.1038/nm0811-91]

24 **Dakshinamurthy AG**, Ramesar R, Goldberg P, Blackburn JM. Infrequent and low expression of cancer-testis antigens located on the X chromosome in colorectal cancer: implications for immunotherapy in South African populations. *Biotechnol J* 2008; **3**: 1417-1423 [PMID: 18956367 DOI: 10.1002/biot.200800144]

25 **Lurquin C**, De Smet C, Brasseur F, Muscatelli F, Martelange V, De Plaen E, Brasseur R, Monaco AP, Boon T. Two members of the human MAGEB gene family located in Xp21.3 are expressed in tumors of various histological origins. *Genomics* 1997; **46**: 397-408 [PMID: 9441743 DOI: 10.1006/geno.1997.5052]

26 **Burgdorf SK**, Fischer A, Myschetzky PS, Munksgaard SB, Zocca MB, Claesson MH, Rosenberg J. Clinical responses in patients with advanced colorectal cancer to a dendritic cell based vaccine. *Oncol Rep* 2008; **20**: 1305-1311 [PMID: 19020707]

27 **Kufer P**, Zippelius A, Lutterbüse R, Mecklenburg I, Enzmann T, Montag A, Weckermann D, Passlick B, Prang N, Reichardt P, Dugas M, Köllermann MW, Pantel K, Riethmüller G. Heterogeneous expression of MAGE-A genes in occult disseminated tumor cells: a novel multimarker reverse transcription-polymerase chain reaction for diagnosis of micrometastatic disease. *Cancer Res* 2002; **62**: 251-261 [PMID: 11782385]

28 **Brasseur F**, Rimoldi D, Liénard D, Lethé B, Carrel S, Arienti F, Suter L, Vanwijck R, Bourlond A, Humblet Y. Expression of MAGE genes in primary and metastatic cutaneous melanoma. *Int J Cancer* 1995; **63**: 375-380 [PMID: 7591235 DOI: 10.1002/ijc.2910630313]

29 **Chen Z**, Li M, Yuan Y, Wang Q, Yan L, Gu J. Cancer/testis antigens and clinical risk factors for liver metastasis of colorectal cancer: a predictive panel. *Dis Colon Rectum* 2010; **53**: 31-38 [PMID: 20010347 DOI: 10.1007/DCR.0b013e3181bdca3a]

30 **Hasegawa H**, Mori M, Haraguchi M, Ueo H, Sugimachi K, Akiyoshi T. Expression spectrum of melanoma antigen-encoding gene family members in colorectal carcinoma. *Arch Pathol Lab Med* 1998; **122**: 551-554 [PMID: 9625425]

31 **Li M**, Yuan YH, Han Y, Liu YX, Yan L, Wang Y, Gu J. Expression profile of cancer-testis genes in 121 human colorectal cancer tissue and adjacent normal tissue. *Clin Cancer Res* 2005; **11**: 1809-1814 [PMID: 15756003 DOI: 10.1158/1078-0432.CCR-04-1365]

32 **Miyazaki M**, Nakatsura T, Yokomine K, Senju S, Monji M, Hosaka S, Komori H, Yoshitake Y, Motomura Y, Minohara M, Kubo T, Ishihara K, Hatayama T, Ogawa M, Nishimura Y. DNA vaccination of HSP105 leads to tumor rejection of colorectal cancer and melanoma in mice through activation of both CD4 T cells and CD8 T cells. *Cancer Sci* 2005; **96**: 695-705 [PMID: 16232202 DOI: 10.1111/j.1349-7006.2005.00093.x]

33 **Scanlan MJ**, Ritter G, Yin BW, Williams C, Cohen LS, Coplan KA, Fortunato SR, Frosina D, Lee SY, Murray AE, Chua R, Filonenko VV, Sato E, Old LJ, Jungbluth AA. Glycoprotein A34, a novel target for antibody-based cancer immunotherapy. *Cancer Immun* 2006; **6**: 2 [PMID: 16405301]

34 **Shebzukhov YV,** Koroleva EP, Khlgatian SV, Belousov PV, Sazykin AY, Kadachigova TS, Pomerantseva EA, Lagarkova MA, Nedospasov SA, Kuprash DV. RAP80/UIMC1 as cancer-associated antigen: alternative splice variants and their immunogenicity. *Cancer Lett* 2007; **255**: 255-262 [PMID: 17562356 DOI: 10.1016/j.canlet.2007.04.013]

35 **Nimmrich I**, Erdmann S, Melchers U, Finke U, Hentsch S, Moyer MP, Hoffmann I, Müller O. Seven genes that are differentially transcribed in colorectal tumor cell lines. *Cancer Lett* 2000; **160**: 37-43 [PMID: 11098082 DOI: 10.1016/S0304-3835(00)00553-X]

36 **Usener D**, Schadendorf D, Koch J, Dübel S, Eichmüller S. cTAGE: a cutaneous T cell lymphoma associated antigen family with tumor-specific splicing. *J Invest Dermatol* 2003; **121**: 198-206 [PMID: 12839582 DOI: 10.1046/j.1523-1747.2003.12318.x]

37 **Scanlan MJ**, Welt S, Gordon CM, Chen YT, Gure AO, Stockert E, Jungbluth AA, Ritter G, Jäger D, Jäger E, Knuth A, Old LJ. Cancer-related serological recognition of human colon cancer: identification of potential diagnostic and immunotherapeutic targets. *Cancer Res* 2002; **62**: 4041-4047 [PMID: 12124339]

38 **Jäger D**, Unkelbach M, Frei C, Bert F, Scanlan MJ, Jäger E, Old LJ, Chen YT, Knuth A. Identification of tumor-restricted antigens NY-BR-1, SCP-1, and a new cancer/testis-like antigen NW-BR-3 by serological screening of a testicular library with breast cancer serum. *Cancer Immun* 2002; **2**: 5 [PMID: 12747750]

39 **Cao J**, Gao T, Stanbridge EJ, Irie R. RBP1L1, a retinoblastoma-binding protein-related gene encoding an antigenic epitope abundantly expressed in human carcinomas and normal testis. *J Natl Cancer Inst* 2001; **93**: 1159-1165 [PMID: 11481388 DOI: 10.1093/jnci/93.15.1159]

40 **Kiniwa Y**, Fujita T, Akada M, Ito K, Shofuda T, Suzuki Y, Yamamoto A, Saida T, Kawakami Y. Tumor antigens isolated from a patient with vitiligo and T-cell-infiltrated melanoma. *Cancer Res* 2001; **61**: 7900-7907 [PMID: 11691810]

41 **He Y**, Wu Y, Mou Z, Li W, Zou L, Fu T, Zhang A, Xiang D, Xiao H, Wang X. Proteomics-based identification of HSP60 as a tumor-associated antigen in colorectal cancer. *Proteomics Clin Appl* 2007; **1**: 336-342 [PMID: 21136683 DOI: 10.1002/prca.200600718]

42 **Uchida N**, Tsunoda T, Wada S, Furukawa Y, Nakamura Y, Tahara H. Ring finger protein 43 as a new target for cancer immunotherapy. *Clin Cancer Res* 2004; **10**: 8577-8586 [PMID: 15623641 DOI: 10.1158/1078-0432.CCR-04-0104]

43 **Shichijo S**, Ito M, Azuma K, Komatsu N, Maeda Y, Ishihara Y, Nakamura T, Harada M, Itoh K. A unique gene having homology with the kinesin family member 18A encodes a tumour-associated antigen recognised by cytotoxic T lymphocytes from HLA-A2+ colon cancer patients. *Eur J Cancer* 2005; **41**: 1323-1330 [PMID: 15939267 DOI: 10.1016/j.ejca.2005.02.025]

44 **Shimokawa T**, Matsushima S, Tsunoda T, Tahara H, Nakamura Y, Furukawa Y. Identification of TOMM34, which shows elevated expression in the majority of human colon cancers, as a novel drug target. *Int J Oncol* 2006; **29**: 381-386 [PMID: 16820880 DOI: 10.3892/ijo.29.2.381]

45 **Koslowski M**, Türeci O, Bell C, Krause P, Lehr HA, Brunner J, Seitz G, Nestle FO, Huber C, Sahin U. Multiple splice variants of lactate dehydrogenase C selectively expressed in human cancer. *Cancer Res* 2002; **62**: 6750-6755 [PMID: 12438276]

46 **Loriot A**, Boon T, De Smet C. Five new human cancer-germline genes identified among 12 genes expressed in spermatogonia. *Int J Cancer* 2003; **105**: 371-376 [PMID: 12704671 DOI: 10.1002/ijc.11104]

47 **de Wit NJ**, Weidle UH, Ruiter DJ, van Muijen GN. Expression profiling of MMA-1a and splice variant MMA-1b: new cancer/testis antigens identified in human melanoma. *Int J Cancer* 2002; **98**: 547-553 [PMID: 11920614 DOI: 10.1002/ijc.10241]

48 **Kim R**, Kulkarni P, Hannenhalli S. Derepression of Cancer/testis antigens in cancer is associated with distinct patterns of DNA hypomethylation. *BMC Cancer* 2013; **13**: 144 [PMID: 23522060 DOI: 10.1186/1471-2407-13-144]

49 **Fratta E**, Coral S, Covre A, Parisi G, Colizzi F, Danielli R, Nicolay HJ, Sigalotti L, Maio M. The biology of cancer testis antigens: putative function, regulation and therapeutic potential. *Mol Oncol* 2011; **5**: 164-182 [PMID: 21376678 DOI: 10.1016/j.molonc.2011.02.001]

50 **Kalejs M,** Erenpreisa J. Cancer/testis antigens and gametogenesis: a review and "brain-storming" session. *Cancer Cell Int* 2005; **5**: 4 [PMID: 15715909 DOI: 10.1186/1475-2867-5-4]

51 **Simpson AJ**, Caballero OL, Jungbluth A, Chen YT, Old LJ. Cancer/testis antigens, gametogenesis and cancer. *Nat Rev Cancer* 2005; **5**: 615-625 [PMID: 16034368 DOI: 10.1038/nrc1669]

52 **Alves PM**, Lévy N, Bouzourene H, Viatte S, Bricard G, Ayyoub M, Vuilleumier H, Givel JC, Halkic N, Speiser DE, Romero P, Lévy F. Molecular and immunological evaluation of the expression of cancer/testis gene products in human colorectal cancer. *Cancer Immunol Immunother* 2007; **56**: 839-847 [PMID: 16960690 DOI: 10.1007/s00262-006-0228-5]

53 **Kanojia D**, Garg M, Gupta S, Gupta A, Suri A. Sperm-associated antigen 9 is a novel biomarker for colorectal cancer and is involved in tumor growth and tumorigenicity. *Am J Pathol* 2011; **178**: 1009-1020 [PMID: 21356354 DOI: 10.1016/j.ajpath.2010.11.047]

54 **Zheng L**, Xie G, Duan G, Yan X, Li Q. High expression of testes-specific protease 50 is associated with poor prognosis in colorectal carcinoma. *PLoS One* 2011; **6**: e22203 [PMID: 21765952 DOI: 10.1371/journal.pone.0022203]

55 **Ladelfa MF**, Peche LY, Toledo MF, Laiseca JE, Schneider C, Monte M. Tumor-specific MAGE proteins as regulators of p53 function. *Cancer Lett* 2012; **325**: 11-17 [PMID: 22664239 DOI: 10.1016/j.canlet.2012.05.031]

56 **Cilensek ZM**, Yehiely F, Kular RK, Deiss LP. A member of the GAGE family of tumor antigens is an anti-apoptotic gene that confers resistance to Fas/CD95/APO-1, Interferon-gamma, taxol and gamma-irradiation. *Cancer Biol Ther* 2002; **1**: 380-387 [PMID: 12432251 DOI: 10.4161/cbt.1.4.11]

57 **Miki K**, Willis WD, Brown PR, Goulding EH, Fulcher KD, Eddy EM. Targeted disruption of the Akap4 gene causes defects in sperm flagellum and motility. *Dev Biol* 2002; **248**: 331-342 [PMID: 12167408 DOI: 10.1006/dbio.2002.0728]

58 **Türeci O**, Sahin U, Zwick C, Koslowski M, Seitz G, Pfreundschuh M. Identification of a meiosis-specific protein as a member of the class of cancer/testis antigens. *Proc Natl Acad Sci U S A* 1998; **95**: 5211-5216 [PMID: 9560255 DOI: 10.1073/pnas.95.9.5211]

59 **Ono T**, Kurashige T, Harada N, Noguchi Y, Saika T, Niikawa N, Aoe M, Nakamura S, Higashi T, Hiraki A, Wada H, Kumon H, Old LJ, Nakayama E. Identification of proacrosin binding protein sp32 precursor as a human cancer/testis antigen. *Proc Natl Acad Sci U S A* 2001; **98**: 3282-3287 [PMID: 11248070 DOI: 10.1073/pnas.041625098]

60 **Romanienko PJ**, Camerini-Otero RD. Cloning, characterization, and localization of mouse and human SPO11. *Genomics* 1999; **61**: 156-169 [PMID: 10534401 DOI: 10.1006/geno.1999.5955]

61 **Loukinov DI**, Pugacheva E, Vatolin S, Pack SD, Moon H, Chernukhin I, Mannan P, Larsson E, Kanduri C, Vostrov AA, Cui H, Niemitz EL, Rasko JE, Docquier FM, Kistler M, Breen JJ, Zhuang Z, Quitschke WW, Renkawitz R, Klenova EM, Feinberg AP, Ohlsson R, Morse HC, Lobanenkov VV. BORIS, a novel male germ-line-specific protein associated with epigenetic reprogramming events, shares the same 11-zinc-finger domain with CTCF, the insulator protein involved in reading imprinting marks in the soma. *Proc Natl Acad Sci U S A* 2002; **99**: 6806-6811 [PMID: 12011441 DOI: 10.1073/pnas.092123699]

62 **Jagadish N**, Rana R, Selvi R, Mishra D, Garg M, Yadav S, Herr JC, Okumura K, Hasegawa A, Koyama K, Suri A. Characterization of a novel human sperm-associated antigen 9 (SPAG9) having structural homology with c-Jun N-terminal kinase-interacting protein. *Biochem J* 2005; **389**: 73-82 [PMID: 15693750 DOI: 10.1042/BJ20041577]

63 **Rajagopalan K**, Mooney SM, Parekh N, Getzenberg RH, Kulkarni P. A majority of the cancer/testis antigens are intrinsically disordered proteins. *J Cell Biochem* 2011; **112**: 3256-3267 [PMID: 21748782 DOI: 10.1002/jcb.23252]

64 **Hassan M,** Watari H, AbuAlmaaty A, Ohba Y, Sakuragi N. Apoptosis and molecular targeting therapy in cancer. *Biomed Res Int* 2014; **2014**: 150845 [PMID: 25013758 DOI: 10.1155/2014/150845]

65 **Hofmann O**, Caballero OL, Stevenson BJ, Chen YT, Cohen T, Chua R, Maher CA, Panji S, Schaefer U, Kruger A, Lehvaslaiho M, Carninci P, Hayashizaki Y, Jongeneel CV, Simpson AJ, Old LJ, Hide W. Genome-wide analysis of cancer/testis gene expression. *Proc Natl Acad Sci U S A* 2008; **105**: 20422-20427 [PMID: 19088187 DOI: 10.1073/pnas.0810777105]

66 **Shi YY**, Wang HC, Yin YH, Sun WS, Li Y, Zhang CQ, Wang Y, Wang S, Chen WF. Identification and analysis of tumour-associated antigens in hepatocellular carcinoma. *Br J Cancer* 2005; **92**: 929-934 [PMID: 15756260 DOI: 10.1038/sj.bjc.6602460]

67 **Kawamoto M**, Tanaka F, Mimori K, Inoue H, Kamohara Y, Mori M. Identification of HLA-A\*0201/-A\*2402-restricted CTL epitope-peptides derived from a novel cancer/testis antigen, MCAK, and induction of a specific antitumor immune response. *Oncol Rep* 2011; **25**: 469-476 [PMID: 21165574]

68 **Okada T**, Akada M, Fujita T, Iwata T, Goto Y, Kido K, Okada T, Matsuzaki Y, Kobayashi K, Matsuno S, Sunamura M, Kawakami Y. A novel cancer testis antigen that is frequently expressed in pancreatic, lung, and endometrial cancers. *Clin Cancer Res* 2006; **12**: 191-197 [PMID: 16397042 DOI: 10.1158/1078-0432.CCR-05-1206]

69 **Ishikawa K**, Kamohara Y, Tanaka F, Haraguchi N, Mimori K, Inoue H, Mori M. Mitotic centromere-associated kinesin is a novel marker for prognosis and lymph node metastasis in colorectal cancer. *Br J Cancer* 2008; **98**: 1824-1829 [PMID: 18506187 DOI: 10.1038/sj.bjc.6604379]

70 **Bera TK**, Saint Fleur A, Lee Y, Kydd A, Hahn Y, Popescu NC, Zimonjic DB, Lee B, Pastan I. POTE paralogs are induced and differentially expressed in many cancers. *Cancer Res* 2006; **66**: 52-56 [PMID: 16397215 DOI: 10.1158/0008-5472.CAN-05-3014]

71 **Bera TK**, Zimonjic DB, Popescu NC, Sathyanarayana BK, Kumar V, Lee B, Pastan I. POTE, a highly homologous gene family located on numerous chromosomes and expressed in prostate, ovary, testis, placenta, and prostate cancer. *Proc Natl Acad Sci U S A* 2002; **99**: 16975-16980 [PMID: 12475935 DOI: 10.1073/pnas.262655399]

72 **Chen YT**, Scanlan MJ, Venditti CA, Chua R, Theiler G, Stevenson BJ, Iseli C, Gure AO, Vasicek T, Strausberg RL, Jongeneel CV, Old LJ, Simpson AJ. Identification of cancer/testis-antigen genes by massively parallel signature sequencing. *Proc Natl Acad Sci U S A* 2005; **102**: 7940-7945 [PMID: 15905330 DOI: 10.1073/pnas.0502583102]

73 **Jungbluth AA**, Chen YT, Stockert E, Busam KJ, Kolb D, Iversen K, Coplan K, Williamson B, Altorki N, Old LJ. Immunohistochemical analysis of NY-ESO-1 antigen expression in normal and malignant human tissues. *Int J Cancer* 2001; **92**: 856-860 [PMID: 11351307 DOI: 10.1002/ijc.1282]

74 **Jungbluth AA**, Busam KJ, Kolb D, Iversen K, Coplan K, Chen YT, Spagnoli GC, Old LJ. Expression of MAGE-antigens in normal tissues and cancer. *Int J Cancer* 2000; **85**: 460-465 [PMID: 10699915 DOI: 10.1002/(SICI)1097-0215(20000215)85: 4<460: : AID-IJC3>3.3.CO; 2-E]

75 **Jagadish N**, Rana R, Mishra D, Kumar M, Suri A. Sperm associated antigen 9 (SPAG9): a new member of c-Jun NH2 -terminal kinase (JNK) interacting protein exclusively expressed in testis. *Keio J Med* 2005; **54**: 66-71 [PMID: 16077255 DOI: 10.2302/kjm.54.66]

76 **Garg M**, Chaurasiya D, Rana R, Jagadish N, Kanojia D, Dudha N, Kamran N, Salhan S, Bhatnagar A, Suri S, Gupta A, Suri A. Sperm-associated antigen 9, a novel cancer testis antigen, is a potential target for immunotherapy in epithelial ovarian cancer. *Clin Cancer Res* 2007; **13**: 1421-1428 [PMID: 17332284 DOI: 10.1158/1078-0432.CCR-06-2340]

77 **Garg M**, Kanojia D, Khosla A, Dudha N, Sati S, Chaurasiya D, Jagadish N, Seth A, Kumar R, Gupta S, Gupta A, Lohiya NK, Suri A. Sperm-associated antigen 9 is associated with tumor growth, migration, and invasion in renal cell carcinoma. *Cancer Res* 2008; **68**: 8240-8248 [PMID: 18922895 DOI: 10.1158/0008-5472.CAN-08-1708]

78 **Garg M**, Kanojia D, Salhan S, Suri S, Gupta A, Lohiya NK, Suri A. Sperm-associated antigen 9 is a biomarker for early cervical carcinoma. *Cancer* 2009; **115**: 2671-2683 [PMID: 19326449 DOI: 10.1002/cncr.24293]

79 **Garg M**, Kanojia D, Suri S, Suri A. Small interfering RNA-mediated down-regulation of SPAG9 inhibits cervical tumor growth. *Cancer* 2009; **115**: 5688-5699 [PMID: 19813278 DOI: 10.1002/cncr.24658]

80 **Kanojia D**, Garg M, Gupta S, Gupta A, Suri A. Sperm-associated antigen 9, a novel biomarker for early detection of breast cancer. *Cancer Epidemiol Biomarkers Prev* 2009; **18**: 630-639 [PMID: 19190149 DOI: 10.1158/1055-9965.EPI-08-0629]

81 **Sinha A,** Agarwal S, Parashar D, Verma A, Saini S, Jagadish N, Ansari AS, Lohiya NK, Suri A. Down regulation of SPAG9 reduces growth and invasive potential of triple-negative breast cancer cells: possible implications in targeted therapy. *J Exp Clin Cancer Res* 2013; **32**: 69 [PMID: 24330581 DOI: 10.1186/1756-9966-32-69]

82 **Garg M**, Kanojia D, Suri S, Gupta S, Gupta A, Suri A. Sperm-associated antigen 9: a novel diagnostic marker for thyroid cancer. *J Clin Endocrinol Metab* 2009; **94**: 4613-4618 [PMID: 19820019 DOI: 10.1210/jc.2009-0703]

83 **Kanojia D**, Garg M, Saini S, Agarwal S, Kumar R, Suri A. Sperm associated antigen 9 expression and humoral response in chronic myeloid leukemia. *Leuk Res* 2010; **34**: 858-863 [PMID: 20138665 DOI: 10.1016/j.leukres.2010.01.017]

84 **Kanojia D**, Garg M, Saini S, Agarwal S, Parashar D, Jagadish N, Seth A, Bhatnagar A, Gupta A, Kumar R, Lohiya NK, Suri A. Sperm associated antigen 9 plays an important role in bladder transitional cell carcinoma. *PLoS One* 2013; **8**: e81348 [PMID: 24349057 DOI: 10.1371/journal.pone.0081348]

85 **Baser E**, Togrul C, Ozgu E, Ayhan S, Caglar M, Erkaya S, Gungor T. Sperm-associated antigen 9 is a promising marker for early diagnosis of endometrial cancer. *Asian Pac J Cancer Prev* 2013; **14**: 7635-7638 [PMID: 24460345 DOI: 10.7314/APJCP.2013.14.12.7635]

86 **Yu P**, Yan L, Zhang H, Lin X, Zhao X. Expression and clinical significance of sperm-associated antigen 9 in patients with endometrial carcinoma. *Int J Gynecol Cancer* 2012; **22**: 87-93 [PMID: 22146769 DOI: 10.1097/IGC.0b013e3182370f2e]

87 **Yi F**, Ni W, Liu W, Pan X, Han X, Yang L, Kong X, Ma R, Chang R. SPAG9 is overexpressed in human astrocytoma and promotes cell proliferation and invasion. *Tumour Biol* 2013; **34**: 2849-2855 [PMID: 23696027 DOI: 10.1007/s13277-013-0845-5]

88 **Jiang J**, Liu Y, Fang W, Liu F. Sperm‑associated antigen 9 promotes astrocytoma cell invasion through the upregulation of podocalyxin. *Mol Med Rep* 2014; **10**: 417-422 [PMID: 24788963 DOI: 10.3892/mmr.2014.2168]

89 **Li H**, Peng Y, Niu H, Wu B, Zhang Y, Zhang Y, Bai X, He P. SPAG9 is overexpressed in human prostate cancer and promotes cancer cell proliferation. *Tumour Biol* 2014; **35**: 6949-6954 [PMID: 24740566 DOI: 10.1007/s13277]

90 **Chen F**, Lu Z, Deng J, Han X, Bai J, Liu Q, Xi Y, Zheng J. SPAG9 expression is increased in human prostate cancer and promotes cell motility, invasion and angiogenesis in vitro. *Oncol Rep* 2014; **32**: 2533-2540 [PMID: 25310386 DOI: 10.3892/or.2014.3539]

91 **Xie C**, Fu L, Liu N, Li Q. Overexpression of SPAG9 correlates with poor prognosis and tumor progression in hepatocellular carcinoma. *Tumour Biol* 2014; **35**: 7685-7691 [PMID: 24801907 DOI: 10.1007/s13277-014-2030-x]

92 **Wang Y**, Dong Q, Miao Y, Fu L, Lin X, Wang E. Clinical significance and biological roles of SPAG9 overexpression in non-small cell lung cancer. *Lung Cancer* 2013; **81**: 266-272 [PMID: 23711689 DOI: 10.1016/j.lungcan.2013.04.021]

93 **Seleit I**, Bakry OA, Samaka RM, Malak MA. Immunohistochemical expression of sperm-associated antigen 9 in nonmelanoma skin cancer. *Am J Dermatopathol* 2015; **37**: 38-45 [PMID: 25033008 DOI: 1097/DAD.0000000000000126]

94 **Volard B**, Krieger S, Planchard G, Hardouin A, Vaur D, Rame JP, Bardet S. Assessment of SPAG9 Transcript in Fine Needle Aspirates of Thyroid Nodules. *Eur Thyroid J* 2012; **1**: 118-121 [PMID: 24783006 DOI: 10.1159/00033892264]

95 **Takahashi N**, Ohkuri T, Homma S, Ohtake J, Wakita D, Togashi Y, Kitamura H, Todo S, Nishimura T. First clinical trial of cancer vaccine therapy with artificially synthesized helper/ killer-hybrid epitope long peptide of MAGE-A4 cancer antigen. *Cancer Sci* 2012; **103**: 150-153 [PMID: 22221328 DOI: 10.1111/j.1349-7006.2011.02106.x]

96 **Matsushita N**, Aruga A, Inoue Y, Kotera Y, Takeda K, Yamamoto M. Phase I clinical trial of a peptide vaccine combined with tegafur-uracil plus leucovorin for treatment of advanced or recurrent colorectal cancer. *Oncol Rep* 2013; **29**: 951-959 [PMID: 23314271 DOI: 10.3892/or.2013.2231]

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

**L-Editor: E-Editor:**

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

|  |  |  |  |  |  |  |  |
| --- | --- | --- | --- | --- | --- | --- | --- |
| **Cancer** | ***SPAG9* mRNA expression** | **SPAG9 Protein expression** | **SPAG9 expression in matched adjacent non-cancerous tissues** | **Serological detection of SPAG9 antibodies** | **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 Papillary Thyroid Cancer (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 G0-G1 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 MMP‑9 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 (FIGO) staging; G0:G0 phase of cell cycle; G1: G1 phase of cell cycle; REPW-1: Human normal prostate epithelial cells; cyclin D1: Cyclin D1protein; cyclin E: Cyclin E protein; SCC: Squamous cell carcinoma.