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Emerging Contributions of Cancer/Testis Antigens to Neoplastic Behaviors

Zane A. Gibbs[1,2] and **Angelique W. Whitehurst**[1,2]

[1]Harold C. Simmons Comprehensive Cancer Center, UT Southwestern Medical Center, Dallas, Texas, USA

[2]Department of Pharmacology, UT Southwestern Medical Center, Dallas, Texas, USA

Abstract

Tumors of nearly every origin activate the expression of genes which is otherwise restricted to gametogenic cells. These genes encode proteins termed cancer/testis (CT) antigens, since expression outside of their naturally immune-privileged site can evoke an immune response. Despite extensive efforts to exploit CT antigens as immunotherapeutic targets, investigation of whether these proteins participate in tumorigenic processes has lagged. Here, we discuss emerging evidence that demonstrates that CT antigens can confer a selective advantage to tumor cells by promoting oncogenic processes or permitting evasion of tumor-suppressive mechanisms. These advances indicate the inherent flexibility of tumor cell regulatory networks to engage aberrantly expressed proteins to promote neoplastic behaviors, which could ultimately present novel therapeutic entry points.

Keywords

cancer/testis antigens; CT genes; CT antigens; cancer germline genes; tumor antigens; CT antigen function

A Brief History of Tumor Antigen Discovery

By the 1970s it was well established that the immune system was capable of recognizing tumor cells as foreign, but the identity of the tumor-specific antigens recognized by T cells was largely unknown. The turning point came in the second half of the decade when Lloyd Old's group developed autologous typing, which permitted clonal selection of antigenic (and non-antigenic) tumor cells using sera obtained from the same patient [1]. Subsequently, Thierry Boon and colleagues used expression cloning with DNA from antigenic melanoma clones to identify gene products that could confer a cellular immune response in non-antigenic counterparts [2]. Remarkably, restoration of an antigen-coding gene, *MAGEA1*

*Correspondence: angelique.whitehurst@utsouthwestern.edu (A.W. Whitehurst).

(the founding member of the “melanoma antigen” family), sensitized the resistant tumor cell clones to destruction by autologous cytotoxic T lymphocytes (CTLs) [2, 3]. Subsequent analysis revealed that *MAGEA1* expression was normally restricted to the testes and placenta, yet detectable in tumors of various origins [4]. In 1995, Michael Pfreundschuh’s group improved the identification of tumor-specific antigens by developing the serological analysis of recombinant cDNA expression libraries (SEREX). Here, a phage display library derived from tumor cDNA is screened with autologous patient sera [5]. This technique facilitated the discovery of numerous tumor antigens, including NY-ESO-1, the most successful target to date for cancer immunotherapy [6–8]. Through these efforts, it soon became clear that *MAGEA1* represented the archetype of a family of genes with a unique testis/tumor-restricted expression pattern. Furthermore, seminal parallel discoveries suggested that their encoded proteins are indeed substrates for antigen processing machinery that leads to the presentation of cleaved peptides on the cell surface by major histocompatibility complex (MHC) molecules (Figure 1A) [9]. As the testes are an immune privileged site, CTLs most likely recognize these peptides as foreign, thereby triggering an anti-tumor immune response [10].

Birth and Growth of the CT Antigen Family

It was at the time of the NY-ESO-1 discovery that Lloyd Old coined the term cancer/testis (CT) antigen to refer to this new family of proteins [7, 11]. This name captured both the unique expression pattern of these genes (termed CT genes) and the antigenic potential of their encoded proteins (Figure 1A). It is important to note that many genes now classified as CT antigens have not been demonstrated to evoke an immune response. This was due in large part to a shift in the discovery platform for CT genes. The availability of expression profiling techniques, beginning in the early 2000s, permitted the *in silico* identification of genes that exhibit the classic testis/tumor-restricted mRNA expression pattern [12–17]. These unbiased analyses also revealed a subset of CT antigens whose mRNA was detectable in normal tissues, typically the brain. Thus, CT antigen classification was further refined to 3 categories: 1) testis-restricted (detectable only in testis and tumor tissues), 2) testis/brain-restricted (detectable in testis, brain, and tumor tissues), and 3) testis-selective (detectable in testis, tumor and 1–2 additional normal tissues) [13]. CT genes are also grouped into subfamilies based on sequence similarity and proximity of their chromosomal location. Interestingly, about half of the CT genes are located on the × chromosome (termed CT-X genes) and are members of multigene families (MAGE, GAGE, PAGE, SSX, CAGE, CSAG1, CTAG, and SPANX families in particular) [18]. Detailed records for most CT antigens can be found in the CT database, an online repository of information regarding the expression and immunogenic properties of CT genes and their encoded antigens, respectively (http://www.cta.lncc.br/) [19]. While the compendium of CT genes and candidate CT antigens has expanded rapidly, the laborious task of demonstrating antigenicity still remains. As such, the terms cancer/germline gene or oncofetal protein have emerged in place of CT antigen. However, it is our opinion that the terms CT antigen (often abbreviated as CTA) and CT gene be preserved as they best capture the unique expression pattern, potential antigenicity, and history of this family. Moving forward, advances in

immunopeptidomics could streamline characterization of CT antigens capable of evoking an immune response and ultimately provide additional immunotherapeutic targets [20–22].

CT Antigens Have Mostly Unknown Cellular Functions

Despite long-standing efforts to exploit the antigenic properties of CT antigens for use in immunotherapy, scant information exists concerning their biological relevance to either spermatogenesis or malignancy. Provocatively, a number of studies have tied individual or sets of CT antigens to poor survival in tumors, suggesting that their expression could promote aggressive tumor growth and/or chemorefractory disease [23–26]. However, ascribing malignant functions to individual CT antigens has proven difficult for several reasons. Many of these proteins lack known motifs or domains from which their activities could be inferred. Additionally, a number of CT antigens have been characterized as intrinsically disordered, making functional predictions based on amino acid sequence alone unreliable [27]. Perhaps the most formidable challenge is that many CT genes are not well conserved, thus precluding traditional *in vivo* genetic studies. Indeed, male reproductive genes have undergone rapid and selective evolution in primates in comparison to other mammals, and many of those encoding CT antigens are no exception [18, 28, 29]. In particular, many CT-X antigens do not have murine counterparts. They are found in normal spermatogonia and are often classified as testisrestricted, but their expression can be readily activated by DNA demethylation in tumors, potentially making them optimal immunotherapeutic targets. However, their contribution to gametogenesis has been difficult to investigate due to a lack of model organisms for genetic studies. On the other hand, non-X CT antigens are typically expressed in spermatocytes and many have murine orthologs. The functions of these non-X CT antigens have been better characterized due to the availability of knockout mouse models. These studies have revealed functional roles in genomic maintenance, meiosis, transcriptional regulation, motility, and energy regulation during spermatogenesis [30]. Notably, CT antigen knockouts often leave animals infertile but otherwise healthy, reinforcing the notion that their inhibition in tumors would have negligible deleterious impacts on normal tissues.

Whether these anomalously expressed proteins could indeed take on functions in a non-native cellular environment and participate in tumorigenesis has been a lingering question since their initial discovery. The functional investigation of CT antigens found to be essential for tumor cell fitness, or those whose expression can predict patient outcome, has the potential to unveil previously unknown mechanisms that promote unbridled growth of cancers (Figure 1B). Indeed, the notion that activation of germline gene expression programs can drive malignancy is a long-held hypothesis first developed by Lloyd Old and colleagues [31]. In the following sections, we highlight recent findings from *Drosophila melanogaster* that appear to support this notion, and discuss the accumulating body of evidence that CT antigens are functional in human cancers and often essential for neoplastic processes.

CT Genes Contribute to Tumorigenesis in *Drosophila melanogaster*

Using a malignant brain tumor model driven by a mutation in the *lethal (3) malignant brain tumor* (*l(3)mbt*) gene, Janic and colleagues found that the expression of Drosophila CT

genes drives tumor growth and that their suppression is sufficient to restrict tumor growth [32]. Notably, these Drosophila CT genes have human orthologs, including *piwi* (*PIWIL1*) and *nanos* (*NANOS1*), many of which are upregulated in human cancers [33]. An RNA interference screen performed using the *l(3)mbt* brain tumor model also recently identified the Drosophila gene *meiotic W68* (*mei-W68*), an ortholog of the human CT gene *SPO11*, as essential for tumor growth [34]. Given the role of SPO11 in the formation of DNA double-strand breaks during meiosis, it is possible that mei-W68/SPO11 contributes to tumorigenesis via modulation of genome stability [30]. However, future work will be required to elucidate the exact mechanism through which this protein supports tumor growth in Drosophila. Studies in human cancers demonstrating the relevance of SPO11 to tumorigenesis are also lacking, despite its reported expression in tumor cells and classification as a CT antigen [19]. Nonetheless, it is becoming clear that using model organisms such as Drosophila is an attractive approach to examine the extent to which activation of germline gene expression programs contributes to tumorigenesis. However, caution must be used in presuming that genetically engineered organisms will activate the same germline genes as humans. The molecular etiology of genetically engineered tumors may bypass the selective pressures encountered during human tumor evolution. Thus, the requirement of specific CT antigens to overcome barriers to transformation may be quite different among species.

CT Antigens Participate in Diverse Cellular Processes in Human Cancer

A number of studies have described individual CT antigens that contribute to neoplastic behaviors of human tumor cells (Table 1) [24, 26, 35–76]. Collectively, these findings have begun to broadly demonstrate the diversity of CT antigen function in human cancer. In many cases, these discoveries are based on large-scale functional genomic screens, proteomic analyses, or gene expression profiling [24, 35, 39, 43, 46, 51–53, 59, 67]. The utility of such approaches in identifying functional CT antigens speaks to the importance of unbiased, hypothesis-generating tools in revealing unanticipated players on the tumorigenic landscape. Interestingly, current evidence indicates that CT antigen function can be organized into three broad categories: transcriptional regulation, mitotic fidelity, and protein degradation (Table 1, Figure 1). A summary of some of these reported functions and their associated cellular processes is represented in Figure 2 **(Key Figure)**.

CT Antigens Can Regulate Tumor Cell Transcriptional Networks

One of the earliest discoveries of a molecular function for a CT antigen in human cancer was reported just over a decade ago by the Bernards group [66]. Analysis of the PRAME protein sequence revealed the presence of a conserved region of nuclear receptor boxes, suggesting a function in modulating nuclear receptor hormone signaling. Indeed, PRAME was characterized as a retinoic acid receptor (RAR) antagonist, preventing its ligand-mediated activation and facilitating recruitment of transcriptionally repressive Polycomb (PcG) proteins to target genomic loci. In this manner, PRAME acts to bypass the tumor suppressive differentiation programs directed by retinoic acid signaling. Hence, efforts aimed at pharmacologically targeting the PRAME-RAR interaction would likely be promising given the general druggability of nuclear receptors [77].

More recently, the C_2H_2 zinc finger transcription factor ZNF165 emerged from a loss-of-function screen as essential for viability and transforming growth factor beta (TGFβ) signaling in triple negative breast cancer (TNBC) [24]. Importantly, TGFβ signaling is highly context-dependent; it is often oncogenic in TNBC but more commonly tumor-suppressive in normal breast epithelia and other breast cancer subtypes (e.g. luminal A/B) [78, 79]. ZNF165 associates with a number of different TGFβ target gene loci to both activate and inhibit their transcription, likely tipping the balance to a more oncogenic signaling program. Given the function of both PRAME and ZNF165, a theme begins to emerge that CT antigens can be engaged to alter transcriptional networks that otherwise serve as gatekeepers to uncontrolled growth.

At least two CT antigens have now been implicated in modulating circadian rhythms in cancer cells via interactions with the CLOCK:BMAL heterodimer, which controls transcription of temporally regulated genes [80]. While the contributions of CLOCK and BMAL to tumorigenesis are not well established, it is becoming clear that disruption of the normal circadian clock is correlated with increased incidence of cancer, as key components of the cell cycle are temporally regulated [80–82]. The CT antigen PASD1, which is evolutionarily related to CLOCK, interacts with BMAL to disrupt its gene regulatory function through molecular mimicry [63]. PIWIL2, which is crucial for piRNA regulation in the developing germ cell, was recently shown to destabilize the CLOCK:BMAL heterodimer and simultaneously block its transcriptional activity in both testis and tumor cells [65, 83]. It is interesting to note that circadian rhythms are absent from the testes, possibly due to the action of CT antigens such as PASD1 and PIWIL2 [84]. Thus, the expression of PASD1 and PIWIL2 in somatic cells may indeed wreak havoc on the normal circadian regulatory network that controls crucial aspects of cellular physiology, such as cell cycle progression.

Of the large MAGE family that consists of more than 60 proteins, twenty-four are classified as CT antigens and display aberrant expression across multiple cancer types [85]. A number of reports suggest a function for this family of CT antigens in transcriptional regulation. Laduron and colleagues found that MAGE-A1, the founding member of the CT antigen family, interacts with the transcription factor SKIP and directs the histone deacetylase HDAC1 to SKIP target genes to facilitate transcriptional repression [86]. Several members of the MAGE family have also been shown to interact with the p53 tumor suppressor to modulate its transcriptional activity, via both HDAC1 recruitment and inhibition of p53 DNA binding [87, 88]. Interestingly, recent reports also suggest that many MAGE proteins interact with the E3 ubiquitin ligase TRIM28 to facilitate degradation of tumor suppressor proteins, including p53 [25, 58, 89]. It is worth noting that the functions of TRIM28, while not completely understood, include widespread transcriptional regulation in addition to its E3 ligase activity [90, 91]. MAGE proteins therefore likely modulate a balance of both transcription and protein degradation to achieve their potent activity as drivers of oncogenic growth.

An exciting, recent finding suggests that long non-coding RNAs are also part of the CT antigen family. Hosono and colleagues recently reported the discovery of a cancer/testis lncRNA (CT-lncRNA), THOR, that is capable of promoting oncogenic growth [92]. Using multiple tumor cell lines and zebrafish as a model organism, the authors demonstrated that

THOR expression is sufficient to enhance malignant growth and is required for melanoma onset in the zebrafish model. THOR interacts with IGF2BP1 and enhances its ability to stabilize target mRNAs. Notably, THOR also interacts with IGF2BP3, a CT antigen previously shown to promote oncogenic growth and HIF signaling, suggesting that THOR may regulate IGF2BP3 activity in a manner similar to IGF2BP1 [24, 53, 54]. Future studies will be able to determine if THOR represents a broader subset of germline-restricted lncRNAs with the capacity to contribute to tumorigenesis via post-transcriptional regulation.

From Meiotic Recombination to Mitotic Fidelity

Numerous CT antigens appear to buttress formation of the mitotic spindle, preventing mitotic and/or genomic catastrophe (Table 1). Kinetochore assembly, which is essential for proper mitosis in rapidly dividing cancer cells, is aided by numerous CT antigens, including TEX14, CASC5, TTK, and NUF2 [40, 61, 71, 93–96]. The CT antigen ACRBP, identified using a synthetic lethal RNA interference screening approach, is essential for bipolar spindle formation in non-small cell lung cancer (NSCLC) and ovarian cancer due to its regulation of NUMA protein accumulation [35, 36]. In addition, at least two CT antigens are implicated in ensuring proper cell abscission during cytokinesis. CEP55 associates with the centralspindlin complex and is required for midbody formation and membrane fusion [41]. MPHOSPH1, on the other hand, interacts with PRC1 to support cytokinesis and drive cell growth in bladder cancer [59]. Notably, cellular or humoral immune responses to TTK, NUF2, ACRBP, CEP55, and MPHOSPH1 have been reported, indicating their potential as immunotherapeutic targets [97–102]. The development of small-molecule inhibitors targeting the functions of these CT antigens is also an attractive approach to increase the effectiveness and simultaneously decrease the toxicity of chemotherapeutic mitotic poisons [51]. For example, multiple ongoing clinical trials are in place to determine the efficacy of small-molecule inhibitors targeting TTK, which is considered to be one of the more promising anti-mitotic therapeutic targets [103]. The CT antigens CCDC110, FMR1NB, NXF2, TCC52, and OIP5 have all also been identified as positive regulators of mitosis in tumor cells, although their exact functions remain unknown [51, 104–106].

A number CT antigens, including SPO11, TEX15, SYCP1/3, and HORMAD1/2, are essential for meiosis [30]. Knowledge of their functions in meiotic recombination has led to the proposal that these CT antigens, among others, can promote oncogenic proliferation and potentially facilitate “meiomitotic” cell division in cancer [52, 107, 108]. Interestingly, at least two meiotic CT antigens have recently been demonstrated to confer an advantage to tumor cells through regulation of DNA damage repair mechanisms and therefore genomic stability. SYCP3 was recently shown to interact with BRCA2 and prevent DNA damage repair mechanisms in tumor cells, which often display severe chromosomal scarring [70]. Expression of another CT antigen, HORMAD1, was found to be highly upregulated in TNBC with high allelic imbalance. The authors implicated HORMAD1 in promoting non-homologous end joining, an error prone DNA repair pathway, and therefore driving genomic instability [52]. Recently, work from our own laboratory has shown that in NSCLC, HORMAD1 can promote homologous recombination to support DNA repair and cell division [26]. These reports suggest that HORMAD1 function in tumors is context-dependent, and that it can exert opposing effects on DNA repair mechanisms to influence

malignant growth in each context. The ability of different tumors to engage the same CT antigen in a capacity suited to a specific genetic background speaks to the extraordinary adaptability of tumor regulatory networks. Importantly, the study of CT antigen function is beginning to reveal the depths and mechanisms of this adaptive capacity.

CT Antigens Regulate Protein Degradation to Antagonize Tumor Suppression Mechanisms

Although several members of the MAGE family of CT antigens are reported to regulate transcription, recent work also suggests a generalizable function for this family in coordinating protein degradation in tumor cells [58, 85]. In particular, the ~170 amino acid, highly conserved MAGE homology domain (MHD) binds RING domain E3 ubiquitin ligases. MAGEC2 and MAGE-A3/6 interact with the E3 ubiquitin ligase TRIM28 to promote proteasome-dependent degradation of the p53 and AMPK tumor suppressors, respectively [25, 58, 89]. MAGE-A4 has also been demonstrated to stabilize the E3 ubiquitin ligase RAD18, thereby promoting trans-lesion synthesis and increasing the capacity of tumor cells to cope with DNA damage [109]. Thus, a general mechanism is emerging for the function of MAGE proteins in regulating E3 ubiquitin ligase activity. Future studies to reconcile the transcriptional and post-translational functions of MAGE proteins will be important for further deducing the significance of MAGE function in tumorigenesis. Outside of the MAGE family, a similar mechanism was described for the CT antigen FATE1, which interacts with the E3 ubiquitin ligase RNF183. Studies have demonstrated that RNF183 is upregulated in tumors and targets the apoptotic proteins BIK and Bcl-XL at the mitochondria for destruction [24]. It is interesting to note that despite FATE1 having no sequence homology with members of the MAGE family, these CT antigens operate through similar mechanisms to prevent tumor-suppressor protein accumulation.

Concluding Remarks

The question as to why tumors express CT antigens has gone unanswered for many decades. Evidence presented herein indicates that their expression may be selected for during tumor evolution to bypass intrinsic proliferation and survival barriers that would otherwise lead to cell cycle arrest, senescence, or death. Continued studies in this untapped discovery space will likely expand our understanding of the remarkable plasticity in the tumor cell regulatory environment to overcome barriers to growth (see Outstanding Questions). However, with over 200 CT antigens annotated and many more candidates identified, it is a daunting task to first identify functionally relevant CT antigens and then delineate their mechanisms of action. High-throughput gain- and loss-of-function screens offer the opportunity to assess the relevance of numerous CT antigens en masse in a variety of contexts. CRISPR gene-editing techniques could serve as a powerful tool in this regard and provide increased fidelity for identifying true positives from such screening approaches. In addition, mining clinical data sets to determine correlative associations among specific CT antigens, patient outcome, and response to therapy can indicate functional relevance and reveal specific disease sites in which to study CT antigen behavior. Further in-depth examination of CT antigen function in distinct tumorigenic processes will undoubtedly provide conceptual breakthroughs regarding the mechanisms that regulate tumor cell growth and proliferation.

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Highlights

- CT antigens have historically been considered promising targets for immunotherapy due to their restricted expression in tumors and immuneprivileged organs, such as the testes.
- The molecular functions of CT antigens in germ cells or tumor cells are difficult to decipher and remain largely unexamined.
- Accumulating evidence suggests that many CT antigens make significant contributions to tumor cell physiology and promote neoplastic behaviors.

Outstanding Questions

- What are the gene expression mechanisms that induce and control the aberrant activation of CT antigens in tumors?
- Why do some tumors express very few CT antigens (e.g. brain, pancreatic), while others express many (e.g. lung, melanoma)?
- Which CT antigens support neoplastic processes and what are the molecular mechanisms by which they do so?
- Are CT antigens capable of initiating tumorigenesis?
- Can CT antigens be directly targeted with single agent therapy or in combination with current targeted/chemotherapeutic agents?

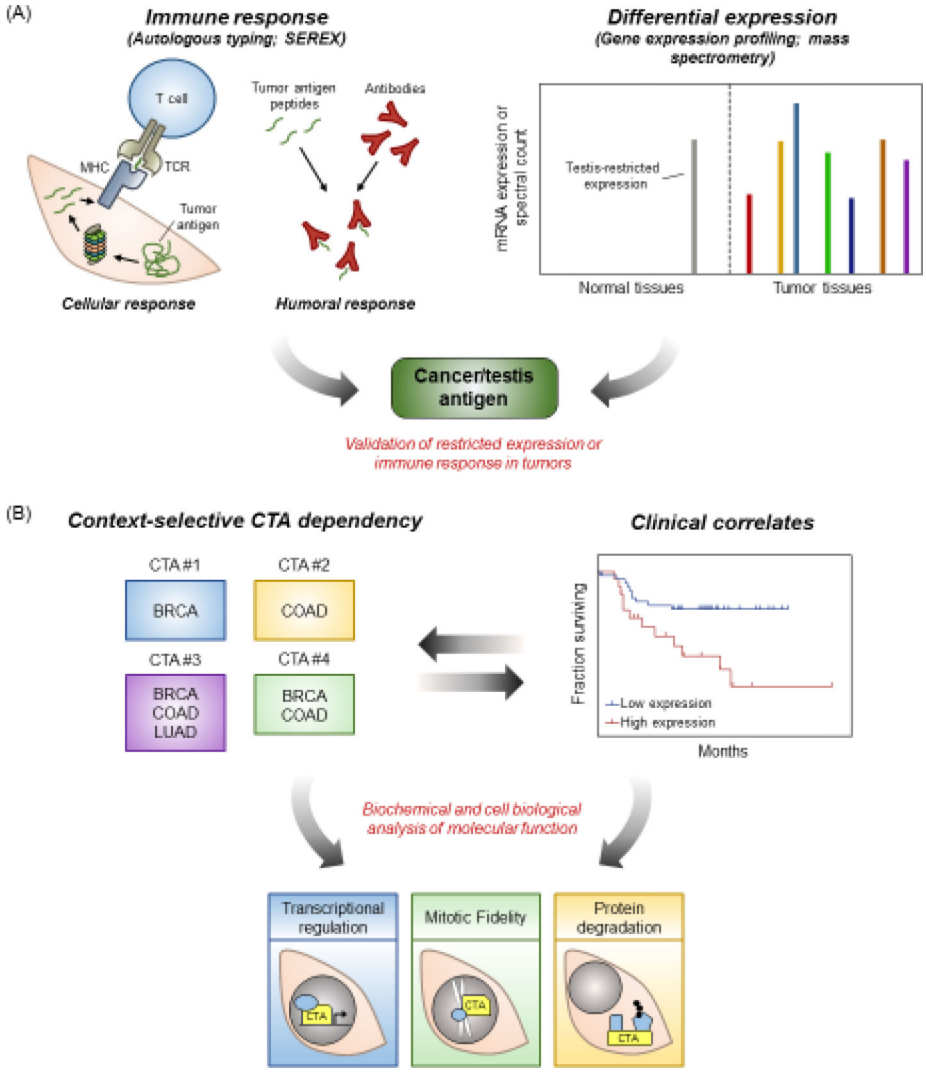

Figure 1. Workflow for the identification and functional characterization of CT antigens.

(A) CT antigens are identified either by their propensity to evoke an immune response (left) or their differential, testis-enriched expression pattern (right). (B) Differential expression analysis and patient survival data can be used to identify appropriate contexts for downstream functional analysis. Abbreviations: MHC, major histocompatibility complex; TCR, T cell receptor; BRCA, breast cancer; COAD, colon adenocarcinoma; LUAD, lung adenocarcinoma.

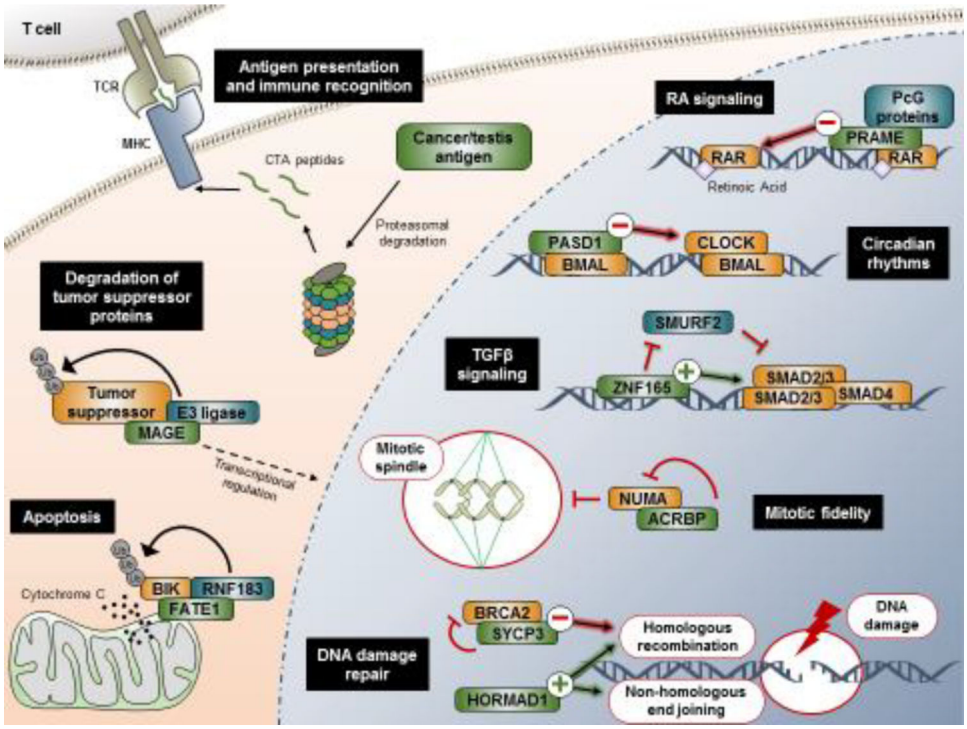

Figure 2. A snapshot of CT antigen behavior in tumors cells.

In addition to their capacity to evoke an immune response through antigen presentation, CT antigens have been demonstrated to contribute to diverse neoplastic behaviors. CT antigens depicted here (in green) serve as key examples of how aberrant expression of these proteins can influence tumor cell regulatory networks. Abbreviations: MHC, major histocompatibility complex; TCR, T cell receptor; Ub, ubiquitin; RA, retinoic acid; PcG, polycomb group; TGFβ, transforming growth factor beta.

Table 1.

CT antigens reported to promote neoplastic behaviors in human tumor cells

Name	Description	Refs
ACRBP	Interacts with NUMA to support mitotic spindle dynamics	[35, 36]
AKAP4	Supports enhanced PKA signaling and oncogenic growth	[37]
ATAD2	Integral to DNA replication in tumor cells and acts as a cofactor for oncogenic MYC	[38, 39]
CASC5	Essential for kinetochore assembly and chromosome segregation	[40]
CEP55	Required for cell abscission during cytokinesis and interacts with PI3K to enhance its activity	[41, 42]
CTAG2	Regulates invasion of breast cancer cells via interaction with pericentrin at the centrosome	[43]
CTCFL	Can activate expression of other CT antigens in cancer and promote alternative splicing to facilitate oncogenic growth	[44, 45]
CTNNA2	Expression of the mutated (truncated) protein promotes invasion and migration	[46]
CCNA1	Enhances VEGF expression and promotes metastasis	[47, 48]
DDX43	Promotes unwinding and translation of SOCS1 mRNA in a manner dependent on its helicase activity, leading to PML suppression and increased growth capacity	[49]
DPPA2	Capable of oncogenic transformation through transcriptional regulation	[50]
FATE1	Facilitates the degradation of the pro-apoptotic protein BIK through interaction with the E3 ubiquitin ligase RNF183	[24]
FMR1NB	Required for successful chromosome segregation and mitosis	[51]
HORMAD1	Regulates DNA damage repair mechanisms	[26, 52]
IGF2BP3	Promotes oncogenic growth through regulation of mRNA stability and mRNA-microRNA interactions	[53, 54]
LUZP4	Modulates mRNA export in melanoma cells	[55]
LY6K	Promotes multiple neoplastic behaviors in breast cancer through regulation of TGFβ signaling	[56]
MAEL	Enhances the lysosome-dependent degradation of the protein phosphatase ILKAP	[57]
MAGEs	Large family of related proteins that form complexes with RING E3 ubiquitin ligases to facilitate oncogenic phenotypes through protein degradation or transcriptional regulation	[58, 85]
MPHOSPH1	Supports cytokinesis via interaction with PRC1	[59]
NUF2	Stabilizes kinetochore-microtubule attachments to support chromosome segregation through interaction with CENP-E	[60–62]
NXF2	Required for successful chromosome segregation and mitosis	[51]
PASD1	Interferes with the circadian clock via interaction with BMAL	[63]
PBK	Enhances JNK1 activity to facilitate oncogenic transformation by H-Ras	[64]
PIWIL2	Interferes with the circadian clock by modulating CLOCK:BMAL stability	[65]
PRAME	Represses retinoic acid (RA) signaling through interaction with RAR	[66]
SPAG9	Supports mitosis through interaction with PLK1 and regulation of FOXK1 transcriptional activity	[67]
SPANXA/C/D	Promotes breast cancer invasion via interaction with lamin A/C	[43]
SSX1/2	Forms a fusion oncoprotein with SS18 and drives synovial sarcoma via re-wiring gene regulation programs through interactions with PRC1.1 and the BAF complex	[68, 69]
SYCP3	Inhibits DNA damage repair mechanisms through interaction with BRCA2	[70]
TEX14	Supports mitosis through regulation of kinetochore-microtubule attachments and the spindle assembly checkpoint	[71]
TFDP3	Regulates cell cycle progression by disrupting the transcriptional activity of E2F proteins	[72]
TSP50	Promotes oncogenic growth through suppression of activin signaling	[73]

Name	Description	Refs
TTK	Essential for the spindle assembly checkpoint and mitosis	[74–76]
ZNF165	Regulates the TGFβ transcriptional network to promote oncogenic signaling in triple negative breast cancer	[24]