

Cancer/Testis Antigens and Colorectal Cancer

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Abstract

Some antigenic proteins are only normally present in male gametogenic tissues in the testis and not in normal somatic cells. When the genes that encode these proteins are expressed in cancerous cells they are referred to as cancer/testis antigen (CTA) genes. Some CTA genes have been proven to encode immunogenic proteins that have been used as successful immunotherapy targets for various forms of cancer. Thus, there has been a focus over the past two decades to characterise their potential applications for the treatment and/or diagnosis of cancer. Colorectal cancer is among the most common forms of cancer and a leading cause of cancer related death worldwide. It is generally regarded as having low levels of CTA gene expression, compared to some other cancers such as malignant melanoma. There is now scope to revisit this dogma, as an ill-defined cohort of colorectal cancers do exhibit CTA gene expression, potentially exposing these tumours to CTA-based therapeutic and/or diagnostic strategies as they are developed.

Keywords: Cancer/testis antigen; Colorectal cancer; Cancer biomarkers; Immunotherapies; Gene expression; Lynch syndrome; Mismatch repair; Microsatellite instability

Abbreviations: APC: Adenomatous Polyposis Coli; CTA: Cancer/Testis Antigen; CRC: Colorectal Cancer; ELISA: Enzyme: Linked Immunosorbant Assay; HNPCC: Hereditary Non-Polyposis Colorectal Cancer; IHC: Immunohistochemistry; MMR: Mismatch Repair; MSI: Microsatellite Instability; PCR: Polymerase Chain Reaction; SEREX: Serological Analysis of Recombinant cDNA Expression Libraries

Introduction

Colorectal cancer (CRC) is among the most common malignancies in both men and women world-wide and a leading cause of cancer related mortality. When treated early by surgical or endoscopic resection, the disease carries a relatively good prognosis, but when it presents in a more advanced stage the prognosis is generally poor. All too often the disease presents at a late stage given the frequent insidious nature of presentation. This latter group of patients are often offered chemotherapy to either palliate symptoms or increase chances of cure when patients are undergoing surgical resection. Radiotherapy is commonly given as adjuvant treatment for tumours within the rectum. Although biological agents are used for selected patients with advanced disease, immunotherapy is not as commonly utilised for the treatment of CRC [1-6]. However, recent advances in our understanding of tumour immunology have reignited interest in such therapeutic strategies across a range of tumour types [7-17]. Cancer/testis antigens (CTAs) have emerged as one group of proteins that have significant immunogenic potential and as such have been heralded as a route towards the development of novel anti-cancer treatments [18-25]. This review summarises current understanding of CTAs and CTA gene expression in CRC and forms a basis to consider future areas for both scientific and clinical exploration.

Colorectal cancer pathways and genetic predisposition

A number of 'pathways' that lead to the development of CRC have been described, but in reality there is a complex overlap between genetic and environmental factors that leads to disease development [26]. The majority of CRCs are sporadic and most of these conform

broadly to the Vogelstein model of carcinogenesis, that is they arise from successive acquired mutations in genes such as *Adenomatous Polyposis Coli (APC)* and *K-ras* and lead to CRC via the 'chromosomal instability pathway' [26,27]. Sporadic cases of CRC usually occur in later life, with an average age at diagnosis of around 70 years and the vast majority of patients being diagnosed over the age of 50 years. The microsatellite instability pathway and CpG Island Methylator Phenotype (CIMP) pathway are the other pathways described, together accounting for approximately one third of all cases of CRC [26,28]. A minority of CRC cases are due to inherited genetic defects that lead to an increased likelihood of developing the disease. Collectively the inherited syndromes associated with CRC account for at least 5% of all cases of the disease, but as yet ill-defined inherited factors may explain a much larger proportion of the overall disease burden [29,30]. Identifying patients who have an inherited disorder predisposing them to CRC is often not easy, and relies on obtaining a detailed family history. However, the diagnosis of an inherited disorder is important as it not only has implications for the management of the disease but also relatives of the individuals who may for instance be offered regular colonoscopic screening or genetic counselling/testing.

Familial adenomatous polyposis (FAP) is an autosomal dominant condition caused by mutation of the *APC* gene that results in loss of normal function leading to the development of hundreds of colonic polyps and the almost inevitable development of CRC before the age of 40 years. Patients known to have this condition usually undergo surgery to remove their entire large bowel before their third decade of life [29].

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The most common inherited disorder associated with CRC is Lynch Syndrome or Hereditary Non Polyposis Colorectal Cancer (HNPCC) [26,31]. Lynch syndrome is also inherited in an autosomal dominant fashion and is caused by germline mutations in DNA mismatch repair (MMR) genes. The common MMR genes that are mutated in Lynch syndrome include: mutL homolog 1 (*MLH1*), mutS homolog 2 (*MSH2*), mutS homolog 6 (*MSH6*) and postmeiotic segregation increased 2 (*PMS2*) [31-33]. Failure of MMR through such gene mutations leads to a state termed microsatellite instability (MSI). Somatic mutations in the same or other MMR genes can lead to CRC via the MSI pathway in much the same way that germline mutations do [33-35]. Mismatches in nucleotide base pairing are common during DNA replication and the MMR system is of vital importance in recognising and repairing these mistakes. Microsatellites are repetitive sequences of DNA that occur throughout the genome and are particularly susceptible to errors during DNA replication [33,36]. If the errors are not corrected in genes involved, for example, in cell growth or replication then this can lead to a phenotype that promotes tumourigenesis [37]. MSI leads to mutations in β -catenin and *TGF β RII* more commonly than genes such as *K-ras* and *p53* that are also associated with CRC [38]. Overall 15-20% of all CRCs develop via the MSI pathway, of which a proportion of patients will come from a family with Lynch syndrome. Failure of MMR, as seen in Lynch Syndrome, leads to an increased susceptibility to cancer more generally. Indeed, Lynch Syndrome is associated with an increased risk of various malignancies, including: endometrial, ovarian, brain, skin as well as tumours of the upper gastrointestinal and urological systems [26]. The Amsterdam criteria and Bethesda guidelines are the two commonly used tools to identify patients at high risk of an inherited genetic disorder [39,40]. Specific genetic testing is then performed to reach a diagnosis [40,41]. The term HNPCC is no longer favoured, largely because it ignores the fact that the condition is associated with an increased risk of many other malignancies and also because these patients can develop polyps within the colon.

Although over half of CRCs develop within the left (or distal) colon and rectum, tumours that develop via the MSI pathway have a propensity to develop within the proximal colon. The exact reasons for this phenomenon are not known but may be related to the level of β -catenin signalling within the colon [42].

The importance of CTA genes

The cancer/testis antigen (CTA) genes have attracted a great deal of interest; particularly in the field of oncology and tumour immunology [18-25]. 'Bona fide' CTA genes have expression restricted to the testes of normal tissues, but are aberrantly expressed in cancer [43,44]. Sub-categories of CTAs, based on expression limited to the brain and testes, and limited expression in normal tissues have been described – termed testis/brain-restricted and testis-selective, respectively [43]. Most of the known CTA genes are X-encoded and many belong to large paralogous gene families (for example, [45]). An additional sub-category of meiosis specific CTA genes – termed mei-CT (or mei-CTA) genes has recently been described [46], many of which are autosomally encoded as the X chromosome becomes transcriptionally silenced in mammalian male meiosis [47]. CTA genes are of importance for two fundamentally distinct reasons. Firstly, there is an immunological barrier known as the blood-testis barrier that generates an immunological privilege within the testis that is mediated via a number of pathways [48,49]; consequently, CTAs are capable of eliciting an autologous immune response. Thus, CTAs can serve as highly restricted cancer-specific antigens, making them exceptionally attractive as diagnostic, prognostic and therapeutic biomarkers, the targeting of which should not induce

deleterious side effects in non-cancerous, healthy cells. Secondly, genes whose products normally serve to drive meiotic chromosome dynamics, germ cell regulation and gametogenesis differentiation may have powerful oncogenic transforming activity (both genetic and epigenetic) if aberrantly expressed in non-germ line, somatic tissues; a possibility which remains largely unexplored.

CTA gene expression in colorectal cancer

Previous studies have indicated that CTA genes are not widely or universally expressed in all tumour types [18-25]. CRCs fall within the tumour group that have limited CTA gene expression. However, this generalised classification does not account for the possibility that there is a sub-group of CRCs that do have a high incidence of CTA gene expression that could provide valuable clinical targets that currently remain unexplored. Here we bring into focus the evidence supporting CTAs as potential biomarkers for at least a cohort of CRCs. Table 1 summarises expression profiles of CTA genes within CRC tissue or cell lines. There is no single, obvious CTA gene that is widely expressed across all CRC tissues, although some of the cited studies were limited in their scope. However, given the patient-specific tailored therapy that advanced immunotherapy approaches represent it could be the case that a cohort of patients might benefit from the therapeutic potential of CTA genes, assuming that CTA gene expression is associated with antigen production that can be therapeutically harnessed. Some tumours have been found to co-express CTA genes [50], and thus, it may be the case that the majority of CRCs do not express any CTA genes, but rather a minority express several. To what extent this is the case currently lacks resolution, but some of the evidence for co-expression in CRCs is given below.

The melanoma associated antigen (*MAGE*) family of CTAs were the first to be described [51]. They have tightly restricted expression limited to the testis in normal tissues and are among the most immunogenic of the known CTAs [45,51-53]. They are encoded by a large paralogous gene family of over 60 genes that are located on the X chromosome [45], and whilst their function in testis germ cells is poorly understood, they are known to possess oncogenic activity (for example, [54]). Several of the *MAGE* gene family members are expressed in CRC (Table 1). Others, such as *MAGE-A12*, *MAGE-B1* and *MAGE-B2* are proposed to have no expression in CRC but this has not been extensively corroborated [55,56]. Burgdorf et al. tested for *MAGE* expression in liver biopsy specimens from nineteen patients with metastatic CRC [57]. In contrast to a previous study showing no *MAGE-A12* expression in primary tumours [55], they found this gene to be expressed in many of the specimens tested [57]. Although the exact frequency of *MAGE-A12* expression was not stated, 47% of the specimens were shown to express all six of the *MAGE* genes tested for. *MAGE-A12* expression was also demonstrated in four CRC cell lines and some primary CRC tissues in a separate study, but insufficient data was provided to give a precise expression frequency [58]. Overall 79% of liver biopsy specimens were found to express at least one of the *MAGE* gene family members [57]. This could imply that more aggressive or advanced forms of colorectal cancer (i.e. that have metastasised) express *MAGE* genes more widely and that they may even be contributing to the metastatic process. Importantly, recent work has demonstrated extensive intra tumour genetic heterogeneity, which extends to metastases vs. primary tumour comparisons [59]. This fundamentally important finding serves to complicate how gene expression profiling is assessed and CTA gene expression profiles should be viewed in this light. The extent of *MAGE* gene co-expression and collective expression of at least one family member within a cohort of primary tumours has not been extensively

CTA gene of interest	% Tissue Expression(number of positive samples/total samples tested)	Matched normal tissue tested?	% Cell Line Expression (number of positive samples/total samples tested)	% Serological detection (positive detection e.g. with ELISA/number of individuals tested)	Comment
AKAP3	(5/6) 83.3% [110]	No	(3/10) 30% [110]		
BCP-20(FBXO39)	(22/57) 38.6% [66]	No	(2/7) 28.6% [32]		Expressed weakly in normal ovary, placenta, lung and prostate
BCP-33 (LOC374973)	(12/57) 21.1% [66]	No			Expressed weakly in normal pancreas
BCP-41(TLL2)	(17/57) 29.8% [66]	No			Weak expression in various normal tissues
BJ-HCC-20	(3/18) 16.7% [111]	Yes			
BORIS	(8/10) 80% [112]	No	(3/4) 75% [33]		
CAGE	(35/47) 74.4% [55,64]	Yes	(2/4) 50% [113]	(7/74) 9.9% [25,27]	Expression seen in some normal colonic tissue (see main text for discussion)
CCDC62-2	(5/40) 12.5% [114]	No		(2/11) 18.2% [114]	
CT16	(1/9) 11.1% [115]	No			CT16 has homology with GAGEfamily
CT45	(36/250) 14.4% [60]	Yes			
cTAGE-1	(6/39) 15.4% [101]	No	See comment		Cell line and tissue expression frequency combined (31 tumours and 8 CRC cell lines used)
cTAGE-5A	(13/39) 33.3% [101]	No	See comment		See comment for cTAGE-1
CTp11	(2/9) 22.2% [116]	No			
DBPC (CONTRIN)	(9/10) 90% [117]	No			Protein production assessed using IHC – no PCR used
FATE(FATE-1, BCP-55)	(3/14) 21.4% [118]	Yes	(0/2) 0% [23]		
FAM46D	(4*/18) 22.2% [119]	No		(0/18) 0% [119]	*Number positive estimated from percentage displayed
GASZ	(4*/18) 22.2% [119]	No		(0/18) 0% [119]	*Number positive estimated from percentage displayed
HAGE	(46/288) 16.0% [60,116,120]	Yes, except [116]			
IGSF11	(6/11) 54.5% [121]	No			
KP-CoT-23 (CCDC83)	[122]			(26/37) 70.3% [122]	
LAGE-1	(68/503) 13.5% [55,60,61,123]	Yes			
LDHC	(3/20) 15% [94]	No			
LEMD1	(17/18) 94.4% [124]	Yes			Used matched normal tissue but data not presented
MAGE-1 (MAGE-A1)	(63/484) 13.0% [55,60,61,65,125]	Yes, except [125]	(7/12) 58.3% [58,65]	(0/25) 0% [126]	[58] – also tested CRC tissue but incomplete data provided
MAGE-2 (MAGE-A2)	(62/338) 18.3% [55,60,65]	Yes	(6/17) 35.3% [58,65,85]		[58] – also tested CRC tissue but incomplete data provided
MAGE-3 (MAGE-A3)	(118/535) 22.1% [55,60,61,65,67,127]	Yes, except [127]	(7/24) 29.2% [65,127]	(0/25) 0% [126]	Note: MAGE-3 has extensive homology to MAGE-6
MAGE-4 (MAGE-A4)	(79/419) 18.9% [60,61,67]	Yes	(4/17) 23.5% [58,65,85]		[58] – also tested CRC tissue but incomplete data provided
MAGE-A5	(17/250) 6.8% [60]	Yes			
MAGE-A6	(69/250) 27.6% [60]	Yes	(2/5) 40% [85]		
MAGE-A10	(1/48) 2.1% [67]	Yes			
MAGE-A12	47%* or more [57] (0/34) 0% [55]	Yes	(4/4) 100% [58]		*See main text for discussion. [58] – also tested CRC tissue but incomplete data provided
MAGE-C1 (CT7)	(4/305) 1.3% [55,60,93,128]	Yes, except [92]			[128] – Identified by IHC in one sample but none by PCR
MAGE-C2 (CT10)	(22/381) 5.8% [60,61,129]	Yes, except [129]	(1/2) 50% [94]		CT10/MAGE-C2 shows extensive homology to CT7/MAGE-C1
NXF2	(2/18) 11.1% [90]	No			
NY-ESO-1	(34/567) 6.0% [55,60,61,67,123,130]	Yes, except [130]		(1/107) 0.9% [61,126]	
OY-TES-1	(2/13) 15.4% [131]	No		(6/58) 10.3% [131]	
PAGE-4	(74/250) 29.6% [60]	Yes			
PASD1	(8*/18) 38.9% [119]	No		(0/18) 0% [119]	*Number positive estimated from percentage displayed

<i>POTE</i>	(7/7) 100% [68]	No			No normal colon tissue analysed in this study
<i>SCP-1</i>	(14/403) 3.5% [60,61,132]	Yes, except [132]			
<i>SPAG9</i>	(58/78) 74.3% [91]	Yes	(2/2) 100% [91]	(38/54) 70.4% [91]	Matched normal tissue obtained in 26 of 78 samples
<i>SPANX</i>	(15/250) 6% [60]	Yes			
<i>SSX-1</i>	(10/561) 1.8% [55,60,61,123,133]	Yes, except [133]			
<i>SSX-2</i>	(37/641) 5.8% [55,60,61,68,123,133,134]	Yes, except [133,134]		(2/99) <2.0%*[73,126]	*Unclear how many sera samples tested in [134]
<i>SSX-4</i>	(50/561) 8.9% [55,60,61,123,133]	Yes, except [133]			
<i>SSX-5</i>	(1/58) 1.7% [133]	No			
<i>TEKT5</i>	(5/10) 50% [135]	No	(4/44) 9.1% [135]		
<i>TPTE</i>	(13/264) 4.9% [60,118]	Yes	(0/2) 0% [118]		
<i>TRAG-3</i>	(6/92) 6.5% [136,137]	Yes but see comment	(3/11) 27.3% [136]		[137] – demonstrated weak expression in one adjacent normal tissue sample and also included two purchased cDNA CRCs not matched.
<i>TSGA10</i>	(1/20) 5% [138]	No		0% [138]	
<i>TSP50</i>	(85*/95) 89.5% [92]	No	(7/7) 100% [92]		*Assessed by IHC in CRC tissue. Weak positivity also in 60% of normal controls.
<i>ZNF165</i>	(6/14) 42.9% [139]	Yes			

Abbreviations: IHC – Immunohistochemistry, PCR – Polymerase Chain Reaction, * – see comment on same row

Table 1: CTA genes that have been demonstrated to have expression in CRC tissue.

investigated for comparison to the Burgdorf study that assessed CRC liver metastases, therefore limiting the conclusions that can be drawn from these findings. The largest set of samples tested found 90 out of 250 CRC specimens to express at least one of six *MAGE-A* genes [60]. Li et al. also tested for the expression of ten well characterised CTA genes, including four members of the *MAGE* family, in a cohort of 121 CRC patients [61]. Over half (56.2%) of patients expressed at least one CTA and around one quarter co-expressed two or more of the CTA genes; the four assessed *MAGE* genes were relatively widely expressed in this cohort. These figures are not dramatically different to the study investigating liver metastatic specimens, which importantly looked at a different set of *MAGE* genes making a direct comparison of limited value [57]. An even higher co-expression frequency (approaching 90%) of one or more of ten *MAGE* genes was seen in a study of eighty CRC specimens [62]. It is interesting to note that *MAGE-8* was found to have the highest expression frequency (44%) and this gene has not been as widely investigated as some of the other *MAGE* genes. Choi and Chang found the expression of *MAGE* and *SSX* genes to be correlated with the presence of liver metastases in CRC [63]. In this study they used primer sets that were complimentary to several *MAGE* (*MAGE-A1-A6*) and *SSX* (*SSX-1-9*) genes; from 37 samples tested a combined expression frequency of 51.4% was found for *MAGE* genes and 32.4% for *SSX* genes.

A phase II trial assessing the use of a therapeutic vaccine based on dendritic cells that have been pulsed with a tumour cell lysate (prepared from a *MAGE*-expressing melanoma cell line) showed some promising results and low toxicity in patients with metastatic CRC [57]. However, in an earlier study a very weak overall clinical response rate of less than 1% was found for the use of cancer vaccines to treat CRC, despite induction of an immune response in around half of patients [6]. However, these vaccines were not all CTA derived and so CTA based vaccines may result in distinct and possibly higher clinical response rates, but this requires formal testing.

Variability in expression between studies and between CTA genes

In Table 1, the frequency of CTA gene expression is combined for the individual genes from data generated in different studies, when more than one study has tested for expression. The actual frequency of expression varies considerably between studies and lack of uniformity in analytical techniques cannot be ruled out as a source of data variation. For example, *CAGE* gene expression was detected in less than one third of CRC specimens by Shi et al. [64] but in over 90% in a separate study [55]. In fact, weak expression was found for the *CAGE* gene in the same proportion of normal colonic samples [55], which questions whether *CAGE* is in fact a CTA gene. Shi et al. [64] also reported that a reduced proportion of the matched normal colonic tissue tested also expressed the *CAGE* gene. It is noteworthy that in one of these studies [55], very weak expression for other well known CTA genes was demonstrated in CRC, which contrasts with the findings of others [56,61,65]. The reasons for the variability in expression of CTA genes found in CRC is not clear but is likely to be related to experimental differences as well as inter- and intra-tumour heterogeneity.

Several reported CTA genes have been demonstrated not to be expressed in CRC in studies with relatively small sample sizes (range 5-34 separate samples) and in general the findings have not been corroborated by multiple or larger scale studies (Table 2). As shown in Table 2, a minority of the genes were shown to be expressed in one or more CRC cell line. Further members of the *MAGE* family have also been investigated to see whether they are expressed in CRC cell lines; in the absence of treatment with a DNA demethylating agent there was no expression of *MAGE-A9*, *MAGE-A11*, *MAGE-B1* or *MAGE-B2* in these samples [66,67]. These low expression frequencies contrast with a markedly high expression frequency of some other CTA genes, notably *AKAP3*, *BORIS*, *CAGE*, *DBPC*, *LEMD1* and *POTE* which were shown to have expression frequencies of 83%, 80%, 74%, 90%, 94% and 100% respectively (Table 1). These studies were all single studies, with the exception of *CAGE* analysis, with relatively few CRC samples

CTA gene of interest	No. of samples tested	Matched normal tissue tested?	% Expression in cell lines (number of positive cell line expression/number of cell lines tested)	Comments
ADAM-2	29 [95,115]	No		ADAM-2 also referred to as CT15
CT9	6 [140]	No		CT9 referred to as bromodomain testis-specific gene (BRDT)
CT17	9 [115]	No		
CT47	6 [141]	No	(3/3) 100% [141]	Not certain from text if CT47 expression detected in all three cell lines. Quantitative PCR and relative expression to testis used to decide if not expressed in tumour tissue.
FTHL17	11 [90]	No		
GAGE-1 to GAGE-8	34 [55]	Yes		Eight GAGE family members tested (i.e. GAGE-1-8)
KU-CT-1	21 [72]	No	(0/7) 0% [72]	Note: 1 out of 18 patients (5.6%) had a positive serological test
MORC	20 [95]	No		
MMA-1A	5 [142]	No	(1/8) 12.5% [142]	Weak expression in cell lines only
MMA-1B	5 [142]	No	(2/8) 25% [142]	Weak expression in cell lines only
PAGE-1	34 [55]	Yes		
RAGE-4	34 [55]	Yes		
SAGE	19 [120]	No		
SCAGE-ac	34 [55]	Yes		
SGY-1	20 [95]	No		
SPO11	20 [95]	No		
TAF2Q	11 [90]	No		
TDRD1	8 [90]	No		
TEX15	8 [90]	No		Expressed in normal brain tissue
TPX-1	20 [95]	No		

Table 2: Well characterised CTA genes that have no evidence of expression in CRC tissue.

tested. *POTE*, for example, was found to be expressed in commercially purchased colon cancer tissue/cDNA [68]. Analysis of normal tissue was excluded but it had been previously reported that *POTE* expression was limited to the prostate, ovary, testis and placenta in normal tissues [69]. However, freshly obtained cancer and (where possible) matched normal tissue should be considered the 'gold-standard' when investigating expression profiles for potential CTA genes. Another of the expressed CTA genes in CRC, *BORIS*, has been shown to have potential as a DNA vaccine, and furthermore *BORIS* may promote DNA demethylation at CTA gene regulatory regions resulting in the activation of the associated gene [70]. Thus, the potential role of *BORIS* in CRC oncogenesis and therapies should be further scrutinised.

Some studies have reported the expression of CTA genes in CRC cell lines but not within CRC tissue. Examples include: *MCAK*, which was expressed in five out of six cell lines tested [71], and *TAG-1*, *TAG-2A*, *TAG-2B*, *TAG-2C*, which were expressed in four, two, one and two out of four CRC cell lines tested respectively [72]. Some of these genes are expressed with high frequency, so further systematic investigation in CRC tissue and matched normal colonic tissue would be worthwhile to establish the significance of these expression profiles. The *MCAK* gene was detected in five out of six CRC cell lines tested but was also weakly detected in normal colonic tissue; it is not stated in the study report how this normal tissue was collected, though it does raise doubt over whether the gene is a *bona fide* CTA gene.

Confirmation of antigen production

Few studies have attempted to detect and quantify the presence of the gene products (i.e. the antigens) within CRC or circulating blood from patients with CRC. This can be done, for example, by looking for the presence of the protein directly within tumour tissue (e.g., immunohistochemistry or Western blotting) or through detection of a serological (immune) response within the body. The latter

method, utilising enzyme-linked immunosorbant assay (ELISA), has been more widely performed and has the advantage of not only indicating antigen presence but also giving some idea of its potential as a biomarker or therapeutic target. NY-ESO-1 was detected strongly by immunohistochemistry (IHC) in one out of twelve (8.3%) CRC specimens tested in one study [61] but in none of ten samples tested in a separate study [73]. A positive serological response against NY-ESO-1 was seen in five of seventy four patients investigated by Scanlan et al. [74] and in one of eighty two patients in another study by Li et al. [61]. This corresponds to an overall serological detection rate of approximately 4%. These figures correlate reasonably well with frequency of gene expression in CRC, which is around 6% (Table 1), suggesting that when the gene is expressed the protein is produced. A significant correlation between *NY-ESO-1* gene expression and more advanced disease stage has also been alluded to [61].

Jungbluth et al. performed IHC using an antibody reactive against various antigens of the MAGE family but had no positive results in fifteen CRC specimens tested [75]. However, positive serological results have been obtained (e.g., against MAGE-A3) with a frequency of approximately 8% [74], suggesting that at least some of the MAGE antigens are aberrantly produced in CRC. NY-ESO-1 and MAGE antigens are amongst the most immunogenic of the CTAs characterised to date and encouraging responses have been seen when used as immunotherapy targets in selected patients [52,76]. Current evidence would suggest that NY-ESO-1 or MAGE proteins are unlikely to be good broad spectrum targets for new diagnostic or therapeutic approaches in all CRC, however, this does not preclude their use for a subset of patients. Indeed a partial clinical response in a patient with metastatic CRC has been seen utilising a vaccine based on the MAGE-A4 antigen [77], which holds promise for the development of further related therapeutic approaches.

Iwata et al. used ELISA to detect anti-CAGE antibodies in the

serum of CRC patients and found two strongly positive results and two weakly positive results; however, they also reported a weak positive in serum from a supposedly cancer-free control individual [78]. The two strong ELISA results corresponded to the presence of the CAGE protein in the tissue, assessed by Western blotting. As with the gene expression analyses (see above), the positive signal in a supposedly cancer-free control sample further question whether CAGE is a testis-restricted CTA, but more importantly reduces the likelihood of it being a useful therapeutic target. However, in the study by Shi et al. [64] the three patients who had a positive serological test (i.e. antibodies against CAGE detectable in their serum) all had more advanced stage disease. This suggests a serological test for CAGE could have potential as a prognostic marker or in a disease monitoring capacity, even if CAGE is demonstrated not to be useful as an immunotherapeutic target in CRC. It is also noteworthy to point out the fact that tissues reported to be 'normal' might have underlying, undiagnosed disease. It has been demonstrated that asymptomatic, apparently healthy individuals frequently carry micro-tumours [79] and Chen et al. reported expression of some CTA genes in some sets of apparently disease-free tissue, but not in others; this suggests that some tissue is susceptible to CTA gene expression before symptomatic or histopathological diagnosis [80]. This observation possibly relates to the fact 'normal' tissue is often obtained *post mortem* from older individuals whose cause of death was not cancer related, but who might have had an underlying undiagnosed cancer.

Global genome demethylation is often seen in cancerous cells and is believed to be responsible for some aberrant gene expression activation [81,82]. The heavily methylated promoter regions of some CTA genes are thought to be responsible, at least in part, for the tight repression of their expression in normal somatic cells [82-84]. The promoter regions of a cohort of CTA genes, including *MAGE-A1*, are often unmethylated in the testes where the genes are normally expressed [84]. Indeed, CRC cell lines show an increase in the expression of various CTA genes when the cultured cells are treated with the demethylating agent 5-azacytidine [85,86]. It has recently been demonstrated that induction of *NY-ESO-1* expression can be achieved *in vivo* resulting in an immune response [86]. Such epigenetic modulation combined with adoptive cell transfer could prove to be a powerful tool in anti-cancer therapy.

Intra- and inter-tumour differential CTA gene expression is likely to have multi-factorial regulation. When CTAs are present, IHC has indicated quite heterogeneous presence within the tumour tissue [67]. Thus, depending on the sampling method, a section of the tumour with a limited CTA distribution may be sampled when other areas of the cancer do express the relevant gene with higher frequency, and this inherent heterogeneity is likely to be a major factor in many tumour types when considering the clinical potential of CTAs [59]. Sharma et al. have demonstrated that γ -irradiation can induce CTA production both within cancer cell lines and biopsies of cancer tissue [87]. Furthermore, this was shown to be associated with an enhanced immune response. It is also interesting to point out that chemo-radiotherapy can be associated with a complete clinical response in patients with rectal cancer [88]. It is possible that enhanced cancer-antigen recognition is, in part, responsible for the complete resolution of tumours seen in some patients. It would be worth exploring whether γ -irradiation can induce the production of CTAs and other cancer-associated antigens in CRCs.

The mei-CTA genes in CRC

A new sub-category of meiosis-specific CTAs has recently been described, termed mei-CT (or mei-CTA) genes, most of which differ

from many of the genes currently analysed in CRC (see above) as they are mostly autosomally encoded [46]. In combined data set meta-analysis of tumour microarray data, none of the meiCT genes were shown to have a significant broad range upregulation in expression for CRCs. However, when individual data sets were analysed some meiCT genes had a significant expression upregulation in CRC; these genes were: *NUT* and *CCDC105* in one data set each, and *TCTE3* in three out of thirteen (23%) individual data sets comparing CRC tissue to normal colonic tissue controls [46]. *SSX-2*, a known CTA gene, was also demonstrated to be upregulated in one of the thirteen data sets for CRC (7.7%); this is a gene that has an overall expression frequency of around 6% in CRC (Table 1). These findings again suggest that specific CTAs could play an important role in a sub-set of CRC patients and be utilised for clinical benefit.

Possible roles in tumorigenesis

CRCs that arise via the MSI pathway, such as those seen in Lynch syndrome, have the propensity to develop in the proximal (right-sided) colon and tend to be larger tumours than their non-MSI counterparts; the generally better prognosis and lower rates of metastases in MSI-positive CRC is not fully understood [33,42,89]. Iwata et al. [78] provide a tantalising piece of evidence to suggest that MSI might be associated with wider CTA gene expression, in that both the patients with CRC in which they demonstrated the presence of anti-CAGE antibodies in the serum had Lynch syndrome. This limited observation opens up the question of whether MSI tumours more commonly express CTA genes. The immuno-suppressive microenvironment within the tumour may protect cells from immune-attack but as soon as they 'escape' the tumour in an attempt to metastasise they might be more susceptible to immune mediated destruction. Thus, MSI tumours might be less likely to seed metastases as expressed CTA genes may generate antigens more readily attacked by the immune system, resulting in a better prognosis for these tumours. Whilst this is a speculative proposal, it illustrates the potential benefits of a detailed and full characterisation of CTA expression in CRCs.

CTA genes are unusual in the fact that a lot of research has now been conducted into their clinical potential, even to the point of conducting clinical trials targeting the antigens produced, when relatively little is known about their function. Many of the known functions appear unrelated to the oncogenic process [90]. This would provide support for the idea that CTA gene activation is a random event and related to the demethylation associated with tumorigenesis [82,83]. The presence of CTAs within a given cancer could have positive as well as negative consequences for the patient; germline genes involved in meiosis when activated could be driving the oncogenic process but conversely could help enhance the immune response against the cancer leading to an improved prognosis/reduced propensity to metastasise. Alves et al. found no significant differences in gene expression or protein presence for some CTAs between metastatic and primary lesions in CRC [67], suggesting the presence of CTAs neither protects nor promotes metastasis. However, others have put forward a list of CTAs that are associated with metastases in CRC [60]. *SPAG9* was shown to be associated with early stages of CRC and not with metastases [91,92], whereas another proposed testis-selective CTA gene, *TSP50*, was associated with poor prognoses in CRC. Future studies may well discover important oncogenic roles for many of the CTAs such as the modulation of p53 by *MAGE* family members [54]. Although serological analysis of recombinant cDNA expression libraries (SEREX) has proved an important method in identifying several new CTA genes [93,94], others have warned of relying too heavily on such

an approach, arguing that gene products “immunologically ignored” in cancer patients may be equally or even superior in a therapeutic setting [95].

Whilst this review has covered many of the CTA genes of potential relevance in CRC, it should be noted that there are further genes that may be of immunological significance in CRC. Whilst *bona fide* CTA genes represent an important category, other genes that have been defined as testis-selective (i.e. weak expression seen in some normal tissues) have been tested in CRC. Examples include: *HSP105* [96,97], *RFX4* variants [98], *GPA34* [99], *RAP80/UIMC1* [100], *TRAG-3* [70], *cTAGE* variants [101], *NY-CO-58/KNSL6* [74], *NW-BR-3* [102], *RBP1L1* [103], *KU-MEL-1* [104], *HSP60* [105], *RNF43* [106], *KIF18A (SW#108)* [107], *TOMM34* [108] and several other antigens identified by SEREX [95]. Although some of these genes might be relevant for separate specific malignancies, HSP105 has demonstrated promise as an immunotherapeutic target in a mouse model of CRC [96]. Furthermore, a phase I clinical trial for advanced CRC using a cancer vaccine developed from antigens encoded by two of these testis-selective genes, in combination with chemotherapy has recently been reported, the results of which showed some limited positive responses [109]. These genes further illustrate the expanse of the potential pool of CTA genes that might provide new clinical tools as we move ever closer to an era of more personalised therapeutics.

Conclusions

We have attempted to collate the current understanding of CTA gene expression and CTA presentation in CRC and although much is already known it remains a relatively under-researched area of translational science research. Although the majority of the well known CTA genes display a generally low expression profile in CRC in accordance with the current dogma, there are some CTA genes that appear to challenge this view. Moreover, there may be a subset of CRCs that express CTA genes more widely and for which the existence of CTAs could be of clinical importance. The specific nature of this cohort of tumours remains unknown. Many studies were conducted some time ago and did not have a specific focus on CRC, nor did they consider CTA gene expression profiles in the context of personalised medicine. The full extent to which CTA genes are expressed and CTAs presented in CRCs and whether they can be utilised as biomarkers or therapeutic targets will be answered in the years to come.

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