CTAs in Triple-Negative Breast Cancer

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Triple-negative breast cancer (TNBC) has been associated with worse prognoses due to the limited treatment options. Thus, there is a need to characterise new biomarkers or treatment targets to improve patient outcomes. Cancer testis antigens (CTAs) are a group of antigens that are preferentially expressed in tumours and exhibit strong immunogenicity, as such, CTAs hold great promise as potential treatment targets and biomarkers in cancer.

Keywords: triple-negative breast cancer ; cancer testis antigens ; pathology

1. Introduction

Breast cancer is a heterogeneous disease that can be classified based on the clinical, morphological and biological characteristics [1][2]. Triple-negative breast cancers (TNBC), a special class of breast cancers that are negative for oestrogen receptor (ER), progesterone receptor (PR) and HER2 (cerbB2), represent 9–17% of all breast carcinomas, depending on the threshold for ER, PR and HER2 positivity [1][3][4][5]. TNBC are also of high histological grade, displaying aggressive clinical behaviour with poorer prognosis and accompanied by frequent metastasis to the brain and lungs, with a shorter time to recurrence and death [3][5].

Cancer testis antigens (CTAs) are tumour antigens that are expressed normally in embryonic stem cells and testicular germ cells and minimally expressed in most other tissues. CTAs are aberrantly found in various cancers, especially advanced cancers with stem-cell-like characteristics [6][7]. CTAs were first identified in 1991 in a study showing that the presence of MAGEA1 caused resistant tumour cell clones to be sensitised to killing by autologous cytotoxic T-lymphocytes [8]. Since then, there has been an explosion of CTA-related research and the discovery of more CTAs, including NY-ESO1, which is the most successful target to date for cancer immunotherapy [9][10]. The number of CTAs identified has also increased exponentially over the years. In 2009, a database was created of about 70 families and more than 200 members of CTAs [11]. With the advent of next-generation sequencing, there has been a huge increase in genomic data. By integrating transcriptomic data from multiple databases, Wang et al. systematically identified 876 new CTAs in 19 cancers [12], and a different research group found an additional 201 new CTAs [13].

2. Expression of CTAs in Triple-Negative Breast Cancer

Receptor tyrosine kinase-like orphan receptor 2 (ROR2), a novel Wnt receptor, belongs to the tyrosine kinase receptor family, which is important in regulating skeletal and neuronal development, cell migration and cell polarity (**Table 1**) [14]. Breast cancer patients, including those with TNBC expressing ROR2, experienced a significantly worse prognosis with shorter overall survival compared to those lacking ROR2 [15].

Table 1. Summary of CTAs in TNBC.

CTAs	Cellular Function	Institute	Cohort	Prevalence of CTAs in TNBC	Type of Assay	Antibodies	Role in TNBC	Ref.
CTAs associated with worse prognosis in TNBC								
A-kinase anchoring proteins (AKAP3)	**Sperm function**	**Breast Cancer Research Centre (Tehran, Iran) [16]**	**Asian**	**20% (*n* = 25)**	**Real-Time Polymerase Chain Reaction (RT-PCR)**		**Loss of expression in TNBC. Breast cancer patients who were positive for AKAP3 had better 5-year disease-free survival.**	**[16] [17]**

CTAs	Cellular Function	Institute	Cohort	Prevalence of CTAs in TNBC	Type of Assay	Antibodies	Role in TNBC	Ref.
Melanoma antigen gene (MAGE)	Not known. May promote tumourigenesis and metastasis.	Italian National Cancer Institute [18]	Caucasian	MAGE-A: 23% (*n* = 44)	IHC	MAGE-A Antibody (6C1)	Frequently overexpressed in TNBC. Higher expression of MAGE-A was reported to define a very aggressive subtype of TNBC and correlated with poor prognosis of patients. MAGE-A3, -A6 and -C2 expression in breast cancers was significantly associated with negative ER or negative PR status, higher-grade tumours and correlated with worse outcomes. MAGE-A10 expression was associated with ER-negative, PR-negative and HER2-negative status.	[18] [19] [20] [21] [22] [23] [24] [25] [26] [27] [28] [29]
		Royal Brisbane Women's Hospital [26]	Caucasian	MAGE-A: 47% (*n* = 65)	IHC	MAGE-A Antibody (6C1), Santa Cruz Biotechnology(USA)		
		Affiliated Tumour Hospital of Xinjiang Medical University [27]	Asian	MAGE-C: 38.2% (*n* = 110)	IHC	Rabbit polyclonal MAGE-C2 Antibody, Sigma-Aldrich (USA)		
		Centre of Breast Cancer of The Fourth Hospital of Hebei Medical University (Shijiazhuang Hebei) [28]	Asian	MAGE-A: 76.5% (*n* = 17)	IHC	MAGE-A Antibody (6C1), Santa Cruz Biotechnology(USA)		
		University Hospital Center Zagreb [25]	Caucasian	MAGE-A: 85.7% (*n* = 49)	IHC	3DA3 Monoclonal Antibody		
		Split University Hospital Centre, Croatia [30]	Caucasian	MAGE-A1 Specific: 69.2% (*n* = 81)	IHC	Monoclonal Antibody 77B		
				Multi-MAGE: 58% (*n* = 81)	IHC	Monoclonal Antibody 57B		
				MAGE-A10: 16% (*n* = 81)	IHC	Monoclonal Antibody 3GA11		
		European Institute of Oncology (Milan, Italy) [19]	Caucasian	MAGE-A: 32% (*n* = 50)	IHC	Antibody cocktail of monoclonal antibodies 6C1, MA454, M3H67 and 57B		
		Copenhagen University Hospital [31]	Caucasian	MAGE-A: 33% (*n* = 78)	IHC	Rabbit polyclonal anti-peptide antibody EP101638 (rab Ab 1982) raised against Mage-4, Eurogentec (Belgium)		
		National Cancer Institute (Milan, Italy) [29]	Caucasian	MAGE-A: 85.7–93% (*n* = 21)	IHC	MAGE-A3 (Clone 60054-1-Ig) Monoclonal Antibody, Proteinthec (USA)		

CTAs	Cellular Function	Institute	Cohort	Prevalence of CTAs in TNBC	Type of Assay	Antibodies	Role in TNBC	Ref.
Mesothelin (MSLN)	GPI-anchored membrane protein	Perelman School of Medicine, University of Pennsylvania [32]	Caucasian	67% (*n* = 99)	IHC	Mesothelin Monoclonal Antibody (clone 5B2), Thermo Scientific (USA)	MSLN is significantly expressed in TNBC compared to non-TNBC and is an independent prognostic marker associated with distant metastasis and worse survival.	[32] [33] [34]
		University of Texas MD Anderson Cancer Center [34]	Caucasian	34% (*n* = 109)	IHC	Mesothelin Monoclonal Antibody (clone 5B2), Novocastra (USA)		
Prostate stem cell antigen (PSCA)	GPI-anchored membrane protein	University Hospital of Dresden, Germany [35]	Caucasian	17% (*n* = 90)	IHC	PSCA antibody MB1	Distribution of PSCA expression among TNBC was comparable to the total population. Patients with PSCA-positive invasive micropapillary carcinoma (IMPC) of the breast had decreased disease-free survival.	[35] [36]
Receptor tyrosine kinase-like orphan receptor 2 (ROR2)	Tyrosine kinase receptor family	University of New South Wales [15]	Caucasian	87% (*n* = 295, breast cancer including triple-negative)	IHC	Human ROR2 polyclonal antibody, Sigma-Aldrich (Australia)	Breast cancer patients including TNBC expressing ROR2 had significantly worse prognoses with shorter overall survival compared to those lacking ROR2.	[15]
Sperm protein associated with the nucleus X-linked (SPANX)	Sperm function	University of Texas Health Science Center [37]	Caucasian	73% (*n* = 15)	IHC	SPANXB1 (#H00728695), Abnova (Taiwan)	SPANXB1 was frequently overexpressed in human primary and metastatic TNBC. In ER-negative patients, elevated SPANX-A/C/D was correlated with shorter distant metastasis-free survival time.	[37] [38]
CTAs associated with better prognosis in TNBC								

CTAs	Cellular Function	Institute	Cohort	Prevalence of CTAs in TNBC	Type of Assay	Antibodies	Role in TNBC	Ref.
New York oesophageal squamous cell carcinoma-1 (NY-ESO-1)	Unknown; might be involved in cell cycle progression and growth	New York Presbyterian Hospital-Weill Cornell Medical Center and UCSF Medical Center [21]	Caucasian	19.2% (*n* = 50)	IHC	NY-ESO-1 Monoclonal Antibody(E978) produced in author's laboratory	Higher expression of NY-ESO-1 was detected in TNBC. NY-ESO-1 expression was correlated with tumour-infiltrating lymphocytes and associated with good prognosis.	[19] [21] [25] [26] [29] [39] [40] [41] [42]
		University Hospital Center Zagreb [25]	Caucasian	10% (*n* = 50)	IHC	NY-ESO-1 Monoclonal Antibody (B9.8.1.1)		
		Roswell Park Cancer Institute [39]	Caucasian	16% (*n* = 168)	IHC	NY-ESO-1 Mouse Monoclonal, Zymed/Invitrogen (USA)		
		Asan Medical Centre, Korea [41]	Asian	9.3% (*n* = 172)	IHC	NY-ESO-1 Monoclonal Antibody (E978), Invitrogen (USA)		
		Royal Brisbane Women's hospital [26]	Caucasian	~20% (*n* = 65)	IHC	NY-ESO-1 Antibody (E978), Santa Cruz Biotechnology(USA)		
		National Cancer Institute (Milan, Italy) [29]	Caucasian	28.6% (*n* = 21)	IHC	NY-ESO-1 Monoclonal Antibody (E978), Invitrogen (USA)		
		European Institute of Oncology (Milan, Italy) [42]	Caucasian	16% (*n* = 50)	IHC	NY-ESO-1 Monoclonal antibody (E978) provided by Ludwig Institute for Cancer Research		
CTAs with oncogenic potential								
Melanoma antigen gene (MAGE)	Not known. May promote tumourigenesis and metastasis.	See Above					Promote tumourigenesis and metastasis via various mechanisms such as acting as master regulator of E3 RING ubiquitin ligase, inhibiting p53 tumour suppressor or by enhancing cell motility.	[19] [20] [21] [22] [23] [24] [25]
New York oesophageal squamous cell carcinoma-1 (NY-ESO-1)	Unknown; might be involved in cell cycle progression and growth	See Above					Might be involved in cellular proliferation and growth.	[9]
Preferentially expressed antigen of melanoma (PRAME)	Membrane-bound protein	National Cancer Institute (Milan, Italy) [29]	Caucasian	85.7–96.6% (*n* = 21)	IHC	PRAME Polyclonal Antibody (Clone NBP1-85418), Novus Boilogicals (USA)	Role in EMT reprogramming. Expression of PRAME was associated with negative ER status.	[43] [44] [45]

CTAs	Cellular Function	Institute	Cohort	Prevalence of CTAs in TNBC	Type of Assay	Antibodies	Role in TNBC	Ref.
Sperm-associated antigen 9 (SPAG9)	Sperm function	National Institute of Immunology, Aruna Asaf Ali Marg, (New Delhi, India) [46]	Asian	NA	IHC	Polyclonal antibody to SPAG9 was prepared in authors' laboratory	Analysis of 100 breast cancer tissues (94 infiltrating ductal carcinomas [IDC], 2 ductal carcinomas in situ [DCIS] and 4 invasive lobular carcinomas [ILC]) revealed that 88% of samples stained positive for SPAG9. Role in invasiveness of breast cancer. Downregulation could reduce invasive potential of TNBC.	[46] [47]
Sperm protein associated with the nucleus X-linked (SPANX)	Sperm function	See Above					Required for metastasis. Interacts with lamin A/C at the inner nuclear membrane and involved in the formation of actin-rich cellular protrusions that reorganise the extracellular matrix.	[37] [38]
Testes-specific protease 50 (TSP50)	Oncogene	Northeast Normal University, (Changchun, China)	Caucasian	NA	IHC	TSP50 Monoclonal Antibody was prepared in authors' laboratory	Analysis of 88 clinical breast cancer tissue microarrays (BR952 and BR1101 from US Biomax, Rockville, MD, USA) revealed that 90.9% of specimens stained positive for TSP50 compared to 10% of adjacent normal tissues. Role in cell growth. Knockdown of TSP50 in breast cancer cells significantly inhibits cellular proliferation. TSP50-positive tumours were associated with negative ER expression and higher grade.	[48] [49]

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CTAs	Cellular Function	Prevalence of CTAs in TNBC	Institute	Cohort	Type of Assay	Antibodies	Role in TNBC	Ref
Cancer/testis antigen 165 (ZNF165)	Gene regulation	90% (n = 10)	Simmons Comprehensive Cancer Center, UT-Southwestern Medical Center, Dallas [50]	Caucasian	IHC	ZNF165 (H00007718), Novus Biologicals (USA)	Enhances growth and survival of human TNBC cells both in vitro and in vivo by regulating TGF-β signalling. Frequently overexpressed in TNBC.	[50] [51]
Tripartite motif containing 27 (TRIM27)	Gene regulation	NA	Simmons Comprehensive Cancer Center, UT-Southwestern Medical Center, Dallas [50]	Caucasian	TCGA		TRIM27 expression was significantly elevated in TNBC compared to normal breast tissue based on TCGA data. Displayed difference in cellular localisation, as it was mainly cytoplasmic in normal breast epithelia and more nuclear in TNBC tissues. Regulates TGFβ-dependent transcription in complex with ZNF165, ZNF446 and SMAD in TNBC.	[50] [51]
Other CTAs with increased expression in TNBC								
MAGE-A8 (HAGE)	Cellular architecture	57% (IF = 4 in TCGA)	National Centre for Tumour Diseases (Heidelberg, Germany) [52]	Caucasian	TCGA		Frequently expressed in TNBC based on in silico analysis.	[52]
MAGE-A3	Cellular architecture	84.7% (n = 17 from commercial vendor)	Johannes Gutenberg University (Mainz, Germany) [53]	Caucasian	IHC	Anti-cMAGE-A3 polyclonal antibody	Frequently expressed in TNBC.	[53]
Chromosome X open reading frame 61, mastermind-like domain containing 1/Kita-Kyu-Shu lung cancer antigen-1 (CXorf61/MAMDL1/KK-LC-1/CT83)	Development of male genitalia	Not known	Kitasato University Medical Center (Japan) [54]	Asian	IHC	Mouse monoclonal antibody was prepared by CLEA Japan (Japan)	Frequently expressed in TNBC based on in silico analysis. Frequently overexpressed in TNBC and tumours without ER positivity.	[52] [54]
Sperm protein 17 (SP17)	Sperm function	47.2% (n = 36)	University of Texas MD Anderson Cancer [55]	Caucasian	IHC	Antibody against SP17	SP17 is frequently expressed in primary breast tumours and in TNBC	[55]

CTAs	Cellular Function	Institute	Cohort	Prevalence of CTAs in TNBC	Type of Assay	Antibodies	Role in TNBC	Ref.
Wilms tumour-1 (WT-1)	Transcription factor	European Institute of Oncology (Milan, Italy) [42]	Caucasian	54% (*n* = 27)	IHC	WT1 Monoclonal Antibody (Clone WT49), Monosan (Netherlands)	Highest expression in TNBC compared to other breast cancer subtypes.	[42]

SPANX-A/C/D was discovered as an important factor for the metastasis of breast cancer cells; it was hypothesised to act via interactions with components of the cytoskeleton at the inner nuclear membrane and is required to produce actin-rich cellular protrusions during modelling of the extracellular matrix (**Table 1**). Sperm-associated antigen 9 (SPAG9) mRNA and protein expression was found in the cytoplasm of all examined breast cancer cells, including TNBC cells (**Table 1**) [47]. Interestingly, the downregulation of SPAG9 ameliorated the invasiveness of TNBC [47].

Testes-specific protease 50 (TSP50) is an oncogene that promotes breast cancer survival, invasion and metastasis via the activation of the NF-κB signalling pathway (**Table 1**) [56]. Knockdown of TSP50 in breast cancer cells significantly inhibited cellular proliferation [49]. The levels of TSP50 together with the expression of p65 and matrix metalloproteinase 9 (MMP9) were analysed in conjunction with clinicopathological features, such as tumour size, pathologic grade, ER and PR levels, in breast cancer tissues [48], and the majority of TSP50+/p65+ tumours (92%) and TSP50+/MMP9+(78%) tumours were negative for ER expression and tended to be of a higher grade [48].

Sperm protein 17 (SP17) is involved in various stages of spermiogenesis, and aberrant SP17 expression has been linked to cancers such as ovarian, oesophagus, central nervous system, multiple myeloma and esthesioneuroblastoma (**Table 1**) [57][58][59][60][61][62]. The exact role of SP17 in cancer cells has not yet been elucidated, with some reports suggesting that SP17 promotes cell–cell adhesion in malignant B-lymphocytes via interaction with heparan-sulphate and enhances cell movement and drug resistance in ovarian cells [63][64]. SP17 was preferentially expressed in breast cancer cell lines and primary breast tumours, including TNBC, compared to non-tumoural breast tissue [55]. Specific anti-SP17 antibodies were also discovered in patients' sera, and the generation of SP17-specific, HLA class I-restricted, cytotoxic T-lymphocytes led to the death of breast cancer cells, opening the possibility that SP17 could be a valid target for TNBC immunotherapy [55].

3. Future Potential Application of CTAs Clinically

Multiple laboratories have utilised mIHC/IF to evaluate biomarkers in several cancers. Systematic review and meta-analysis of tumour patients from 8135 patients revealed that mIHC/IF had superior diagnostic accuracy in predicting clinical response to anti-PD-1/PD-L1 therapy than PD-L1 IHC, tumour mutational burden or gene expression profiling [65]. An optimised mIHC /IF protocol was developed for PD-L1 testing in TNBC which demonstrated good correlation with conventional IHC, thus providing further evidence for the feasibility of incorporating mIHC/IF in clinical practice [66]. A recent multisite study comparing mIF on PD-1/PD-L1 axis on tonsil and breast carcinoma and non-small cell lung cancer (NSCLC) demonstrated good reproducibility and sensitivity across multiple institutions which included Johns Hopkins University, Yale University, MD Anderson Cancer Center, Earle A. Chiles Research Institute, Akoya Biosciences and Bristol-Myers Squibb [67]. Standardisation, validation and reproducibility of end-to-end workflow across multiple sites and clinical laboratory processes are important to promote translation of mIHC/IF technology to clinical practice. The evidence from feasibility studies have been encouraging, and more research could be done in this area to translate emerging technologies to clinical practice. Recently, a high throughput system to analyse tissue microarrays of breast cancer samples using multiplexed microfluidic IHC to generate biomarker barcodes have been developed [68]. The biomarker barcode of breast cancer patient-derived tissue microarrays was compared to traditional method of breast cancer diagnosis, thus opening the possibility of high-throughput screening with diagnostic capability.

CTAs such as NY-ESO-1, MAGE, MSLN, SPANXB1, PRAME, ZNF165, TRIM27, KK-LC-1 , SP17 and WT-1 were found to be enriched in TNBC compared to non-TNBC tissue (**Table 1**). The specific enrichment of CTAs opens the possibility of CTAs as targets for personalised treatments or for identifying subtypes of patients with better or worse prognosis. In addition, CTAs are immunogenic, thus providing opportunities for development of therapeutic vaccines and are attractive targets for immunotherapy. Given the growing evidence of their roles in TNBC, CTAs are potential targets for therapeutic intervention. Several clinical trials are currently being conducted to evaluate the effectiveness of CTAs, such as NY-ESO-1, MAGE, MSLN, PRAME, PSCA, ROR2 and WT1, as treatment targets in breast cancers and other solid tumours. CTAs

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are being widely developed as cancer vaccine, T-cell immunotherapy and antibody-based therapy in breast cancer including TNBC. Other CTAs are also evaluated in clinical trials as single therapy or combination therapy in other cancer types, highlighting the attractiveness of CTAs as potential therapeutic strategy for cancers [69].

Further research could be performed to analyse expressions of CTAs with different immune infiltrates and correlate with clinicopathological characteristics, given the growing evidence of CTA having immunogenic and oncogenic properties in TNBC and promoting tumourigenesis or invasiveness (**Table 1**). Several CTAs are co-expressed in TNBC, and further research could be performed to delineate different CTAs molecular signatures and its prognostic value for TNBC clinically. More research could be performed to understand mechanistically the underlying biological and cellular functions of CTAs in TNBC, as well as the relationships between CTAs and hormone receptor or HER2 signalling pathways. Current evidence suggests that some CTAs reduced the effectiveness of responses to chemotherapy, and a detailed understanding of the important signalling pathways in TNBC could help in designing more efficacious drugs.

A key aspect of investigation is whether CTAs are aberrantly expressed in breast tissue before malignancy occurs and if they could be used as potential biomarkers for TNBC. This would help clinicians to identify women at high risk for cancer development, thus improving screening protocols for the early detection of TNBC (**Figure 1**).

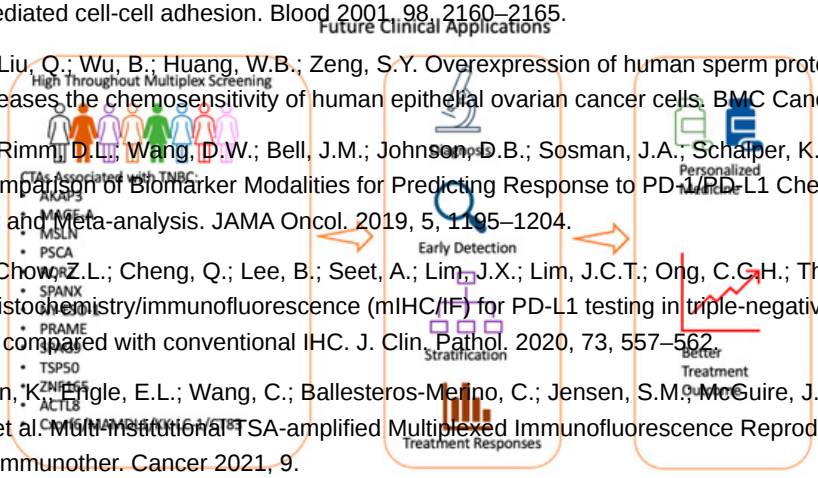

Figure 1. Potential workflow in screening for CTAs in TNBC. The expression of CTAs in TNBC provides great promise for enhancing current screening, diagnosis and treatment methods. TNBC: triple-negative breast cancer; CTA: cancer-testis antigen.

4. Conclusions

TNBC frequently expresses CTAs, and some CTA expression correlates with overall survival and prognosis. CTAs show biased expression in cancer and robust immunogenicity, thus serving as ideal targets for cancer immunotherapy. Multiple clinical trials have been conducted or are currently on-going to investigate the role of CTAs as treatment targets in advanced cancers, such as TBNC. Further research could be conducted to delineate the mechanism of action of CTAs in TNBC, increasing the efficacy of CTAs or in combination with other immunotherapies, identifying patients that would benefit most from the treatment and devising better drug delivery. With the collation of more data, CTAs may also be incorporated in routine screening protocols for TNBC.

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