Discovery of a Novel Cancer-Specific Antigen for Therapeutic Targeting using the Oncotope Platform

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BACKGROUND

- Identifying suitable antigenic targets for cancer immunotherapy is challenging due to cancer's complex biology and the evasive strategies employed by cancer cells (1).
- Current approaches to antigen discovery in cancer immunotherapy are time-consuming and cost-prohibitive.
- The Oncotope Platform, developed by our team, swiftly and comprehensively identifies cancer-specific cell surface targets absent in normal cells, offering a groundbreaking approach to target discovery (2).
- Using the Oncotope Platform, our team has identified several untargeted cell surface antigens on Triple Negative Breast Cancer, including SLC39A7/ZIP7.
- SLC39A7/ZIP7, a zinc transporter located in the Endoplasmic Reticulum (ER) , regulates cellular zinc levels crucial for gene transcription, cell growth, and metabolism (3).
- Elevated zinc levels in breast tumors highlight ZIP7's importance in cancer biology, with implications in modulating zinc levels, particularly in antihormone-resistant cells (3,4).
- Despite its significance, **SLC39A7/ZIP7's cell surface expression in breast cancer cells or other cancer types hasn't been documented**, and its suitability as a cell surface antigen for therapeutic antibody strategies such as ADCs, bispecific antibodies, or CAR therapies hasn't been established.

METHODS AND MATERIALS

1. **Generation of Polyclonal Xeno-Antibodies**: Triple Negative Breast Cancer (TNBC) cells (Hs578T) were injected into rabbits to generate polyclonal xeno-antibodies. Utilizing the proprietary Oncotope platform, antibodies specifically targeting TNBC cells were isolated, excluding those binding to normal breast epithelial cells derived from the same patient (Figure 1).
2. **Protein Microarray Analysis:** Total Anti-Breast Cancer Serum (before filtration) and Oncotope Platform Filtered Anti-Breast Cancer Serum were reacted with approximately 30,000 human proteins on a Protein Microarray (Yamaha/Tuning Fork Bio).
3. **Validation of SLC39A7/ZIP7 (ONCA 001) Presence on Breast Cancer Cell Surface:** Cytotoxicity Assays: Commercially available rabbit polyclonal anti-SLC39A7 antibodies were added to cultures of both Breast Cancer Cells & Normal Breast Epithelial Cells (Table 1) plus a Secondary Antibody Drug Conjugate (ADC), aOIgG-NC-MMAF (Moradec). Following a 48-hour incubation period, cell viability was assessed using the CellTiter Glo assay.

Flow Cytometry: Sera or Antibodies were added to cultures of both Breast Cancer Cells Normal breast epithelial Cells from the same tissue type from the same individual and incubated for 1 hour followed by addition of Alexa-488 conjugated Secondary Donkey anti-Rabbit antibody. After 30 minutes Incubation with the secondary antibody, cells were analyzed on an Attune Flow cytometer.

Table 1. BREAST CANCER CELL LINES

Breast Cancer Cell line	SLC39A7 mRNA expression	SUBTYPE	Receptor expression
HCC 1419	YES	**HER2 POSITIVE**	HER2
MDA-MB-415	YES	**LUMINAL A - ER+ PR +/- HER2 -**	ESR1
HCC202	YES	**HER2 POSITIVE**	HER2
CAMA-1	YES	**LUMINAL A - ER+ PR +/- HER2 -**	ESR1
UACC-893	YES	**HER2 POSITIVE**	HER2
HCC1954	YES	**HER2 POSITIVE**	HER2
BT-549	YES	**TRIPLE NEGATIVE B**	TNBC
Hs578T	YES	**TRIPLE NEGATIVE B**	TNBC
MDA-MB-436	NO	**TRIPLE NEGATIVE A**	TNBC
MDA-MB-468	NO	**TRIPLE NEGATIVE A**	TNBC

SLC39A7/ZIP7 Novel Breast Cancer Surface Antigen

Figure 1. GENERATION OF CANCER SPECIFIC POLYCLONAL ANTIBODIES

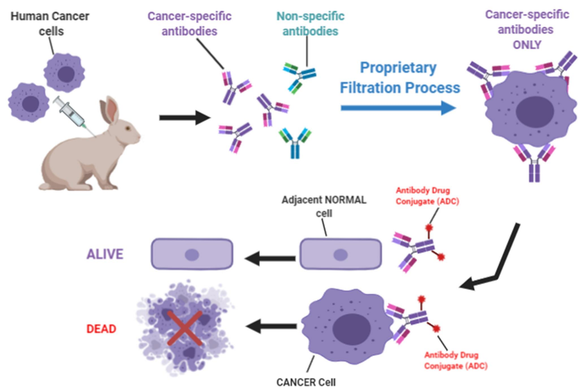


Figure 2. FLOW CYTOMETRY: ANTIBODIES TO SLC39A7/ZIP7 (ONCA 001) BIND CANCER CELLS BUT NOT NORMAL CELLS

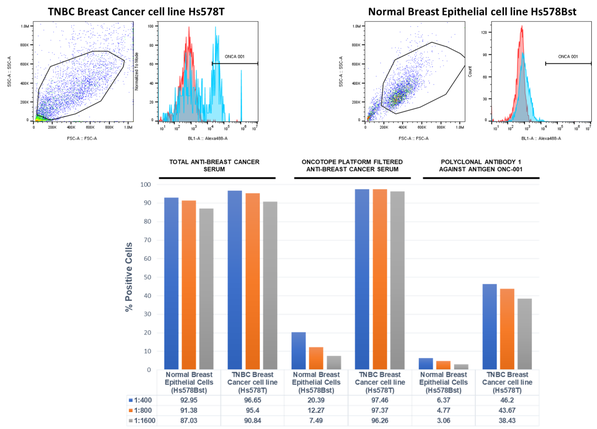


Figure 3. TARGETING SLC39A7/ZIP7 (ONCA 001) KILLS BREAST CANCER CELLS WHILE SPARING NORMAL CELLS FROM THE SAME PATIENT

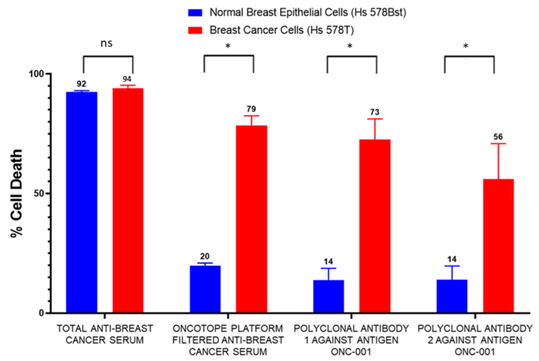
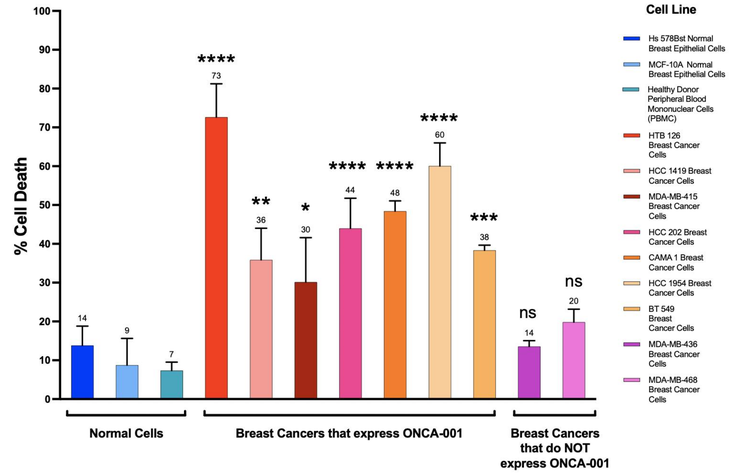


Figure 4. ANTI- SLC39A7/ZIP7 (ONCA 001) POLYCLONAL ANTIBODY KILLS MULTIPLE BREAST CANCER CELL LINES



RESULTS

1) Identification of SLC39A7/ZIP7 as a Potential Target: Through the Oncotope Platform, 8 unique antigens with significantly higher affinity binding to TNBC cells were identified, among which SLC39A7/ZIP7 had the strongest binding.

2) Validation of SLC39A7 Presence on Breast Cancer Cell Surface: SLC39A7 was found exclusively on the surface of breast cancer cells, and not normal cells. The presence of SLC39A7 was confirmed through the use of commercially available rabbit polyclonal anti-SLC39A7 antibodies in cell cultures, followed by assessment of flow cytometric analysis and cell viability (Figure 2 & 3). Statistical analysis revealed statistically significant cell death exclusively in breast cancer cell lines, while normal breast epithelial cells remained unaffected. Statistics were calculated in comparison to Hs 578Bst Normal Cells. ns, not significant. * $p < 0.05$, ** $p < 0.01$, *** $p < 0.001$. **** $p < 0.0001$.

3) Evaluation Across Breast Cancer Subtypes: Investigation extended to confirm SLC39A7 presence across various breast cancer subtypes, including Luminal A, Luminal B, HER2 Positive, and Triple Negative. Two breast cancer cell lines showed no mRNA expression of SLC39A7, while the remaining eight had mRNA expression (Table 1).

Flow cytometry and cell cytotoxicity assays confirmed the cell surface expression of SLC39A7 in the eight breast cancer cell lines expressing SLC39A7 mRNA (Figure 4).

4) Selective Targeting Potential: Minimal cell death was observed in breast cancer cell lines lacking SLC39A7 mRNA expression, as well as in normal breast epithelial cell lines and PBMCs, indicating selective targeting potential (Figure 4).

CONCLUSIONS

Our research marks a significant milestone as we are the **first to reveal the cell surface expression of SLC39A7/ZIP7 in various types of breast cancer cells, distinctly absent in healthy breast epithelial cells and peripheral blood mononuclear cells (PBMC)** from individuals without the disease. Importantly, **our findings implicitly demonstrate the therapeutic targeting SLC39A7/ZIP7 on various breast cancer types using ADCs** (Antibody Drug Conjugates), offering a precise and selective approach that avoids unintended interactions with adjacent healthy breast epithelial cells and other non-cancerous cell types. Furthermore, **the identification of SLC39A7/ZIP7's cell surface expression across diverse breast cancer cell types establishes it as a promising biomarker for breast cancer diagnosis.**

References

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