Adipocyte–Tumor Cell Interactions Drive Aggressiveness in Triple-Negative Breast Cancer

Tiziana Triulzi, PhD[1], Michela Francesconi[1], Serena Iannicelli[1], Viola Regondi[1], Loris De Cecco, PhD[2], Elda Tagliabue, PhD[1], Serenella M. Pupa, PhD[1], and Andrea Vingiani, MD[3]

[1]Microenvironment and Biomarkers of Solid Tumors, [2]Integrated Biology of Rare Tumors, Department of Experimental Oncology, [3]Pathology Unit 2, Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy

Introduction

Triple-negative breast cancer (TNBC) is a particularly aggressive subtype of breast cancer, representing approximately 10–20% of all cases.[1]

It is distinguished by a unique tumor microenvironment in which immune components play a critical prognostic role. [2-3]

Beyond immune cells, various stromal elements contribute to cancer progression, with tumor cells actively shaping their microenvironment through bidirectional interactions that influence the functional phenotype of these surrounding cells.[4-5]

Among stromal components, adipocytes, the predominant stromal cells in breast tissue, have gained increasing attention.[6-7]

We recently demonstrated a pro-tumorigenic effect of AT-TNBC crosstalk in vitro.[8]

This study aimed to determine whether interactions between TNBC cells and adipocytes promote cancer aggressiveness

Methods and Materials

In vitro studies:

ASC52telo human adipose-derived mesenchymal stem cells were differentiated into mature adipocytes (MA) and subsequently exposed to conditioned medium (CM) collected from human TNBC cell lines for 7 days. Adipocytes were then evaluated for triglyceride (TG) accumulation using Oil Red O staining, as well as for the expression of adipocyte-specific markers by qPCR or RNA-seq.

Tumor cells cultured in adipocyte-derived CM were analyzed for the expression of cancer stem cell (CSC) markers by qPCR and flow cytometry.

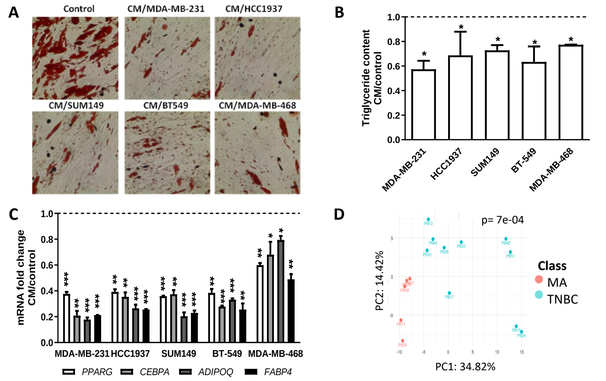
Patient cohorts:

Tumor–adipocyte crosstalk in patient samples was evaluated using E&E-stained sections from two cohorts:

- 92 primary TNBC cases surgically treated at our Institute between 2007 and 2010.
- 60 primary TNBC cases surgically treated at our Institute between 2002 and 2006, for which gene-expression data were available.

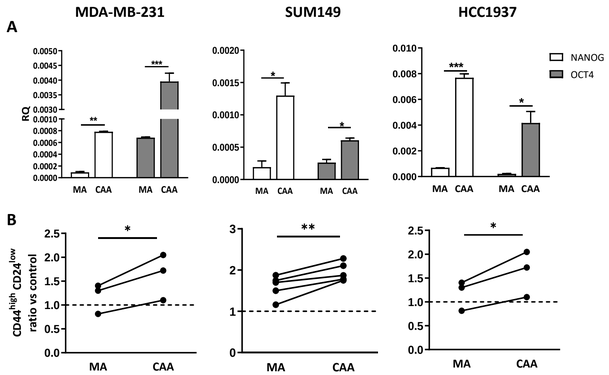
Results

In vitro studies: Adipocytes are reprogrammed by TNBC cells



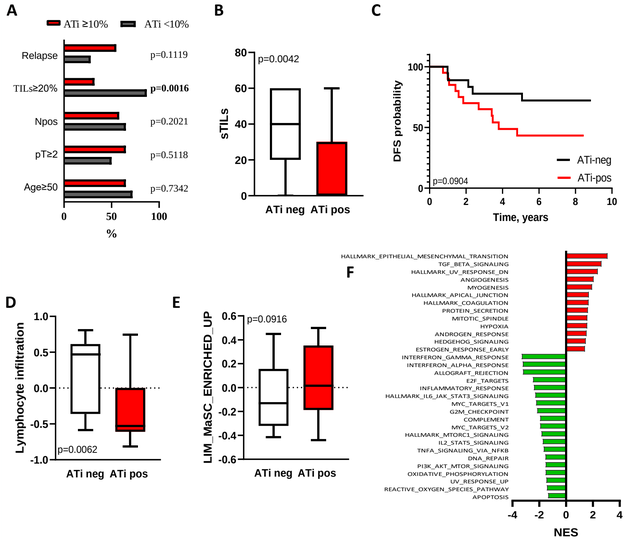
A-B) Representative Oil Red O-stained lipid droplets (A) and TG quantification (B) in ASC52 adipocytes cultured for 7 days with CM from TNBC cells or RPMI (Control). **C)** Expression levels of common adipocyte genes in adipocytes, treated as described in A, as evaluated by qRT-PCR. *B2M* was used as a housekeeping gene. * $P < 0.05$, ** $P < 0.01$ *** $P < 0.0001$ by paired Student's *t*-test. **D)** PCA of RNAseq of adipocytes treated as in A. MA: mature adipocytes treated with RPMI as control; TNBC: adipocytes treated with CM from MDA-MB-231 and HCC1937 cells.

TNBC are modified by adipocytes acquiring stem cell-like properties



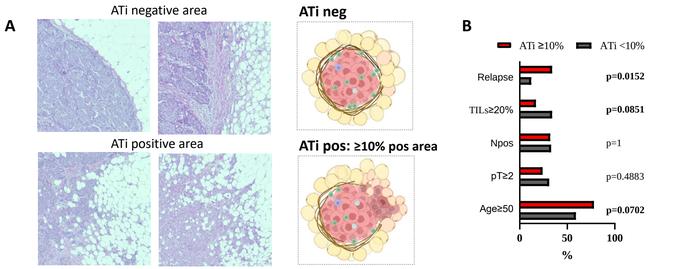
A) Relative expression levels (RQ) of selected genes associated with CSC-like phenotype (NANOG and OCT4) in TNBC cells treated with CM of MA and cancer associated adipocytes (CAA) derived from culture of adipocytes with TNBC CM. **B)** CD44high/CD24low subset in tumor cells treated with CM of MA and CAA vs control cells (treated with control medium, dotted line), as evaluated by flow cytometry. * $P < 0.05$, ** $P < 0.01$ *** $P < 0.001$ by paired Student's t test.

ATi associates with patient prognosis (cohort 2) and aggressive tumor features



A) Frequency of clinicopathological features in the TNBC cohort 2 stratified by ATi score. N, lymph node involvement; pT, pathological tumor size; TILs, tumor-infiltrating lymphocytes. p-values by Fisher's exact test. **B)** Association between ATi and TILs as continuous variable. p-value by Student's t test. **C)** Kaplan–Meier curve showing patient DFS probability stratified by ATi score (cut-off: 10%). p-value by log-rank test. **D-E)** Association between ATi and the lymphocyte infiltration signature[9] (D) and Lim_MaSC signature[10] (E) as evaluated by ssGSEA in GSE86945 dataset.[11] p-value by Student's t test. **F)** Hallmark pathways significantly enriched in TNBC according to ATi, as evaluated by fGSEA. Red: pathways enriched in ATi-pos TNBC. Green: pathways enriched in ATi-neg TNBC. NES: normalized enrichment score.

Patients: Adipose Tissue infiltration (ATi) score is associated with poor prognosis in TNBC patients (cohort 1)



A) Representative E&E images of ATi negative and positive area and cartoon of ATi neg and ATi pos tumors. **B**) Frequency of clinicopathological features in the TNBC cohort 1 stratified by ATi score. N, lymph node involvement; pT, pathological tumor size; TILs, tumor-infiltrating lymphocytes. p-values by Fisher's exact test. **C**) Kaplan–Meier curve of patient disease-free survival (DFS) probability stratified by ATi score dichotomized with the best cut-off (10%). p-value by log-rank test. HR above is relative to Cox analysis using ATi as continuous variable. **D**) Forest plots of the association between ATi score and DFS adjusted for other clinicopathological variables, in bivariate analyses.

Conclusions

Overall, our findings strongly indicate that the interaction between tumor cells and adipocytes contributes to the aggressiveness of TNBC, underscoring the importance of further investigating the underlying mechanisms for potential therapeutic targeting

Contact

Tiziana Triulzi
Fondazione IRCCS Istituto Nazionale dei Tumori, Milan, Italy
Email: tiziana.triulzi@istitutotumori.mi.it
Website: https://www.istitutotumori.mi.it/persone/tiziana-triulzi
Phone:+390223905121

References

1. Metzger-Filho O et al. Dissecting the heterogeneity of triple-negative breast cancer. JCO 2012, doi: 10.1200/JCO.2011.38.2010.
2. Zhang J et al. Immune-related biomarkers in triple-negative breast cancer. Breast Cancer 2021, doi: 10.1007/s12282-021-01247-8.
3. Adams S et al. Prognostic value of tumor-infiltrating lymphocytes in triple-negative breast cancers from two phase III randomized adjuvant breast cancer trials: ECOG 2197 and ECOG 1199. JCO 2014, DOI: 10.1200/JCO.2013.55.0491.
4. Tu KJ et al. The tumor microenvironment of 14,837 breast cancers is associated with clinical outcome independently of genomic subtypes. Cell Rep Med 2025, DOI: 10.1016/j.xcrm.2025.102450.
5. Wu SZ et al. Stromal cell diversity associated with immune evasion in human triple-negative breast cancer. EMBO J 2020, doi: 10.15252/embj.2019104063..
6. Rybinska I et al. Adipocytes in Breast Cancer, the Thick and the Thin. Cells 2020 doi: 10.3390/cells9030560
7. Rybinska I et al. Cancer-Associated Adipocytes in Breast Cancer. IJMS 2021 doi: 10.3390/ijms22073775
8. Rybinska I et al. SAA1-dependent reprogramming of adipocytes by tumor cells is associated with triple negative breast cancer aggressiveness. IJC 2024 doi: 10.1002/ijc.34859
9. Charoentong P et al. Pan-cancer immunogenomic analyses reveal genotype-immunophenotype relationships and predictors of response to checkpoint blockade. Cell Rep 2017, doi: 10.1016/j.celrep.2016.12.019.
10. Lim E et al. Transcriptome analyses of mouse and human mammary cell subpopulations reveal multiple conserved genes and pathways. Breast Cancer Res 2010, doi: 10.1186/bcr2560
11. Romero-Cordoba SL et al. Comprehensive omic characterization of breast cancer in Mexican-Hispanic women. Nat comm 2021, doi: 10.1038/s41467-021-22478-5.

Supported by AIRC

This presentation is the intellectual property of the author/presenter. Contact them at tiziana.triulzi@istitutotumori.mi.it for permission to reprint and/or distribute.

© Poster Template by Genigraphics® 1.800.790.4001 www.genigraphics.com