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# Adipocyte-Derived Conditioned Medium Influences the Malignant Phenotype of Human Breast Cancer-Associated Fibroblasts

**Maria Francesca Fiorita[1,2]*, Giuseppina Augimeri[1,2], Luca Gelsomino[1,2], Cinzia Giordano[1,2,3], Daniela Bonofiglio[1,2], Ines Barone[1,2], Stefania Catalano[1,2,3]**

*[1]Department of Pharmacy, Health and Nutritional Sciences, University of Calabria, Rende, Italy; [2]Centro Sanitario, University of Calabria, Rende, Italy;*

*[3]Clinical Laboratory Unit, A.O. "Annunziata", Cosenza, Italy*

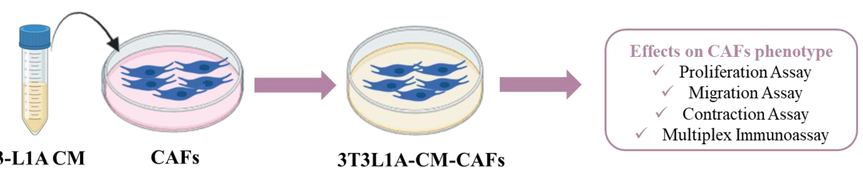
**email: mariafrancesca.fiorita@unical.it*

## INTRODUCTION & AIM

Obesity represents a global health concern, leading to the development of several types of malignancy, including breast cancer (BC).[1] In the obese state, adipocytes become hypertrophic, releasing elevated amounts of fatty acids, adipokines and pro-inflammatory cytokines, which support BC progression.[2] The secreted factors from adipocytes establish paracrine network with BC cells and the components of the tumor microenvironment (TME), creating a pro-tumoral milieu.[3] Cancer-associated fibroblasts (CAFs) represent the main population within the TME.[4] However, whether their phenotype might be influenced by the secreted factors from adipocytes is not fully understood. Here, we aimed to investigate whether the conditioned medium of mature 3T3-L1 adipocytes (3T3-L1A) influences human breast CAF phenotype.

## METHOD

CAFs were isolated from breast tumor biopsies. Conditioned medium (CM) from adipocytes was collected after incubating mature murine adipocytes (3T3-L1A) in 3% charcoal-stripped fetal bovine serum for 36-48 hours and used in co-culture systems with CAFs. MTT, Boyden Chamber Migration, Contraction, Phalloidin Staining Assays and Multiplex Bead-Based Immunoassay were performed to assess the phenotypic features of 3T3-L1A CM-treated CAFs.




## RESULTS & DISCUSSION

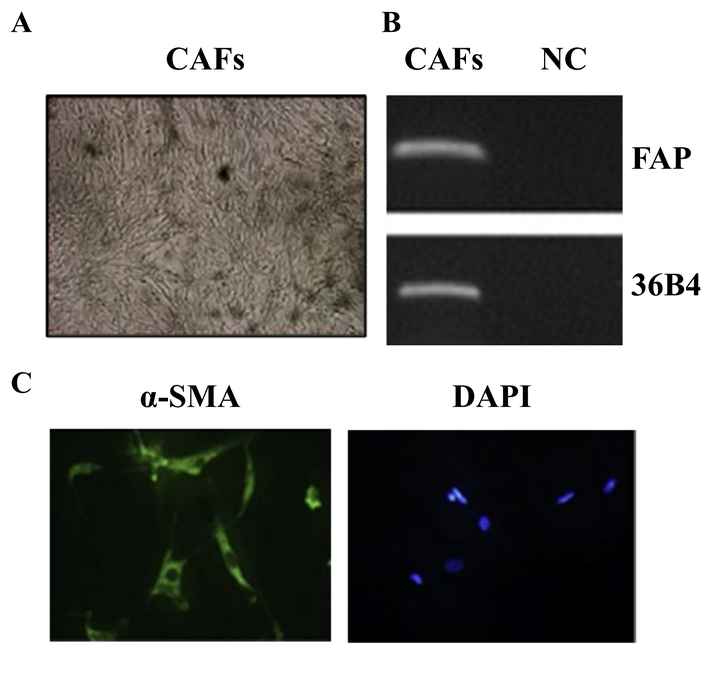



Figure 1. Characterization of human breast CAFs. **(A)** Phase-contrast microscopy image of human breast CAFs.

**(B)** qRT-PCR analysis of CAF-related markers, including fibroblast activation protein (FAP), with negative control (NC).

**(C)** Immunofluorescence staining of α-SMA (green) and DAPI (blue) in CAFs.

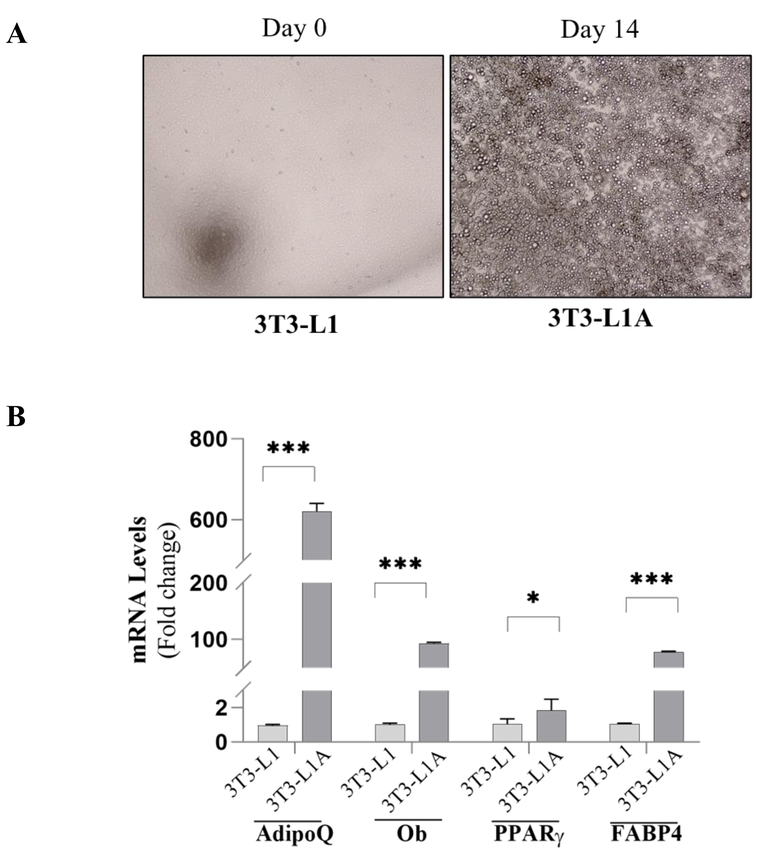



Figure 2. Characterization of mature 3T3-L1A. **(A)** Phase-contrast microscopy images of 3T3-L1 cells during adipogenic differentiation at day 0 and day 14. **(B)** qRT-PCR analysis of adipocyte-related markers, including adiponectin (AdipoQ), leptin (Ob), peroxisome proliferator-activated receptor gamma (PPARγ), and fatty acid-binding protein 4 (FABP4).

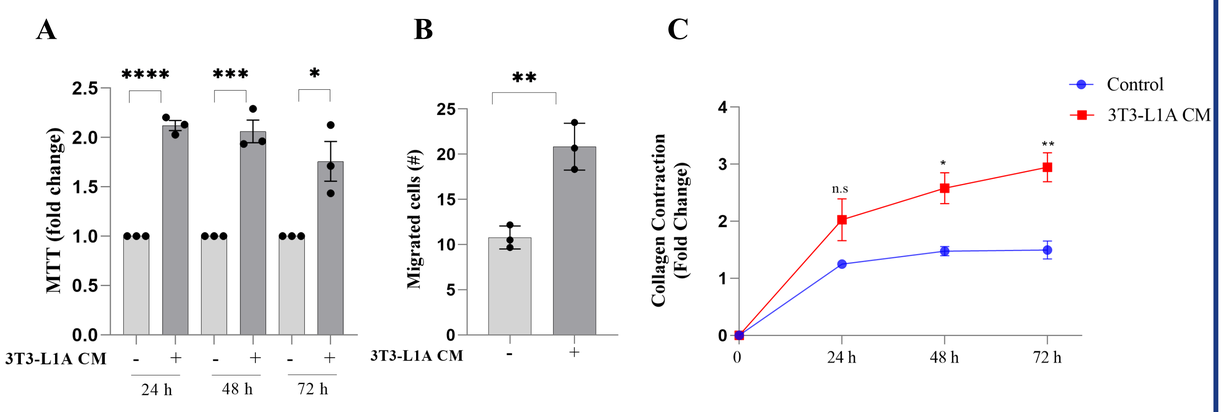



Figure 3. Effects of 3T3-L1A Conditioned Medium (CM) on Growth, Motility, Contraction and F-actin Organization of CAFs. **(A)** MTT Assay in CAFs treated (+) or not (-) with 3T3-L1A-derived CM for 24, 48, 72 h. **(B)** Transmigration assays in CAFs treated as indicated for 24 h. **(C)** Contraction assays in CAFs treated as indicated for 24 h, 48 h, 72 h. **(D)** Immunofluorescent staining of F-actin (green) and DAPI (blue) in CAFs treated as indicated for 48 and 72 h. *, $P < 0.05$; **, $P < 0.01$. ***, $P < 0.001$; **** $P < 0.0001$; n.s. not significant.

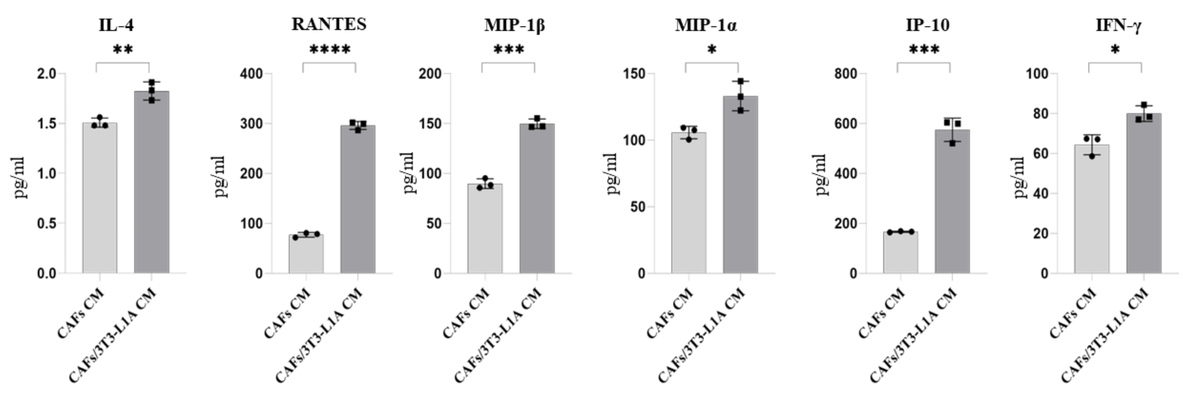



Figure 4. Cytokine, Chemokine and Growth Factors Profiles in CAFs CM and in CAFs/3T3-L1A CM. Concentrations of deregulated mediators in CAFs CM and in CM of CAFs previously treated with 3T3-L1A CM measured by Multiplex Human Cytokine, Chemokine, and Growth Factor Kit. Values are expressed in pg/mL (mean ± SD). *p<0.05;**p<0.01;***p<0.001;****p<0.0001. IL-4:Interleukin-4; RANTES: Regulated upon Activation Normal T Cell Expressed and Secreted; MIP-1β: Macrophage Inflammatory Protein-1 Beta; MIP-1α: Macrophage Inflammatory Protein-1 Alpha; IP-10: Human Interferon Inducible Protein 10; IFN-γ: Interferon-γ.

## CONCLUSIONS

- ✓ Secreted factors from 3T3-L1A affect the phenotypic features of human breast CAFs, increasing their viability and their migratory and contractility capacities.
- ✓ 3T3-L1A CM induced the upregulation of several pro-tumoral secreted factors in CAFs, with RANTES among the most markedly upregulated.

## FUTURE WORK/REFERENCES

Evaluation of the role of the chemokine RANTES as a potential mediator of the impact of adipocyte-conditioned medium on the CAF phenotype.

**References:**

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