The role of MARCKS in inflammation in inflammatory breast cancer using *in vitro* and *in silico* studies

M. Manai[1,2,3*], I. Brahmi[1*], N. Akkari[1,4], O. Bejar[1,4], N. Boughazala[1,5], M. Selmi[1], H. Aouadi[1,6], N. Bayou[2], S. Kristou[1,7], C. Reduzzi[2], Mo. Manai[8], H. Boussen[9], M. Cristofanilli[2], F. Bertucci[3]

1 Laboratory of Transmission, Control, and Immunobiology of Infections, LR11IPT02 (LTCII), Pasteur Institute of Tunis, Tunis, Tunisia; 2 Department of Medicine, Hematology/Oncology division, Weill Cornell Medicine, New York, NY, United States;3 Predictive Oncology Laboratory, Marseille Cancer Research Center, Institut Paoli-Calmettes, Aix-Marseille University, « Equipe labellisée Ligue Contre le Cancer », 13009 Marseille, France; 4 Higher Institute of Medical Technologies of Tunis, University of Tunis El-Manar, Tunis, Tunisia; 5 Human Genetics Laboratory (LR99ES10), Faculty of Medicine of Tunis, University of Tunis El-Manar, Tunis 2092, Tunisia; 6 National Engineering School of Sfax, Sfax, Tunisia; 7 Higher School of Health Sciences and Technology of Tunis, University of Tunis El-Manar, Tunis, Tunisia; 8 Mycology, Pathologies and Biomarkers Laboratory (LR16ES05), Faculty of Sciences of Tunis, University of Tunis El Manar, Tunis 2092, Tunisia; 9 Abderrahman Mami Hospital, Medical Oncology Department, Ariana, Tunisia; *These authors should be considered as co-first authors

Abstract

Inflammatory Breast Cancer (IBC) is a rare and aggressive form of breast cancer marked by high metastatic potential and a strongly inflammatory tumor microenvironment (TME). This study investigated the role of the Myristoylated Alanine-Rich C Kinase Substrate (MARCKS) protein and related inflammatory pathways in IBC through combined *in vitro* and *in silico* approaches. *MARCKS* inhibition in IBC cell lines reduced cell migration and pro-tumor activity under inflammatory conditions. Gene and protein expression analyses revealed that MARCKS suppression modulated key inflammatory regulators, including NF-κB and its inhibitors, suggesting a link between MARCKS activity and inflammation-driven tumor behavior. *In silico* analyses further highlighted alterations in major signaling pathways such as PI3K/AKT and JAK/STAT. Together, these findings underscore the central role of MARCKS in IBC aggressiveness and support its potential as a therapeutic target.

Introduction

Inflammatory breast cancer (IBC) is a rare but highly aggressive form of breast cancer, representing up to 7% of cases in Tunisia. It is characterized by early lymph node metastases, rapid progression, and a highly inflammatory tumor microenvironment. The protein MARCKS, a regulator of cell migration and pro-inflammatory cytokines, is significantly overexpressed in IBC, suggesting its role in activating inflammatory pathways. Using combined *in silico* and in vitro approaches, we investigated MARCKS function in IBC (SUM149, KPL4) and non-IBC (nIBC) (MDA-MB-231) cell lines, assessing its impact on migration and inflammatory signaling (NF-κB, pSTAT3, and EGFR). Our findings highlight MARCKS as a central mediator of IBC inflammation and a potential therapeutic target in IBC.

Methods and Materials

IBC cell lines (SUM149, KPL4) and the nIBC cell line (MDA-MB-231) were used for *in vitro* studies. *MARCKS* expression was silenced via siRNA transfection (si-MARCKS). To induce an inflammatory environment, cells were treated with LPS (1µg/mL). The cells' migration ability was assessed using Scratch Wound Healing Assays. For molecular analysis, Examining key signaling pathways affected by MARCKS inhibition using Western Blotting. Quantifying the expression of target genes via qRT-PCR to compare the effects of siRNA between IBC and nIBC cells. Public microarray datasets (GSE5847, GSE22597, and GSE23720) were selected from the GEO database and processed using RMA normalization for data comparability. Differentially Expressed Genes (DEGs) between Inflammatory Breast Cancer (IBC) and nIBC samples were identified using the "limma" package (with criteria : adj. p<0.05 and |log 2 FC|>1). Transcriptional signatures were visualized using PCA, volcano, and UpSet plots. Subsequent enrichment analysis (e.g., KEGG pathways) was performed to identify relevant biological signaling pathways.

Results

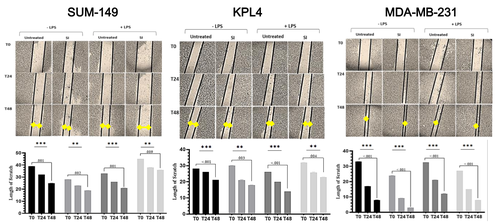


Figure 1 : MARCKS inhibition modulates IBC cells migration. LPS stimulation strongly enhances cell migration, whereas MARCKS silencing by siRNA markedly reduces it, underscoring its pivotal role in motility. Following MARCKS inhibition, wound closure is significantly slower in SUM149 ($p < 0.001$) and KPL4 ($p < 0.001$) compared to MDA-MB-231. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, ns: p > 0.05.

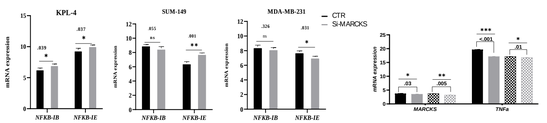


Figure 2 : The regulation of NF-κB inhibitors and TNFα expressions in IBC cell lines following the inhibition of MARCKS. MARCKS inhibition alters NF-κB inhibitor isoform expression, significantly upregulating *NF-κB-IE* in IBC cells (KPL4, SUM149) but induced its downregulation in nIBC cells (MDA-MB-231). TNFa was downregulated after MARCKS inhibition. highlighting a distinct regulatory effect in IBC. ***p ≤ 0.001, **p ≤ 0.01, *p ≤ 0.05, ns: p > 0.05.

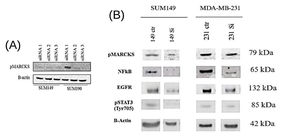


Figure 3 : MARCKS inhibition downregulated key signaling pathway in IBC cells. Only treated SUM149 cells showed downregulation of NFkB, EGFR, and pSTAT3 (Tyr705) proteins, whose role is well established in the promotion of chronic inflammation and tumor proliferation and found to be associated with IBC patients samples.

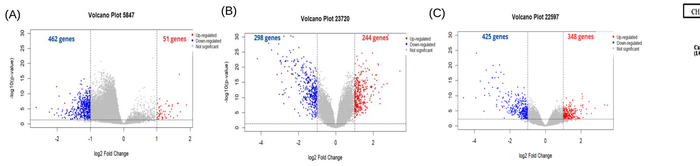
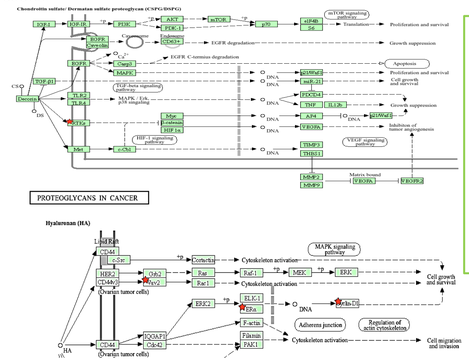


Figure 4 : Identification of differentially expressed genes. Comparative transcriptomic analysis reveals significant up- and downregulation of genes in IBC versus nIBC samples, highlighting substantial molecular variability among IBC cases.



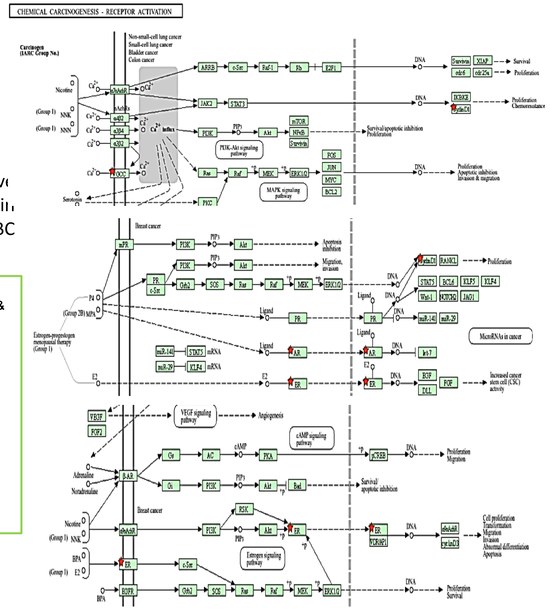


Figure 5 : Enrichment mapping. KEGG analysis reveals that core alterations in IBC, including perturbations in PI3K/Akt, MAPK/ERK, RTKs, Cyclin D1, and ER/AR, drive aggressiveness by promoting proliferation, migration, inflammation, and CSC plasticity.

Discussion and conclusions

This study demonstrated that *MARCKS* inhibition significantly reduces the migratory and pro-tumorigenic capacity of IBC cells *in vitro*. Furthermore, MARCKS inhibition acts as as a key regulator of downstream signaling pathways known to be associated to inflammation like NF-κB, pSTAT3, and EGFR. Using RT-qPCR, *MARCKS* inhibition selectively regulates NF-κB in IBC through IκB isoforms, confirming its key role in tumor-associated inflammation. Transcriptomic analysis revealed an aggressive molecular signature in IBC, marked by the concurrent activation of major signaling axes, including MAPK, JAK/STAT, and PI3K/AKT pathways. These findings confirm the intrinsic role of inflammation in IBC aggressiveness and highlight several targetable molecular and inflammatory mechanisms for personalized therapeutic strategies. The results reinforce previous data on MARCKS overexpression in IBC, solidifying its pronounced role in driving disease aggressiveness and associated inflammation.

Contact

Laboratory of Transmission, Control, and Immunobiology of Infections, LR11IPT02 (LTCII)
Pasteur Institute of Tunis, University of Tunis El-Manar, Tunis, Tunisia;
E-mail: marwa.manai@pasteur.utm.tn;
Tel.: +216-51495876;
ORCID: 0000-0003-4037-340

References

1. Wang X, Semba T, Phi LTH, Chaintikun S, Iwase T, Lim B, Ueno NT. Targeting Signaling Pathways in Inflammatory Breast Cancer. Cancers (Basel). 2020 Sep 1;12(9):2479;
2. Manai M, ElBini-Dhouib I, Finetti P, Bichiou H, Reduzzi C, Aissaoui D, Ben-Hamida N, Agavnian E, Srairi-Abid N, Lopez M, Amri F, Guizani-Tabbane L, Rahal K, Mrad K, Manai M, Birnbaum D, Mamessier E, Cristofanilli M, Boussen H, Kharrat M, Doghri R, Bertucci F. MARCKS as a Potential Therapeutic Target in Inflammatory Breast Cancer. Cells. 2022 Sep 19;11(18):2926.

PS5-04-12

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