UNIVERSITÀ POLITECNICA DELLE MARCHE

DIPARTIMENTO DI SCIENZE DELLA VITA E DELL'AMBIENTE

Corso di Laurea in Scienze Biologiche

Ruolo del Rame nel cancro al seno HER2 negativo
una nuova prospettiva diagnostica

Role of Copper on HER2-negative breast cancer
a new diagnostic perspective

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Tesi di Laurea di:

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Sessione autunnale

Role of Copper on HER2-negative breast cancer

What is the objective of the research?

Through various experiments it is intended to prove that the bioavailability of copper has a significant impact on the dissemination of breast cancer

Why?

- To better understand the biology of cancer
- To develop new therapeutic strategies

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ORIGINAL ARTICLE

Check for updates

AKT-driven epithelial-mesenchymal transition is affected by copper bioavailability in HER2 negative breast cancer cells via a LOXL2-independent mechanism

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Abstract

Background The main mechanism underlying cancer dissemination is the epithelial to mesenchymal transition (EMT). This process is orchestrated by cytokines like TGFβ, involving "non-canonical" AKT- or STAT3-driven pathways. Recently, the alteration of copper homeostasis seems involved in the onset and progression of cancer.

Methods We expose different breast cancer cell lines, including two triple negative (TNBC) ones, an HER2 enriched and one cell line representative of the Luminal A molecular subtype, to short- or long-term copper-chelation by triethylenetetramine (TRIEN). We analyse changes in the expression of EMT markers (E-cadherin, fibronectin, vimentin and αSMA), in the levels and activity of extracellular matrix components (LOXL2, fibronectin and MMP2/9) and of copper homeostasis markers by Western blot analyses, immunofluorescence, enzyme activity assays and RT-qPCR. Boyden Chamber and wound healing assays revealed the impact of copper chelation on cell migration. Additionally, we explored whether perturbation of copper homeostasis affects EMT prompted by TGFβ. Metabolomic and lipidomic analyses were applied to search the effects of copper chelation on the metabolism of breast cancer cells. Finally, bioinformatics analysis of data on breast cancer patients obtained from different databases was employed to correlate changes in kinases and copper markers with patients' survival.

Results Remarkably, only HER2 negative breast cancer cells differently responded to short- or long-term exposure to TRIEN, initially becoming more aggressive but, upon prolonged exposure, retrieving epithelial features, reducing their invasiveness. This phenomenon may be related to the different impact of the short and prolonged activation of the AKT kinase and to the repression of STAT3 signalling. Bioinformatics analyses confirmed the positive correlation of breast cancer patients' survival with AKT activation and up-regulation of CCS. Eventually, metabolomics studies demonstrate a prevalence of glycolysis over mitochondrial energetic metabolism and of lipidome changes in TNBC cells upon TRIEN treatment.

Conclusions We provide evidence of a pivotal role of copper in AKT-driven EMT activation, acting independently of HER2

DISSEMINATION OF BREAST CANCER

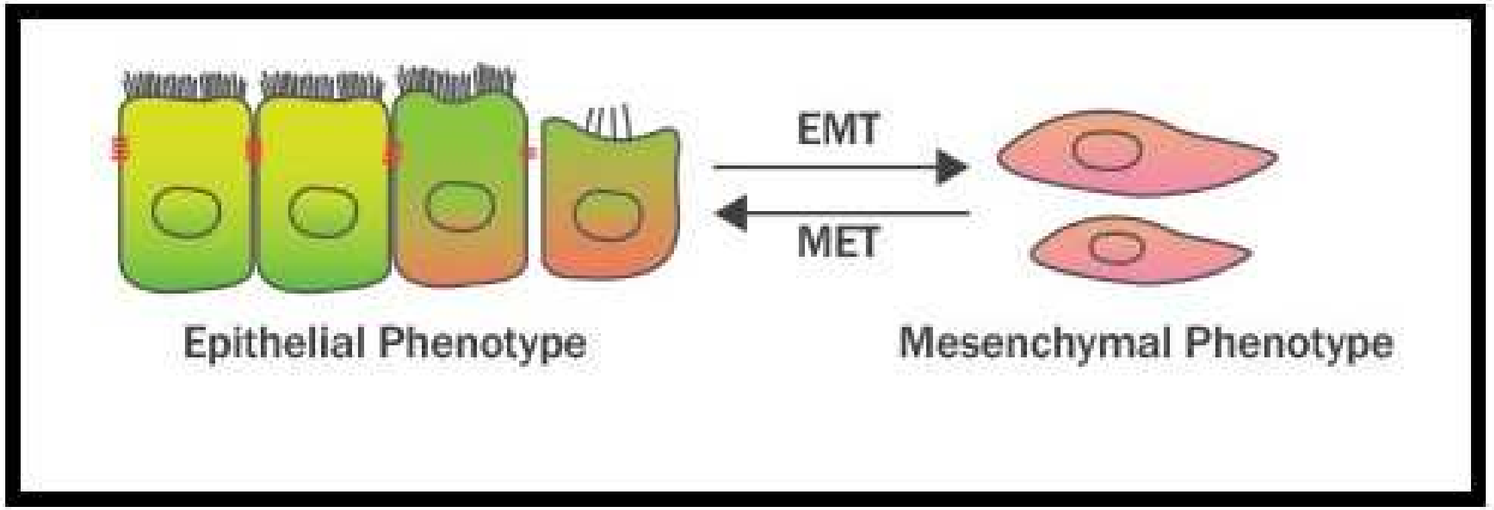


Fig. 1 FertilityCenter. (s.d.). FertilityCenter – Informazioni scientifiche. https://www.fertilitycenter.it/oncologia/emt-epithelial-mesenchymal-transition

1. **EMT**
   - TGFβ and its canonical and non-canonical pathways: AKT and STAT3

2. **Rimodelling of the extracellular matrix (ECM)**

ROLE OF COPPER

In breast carcinoma, there is an increase in:

LOXL2

It depends on the presence of copper

CTR1

It’s the main copper transport

ATP7A/B

They use ATP to transport copper

METHODS EMPLOYED:

- Different cell lines
- TRIEN: copper chelator

- Western Blot and Immunofluorescence: analysis of the expression of specific markers
- RT-qPCR: mRNA quantification of these genes
- Boyden chamber and Scratch assay: analysis of migratory capacity
- Metabolic analysis
- Bioinformatic analysis

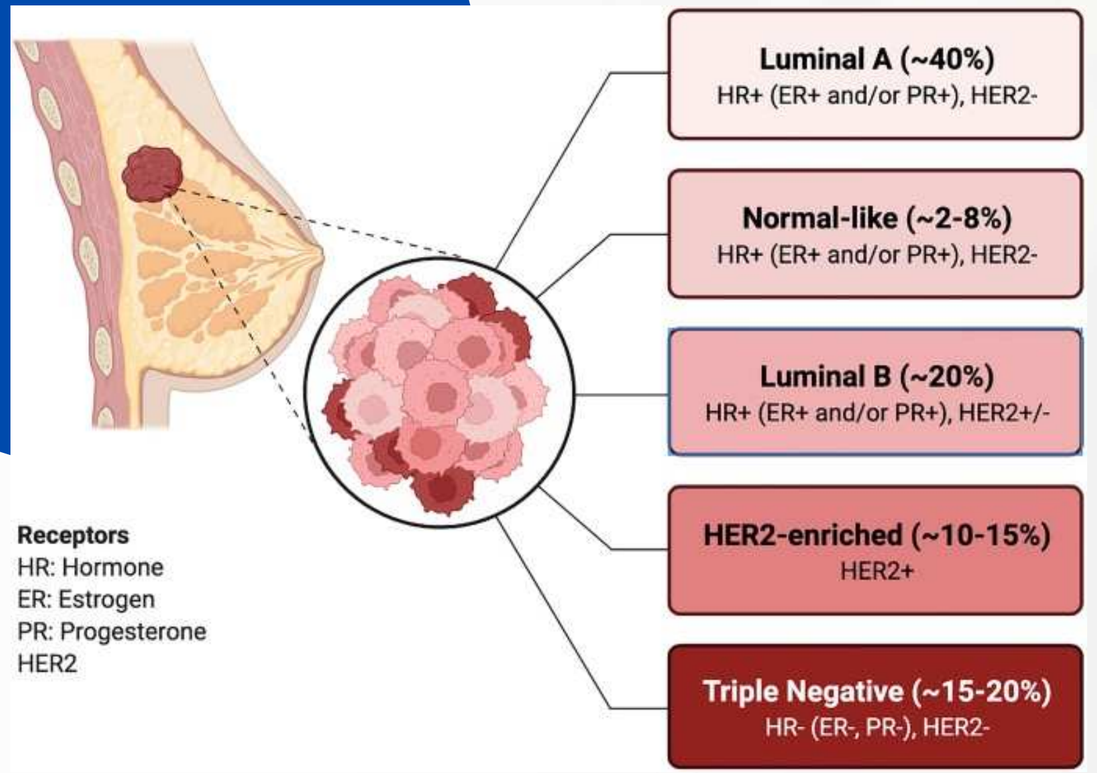


Fig.2 Approcci terapeutici per il cancro al seno: review. (s.d.). Medicina in Biblioteca. https://blogpinali.wordpress.com/2023/03/20/approcci-terapeutici-per-il-cancro-al-seno-review/

RESULTS AFTER 24H

Copper bioavailability influences epithelial and mesenchymal markers

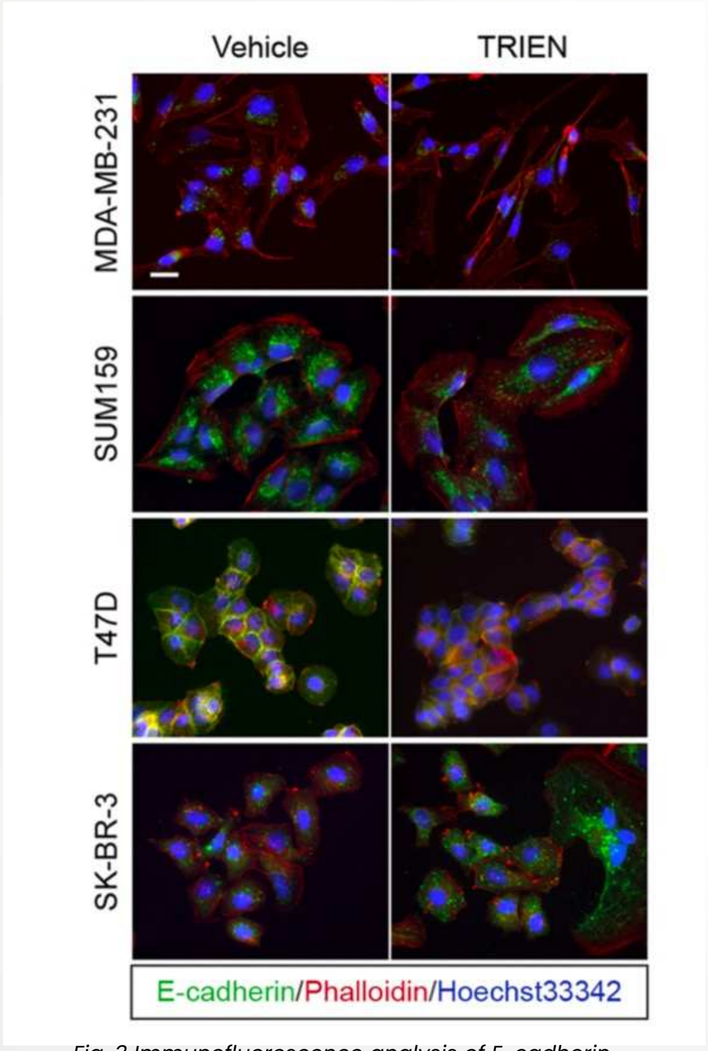
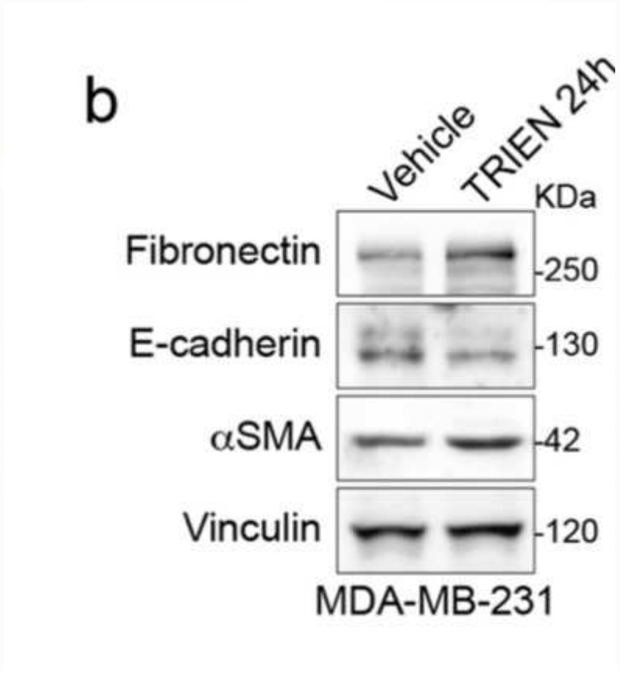


Fig. 3 Immunofluorescence analysis of E-cadherin



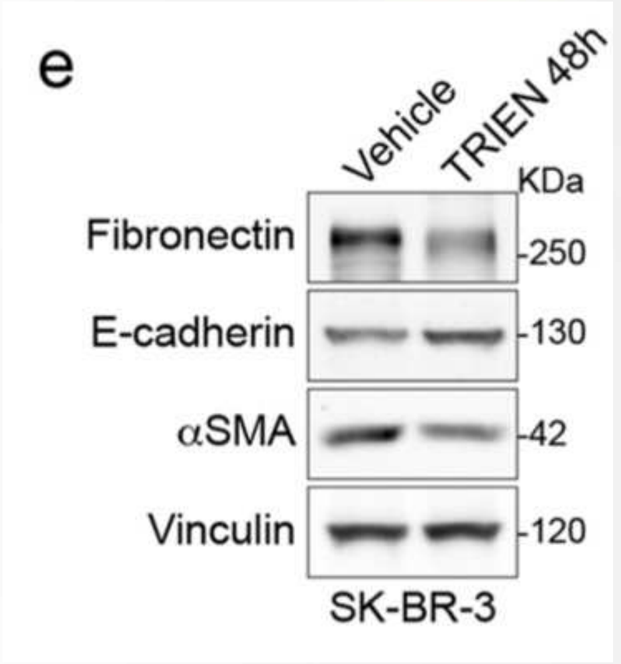
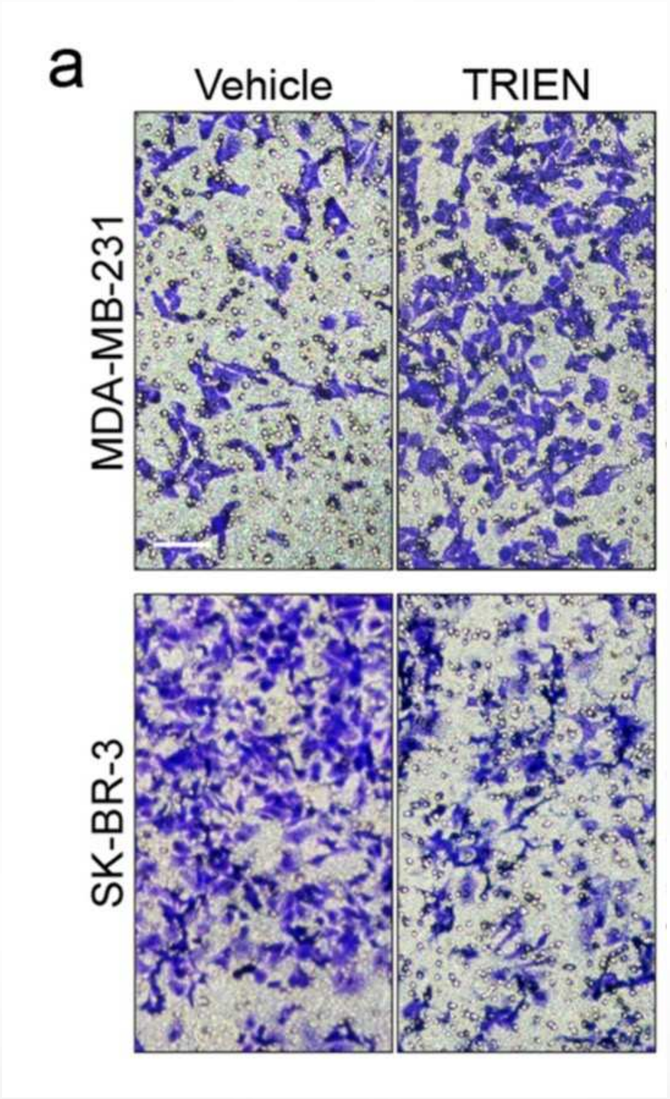


Fig.4 Levels of epithelial and mesenchymal markers, analysed by Western Blot

TRIEN had different effects depending on the cell line

RESULTS AFTER 24H

TRIEN has effects on cell migration



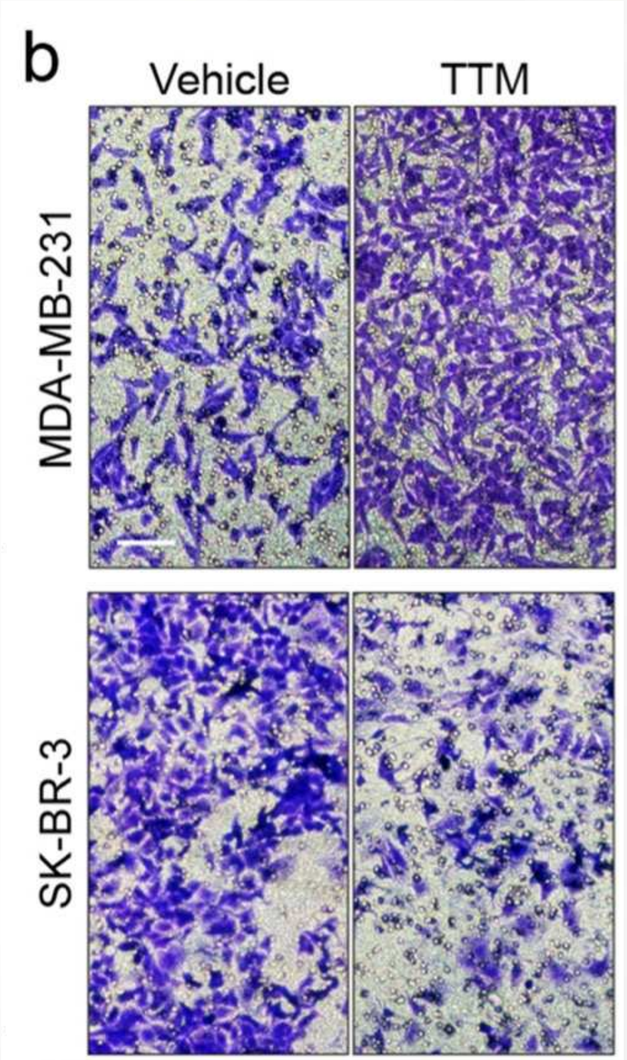
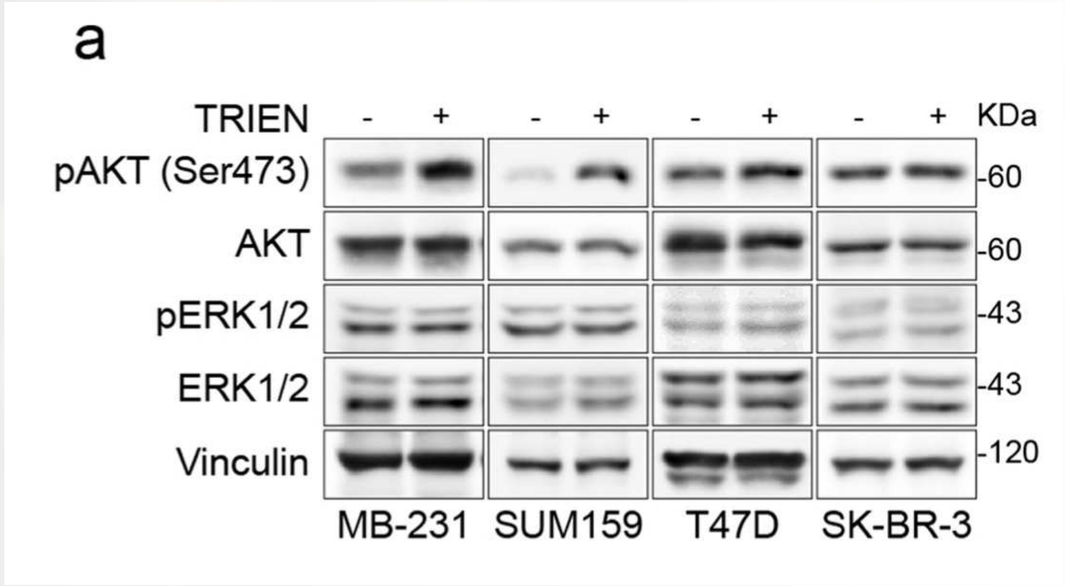
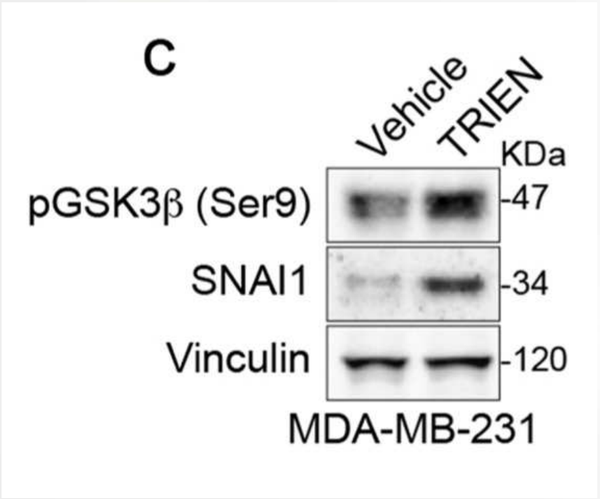


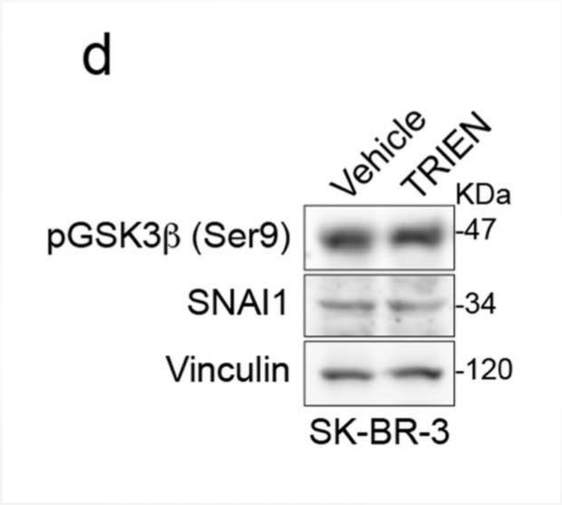
Fig. 5 Boyden chamber assay after TRIEN and TTM treatment

RESULTS AFTER 24 H

Copper chelation affects EMT







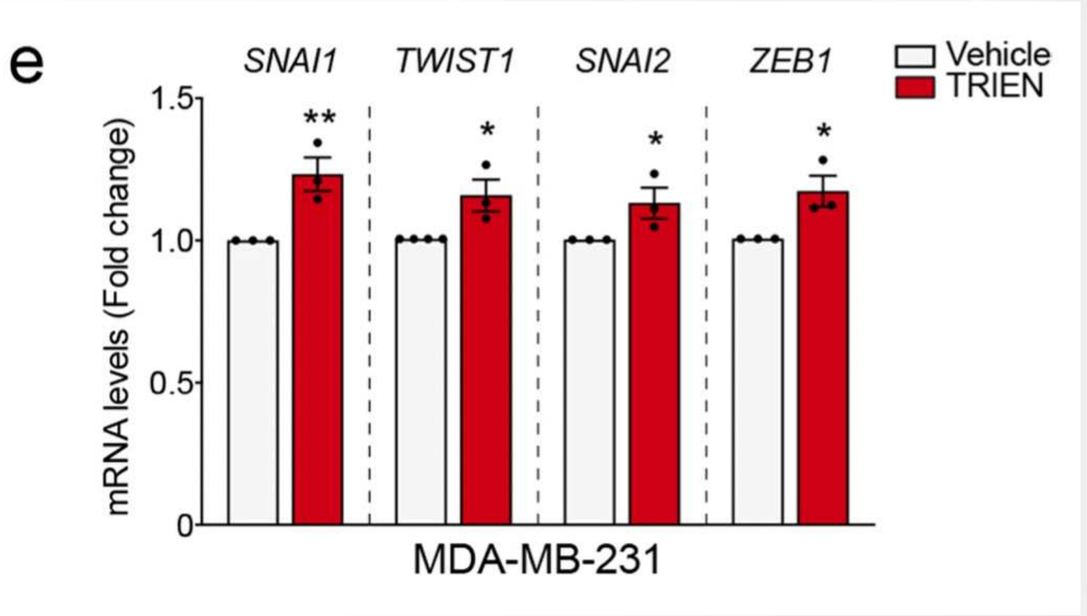


Fig. 7 RT-qPCR of EMT transcription factor values

Fig. 6 Western blot of pAKT, GSK3β and SNAI1 levels

RESULTS AFTER 6 DAYS

TRIEN opposes TGFβ-induced EMT

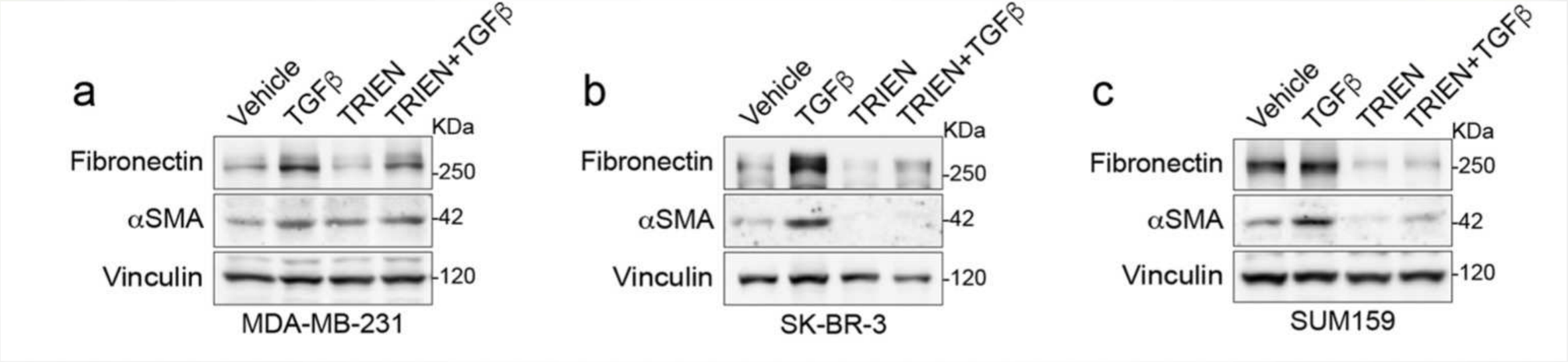


Fig. 8 Western Blot of specific markers

RESULTS AFTER 6 DAYS

TRIEN opposes TGFβ-induced EMT

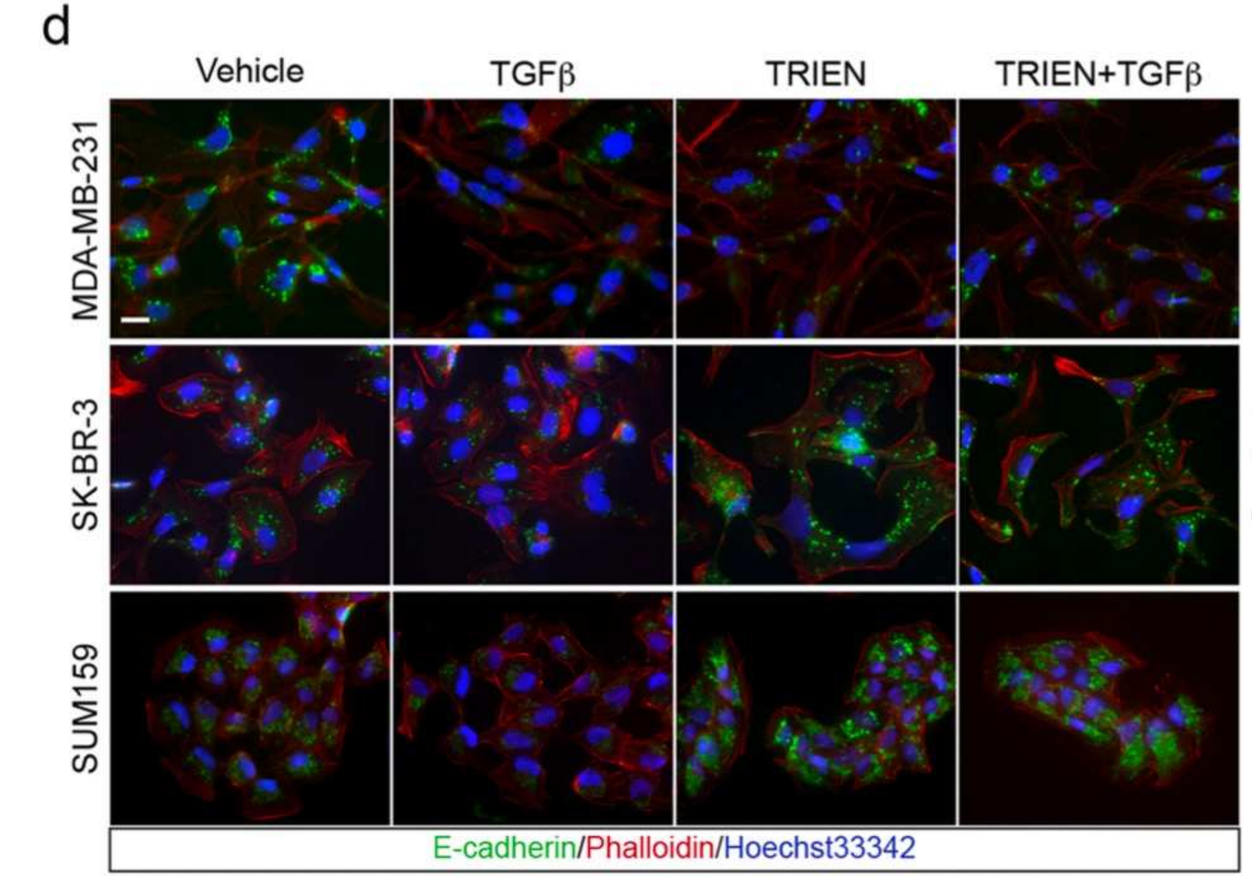
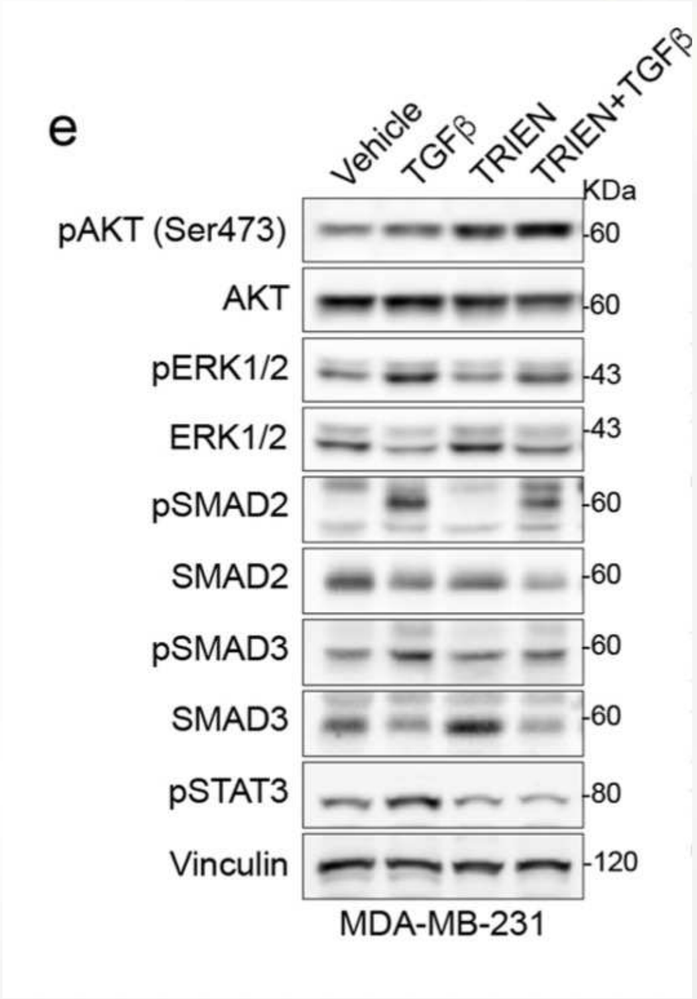


Fig. 9 Immunofluorescence and Western Blot of specific markers



RESULTS AFTER 6 DAYS

TRIEN has effects on extracellular matrix rimodelling

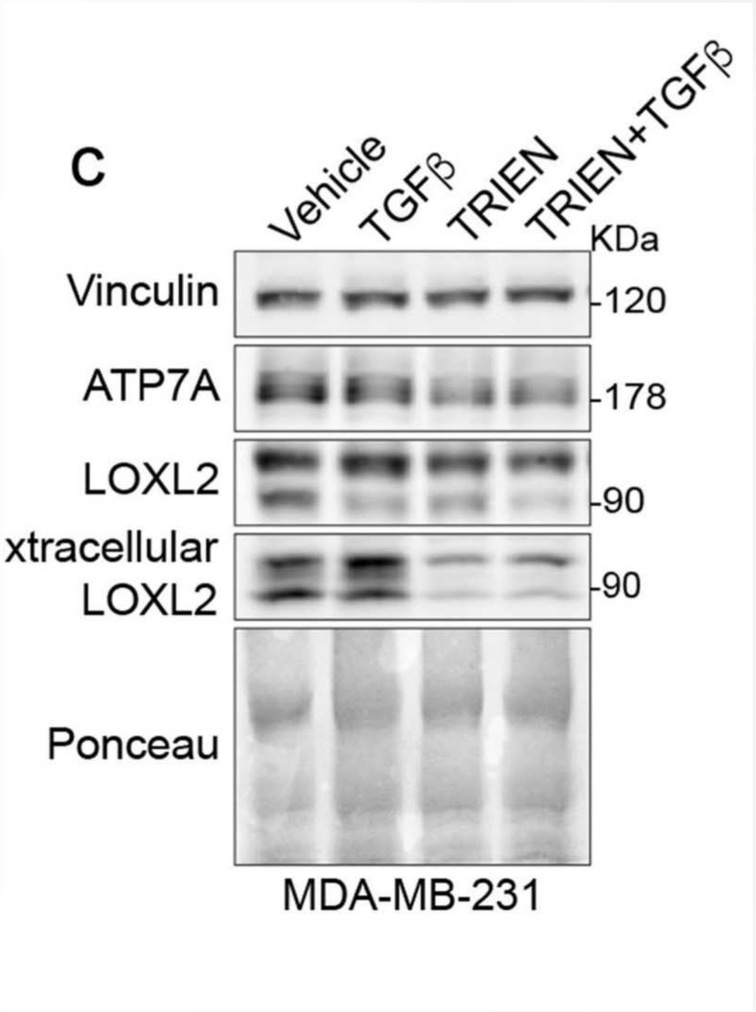


Fig. 11 Western Blot of ATP7A and LOXL2 levels

RESULTS AFTER 6 DAYS

TRIEN influences cell metabolism

Metabolism	Metabolite	Control	TGFβ	TRIEN	TRIEN +TGFβ
Glucose/pyruvate metabolism	Lactic acid	39.52±0.21	37.36±4.73	33.13±3.19	33.05±1.12
One carbon metabolism	Formic acid	10.89±3.41	10.61±1.80	17.28±5.22	15.20±1.79
Nucleotide metabolism	ATP+ADP	1.23±0.08	1.53±0.24	0.61±0.06	1.08±0.22
	NAD+NADP	0.40±0.09	0.90	0.32	0.29±0.07
Aminoacids metabolism	L-phenylalanine	5.15±0.67	5.82±1.02	5.63±0.25	6.31±0.84
	L-tyrosine	1.26±0.19	1.53±0.35	1.16±0.14	1.53±0.12
	L-histidine	0.14±0.11	0.24±0.18	0.11±0.02	0.21±0.04
	L-glycine	2.62±0.24	2.58±0.09	2.21±0.25	2.90±0.06
	L-glutamic acid	6.26±0.38	5.84±0.30	6.83±0.02	6.84±0.52
	L-glutamine	1.94±0.43	2.36±0.16	2.45±0.11	2.69±0.11
	L-isoleucine	0.96±0.01	1.32±0.17	0.94±0.03	1.12±0.01
	L-aspartic acid	1.12±0.49	1.64±0.40	1.22±0.11	1.52±0.19
	L-alanine	3.38±0.17	3.55±0.02	3.41±0.29	3.73±0.12
Redox balance metabolism	Glutathione	3.61±0.44	3.11±0.10	3.55±0.09	3.15±0.10
	Taurine	2.32±0.33	2.92±0.34	2.38±0.13	2.48±0.22
Lipid metabolism	GPC+PCho+Cho	7.11±0.21	6.17±0.69	5.73±0.38	4.74±0.22
	Myo-inosytol	5.32±0.20	4.91±0.22	5.45±0.13	4.97±0.33
Lipid and aminoacid metabolism	Acetic acid	1.92±0.26	2.05±0.45	2.58±0.38	2.42±0.22
Krebs cycle	Succinic acid	0.37±0.03	0.34±0.03	0.32±0.01	0.32±0.02
Ornithine cycle	Polyamines	4.46±0.72	5.66±0.68	4.83±0.27	5.45±0.44

Fig. 12 NMR for metabolite levels

AKT expression correlates with patient survival

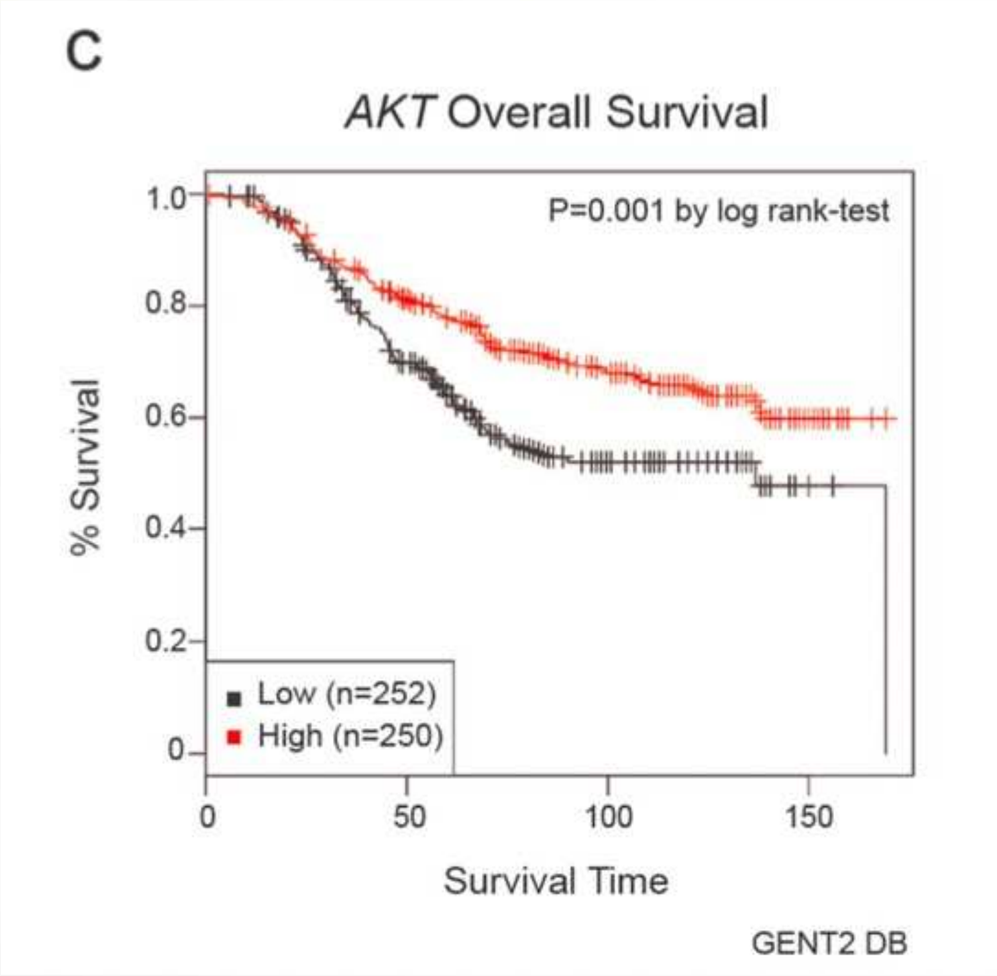
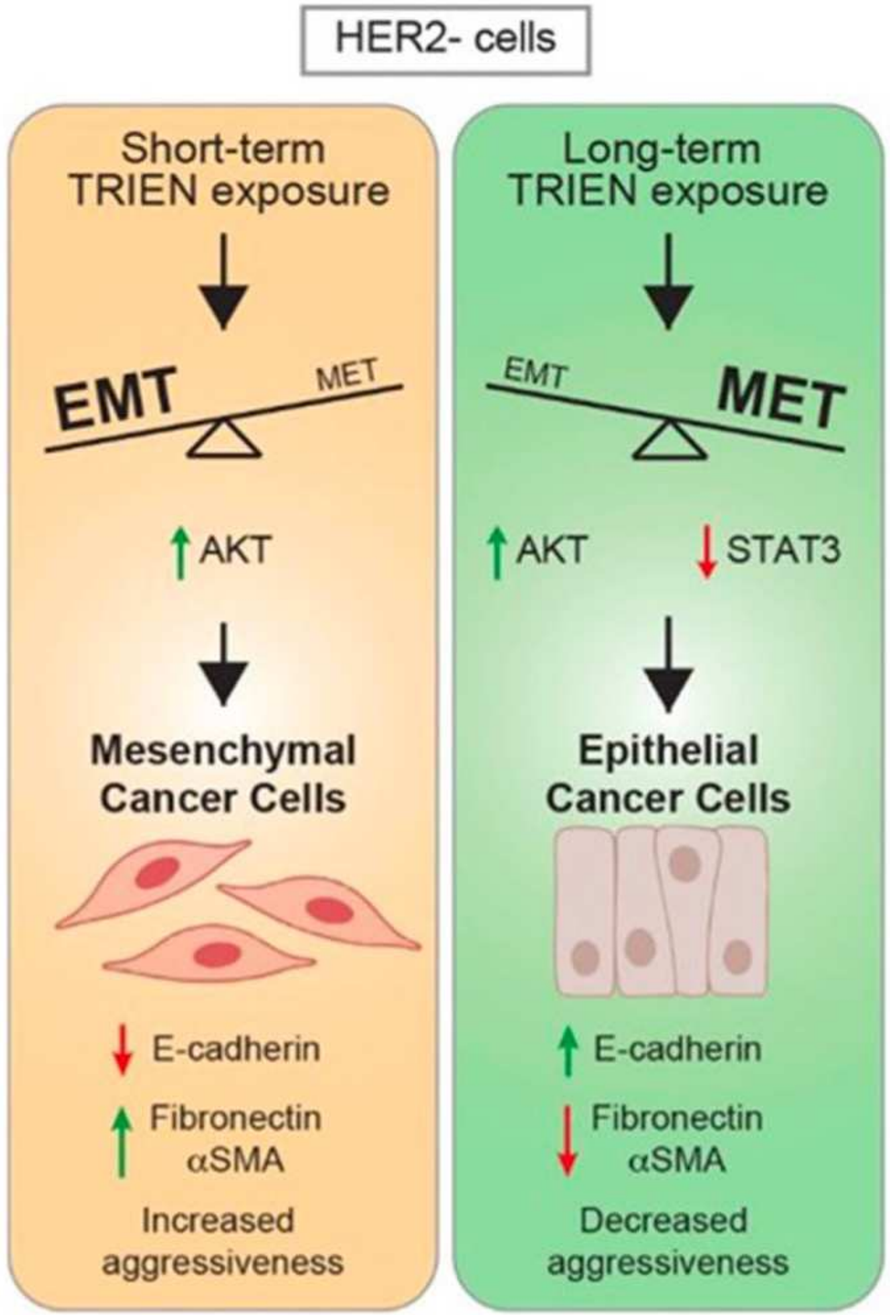


Fig. 13 Bioinformatic analysis of AKT levels related to patient survival rate

WHAT DO THESE RESULTS MEAN?

- TRIEN doesn't affect the primary tumour mass but prevents the formation of metastases.
- There is a persistent activation of AKT and repression of STAT3 signaling.
- There is an agonistic interaction between TRIEN and TGFβ
- **The amount of copper in HER2 negative cells influences the ability of AKT to activate EMT**



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RIASSUNTO ESTESO

Lo studio si concentra sul ruolo del rame nella progressione del tumore al seno, analizzando come la chelazione del rame possa influenzare vari aspetti del comportamento delle cellule tumorali, in particolare l'EMT.
Nello specifico, le diverse linee cellulari sono state trattate con il TRIEN e sono state sottoposte a delle analisi per valutare l'espressione di marcatori proteici, la migrazione cellulare, l'espressione di specifici geni e il profilo metabolico.
Si evidenzia che la riduzione dei livelli di rame ha effetti variabili a seconda della linea cellulare considerata. In particolare, nelle cellule HER2-, è stato osservato che la chelazione del rame inizialmente aumenta la capacità di invasione, ma a lungo termine, riduce l'aggressività delle cellule.
Infatti, i cambiamenti osservati potrebbero essere legati all'attivazione differenziale della chinasi AKT e alla repressione della segnalazione STAT3. DI conseguenza, l'effetto complessivo del TRIEN potrebbe condurre a una riduzione dell'aggressività tumorale e a una migliore risposta ai trattamenti.
Ciò significa che la manipolazione dei livelli di rame potrebbe avere implicazioni significative nelle nuove strategie terapeutiche per il cancro al seno

THANK YOU