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Stage-dependent epigenetic and anti-inflammatory effects of sprouts, microgreens, and baby leaves of *Broccolo nero*: a multi-omics perspective for sustainable nutrition

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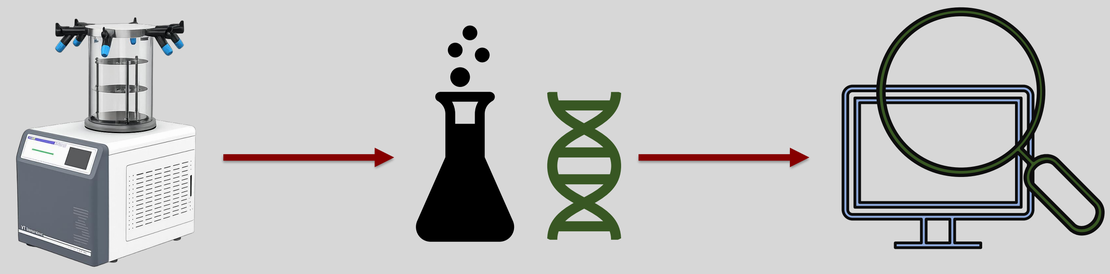
BACKGROUND

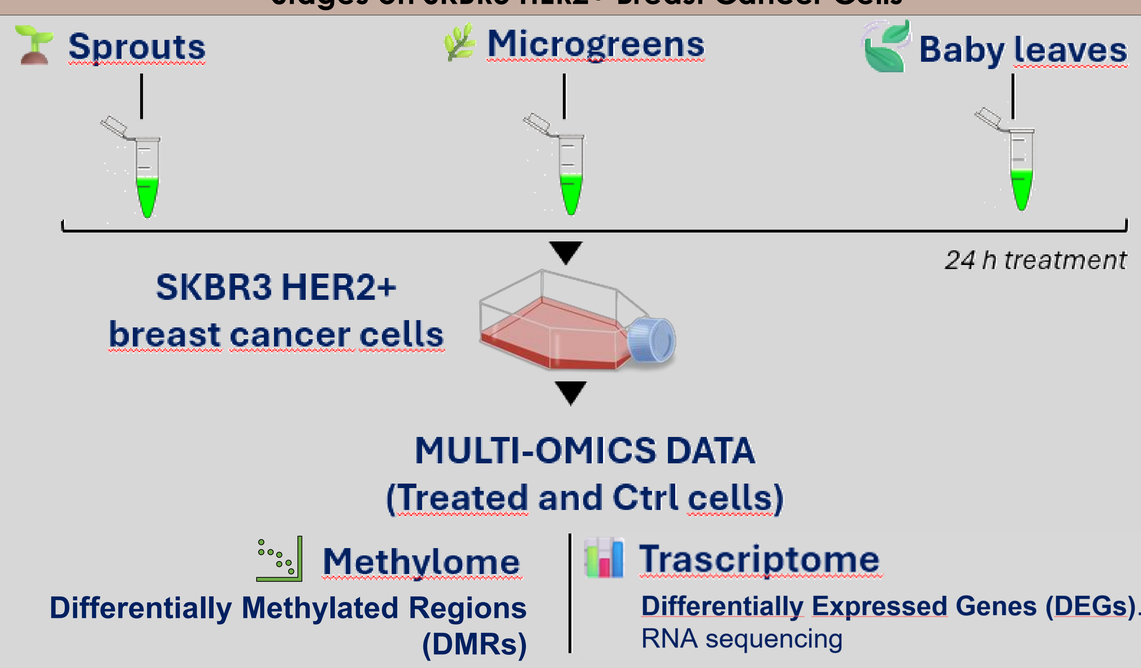
Broccolo nero (BN) extract of sprouts (SPs), microgreens (MGs), and baby leaves (BLs), are increasingly recognized as sustainable "superfoods" with potential benefits for human health and disease prevention [1]. Their rapid growth, low-input cultivation, and accessibility make them attractive for both producers and consumers, aligning with current priorities in sustainable nutrition. Despite growing interest, limited evidence exists on the molecular mechanisms underlying their anti-inflammatory effects, particularly at the level of gene regulation. In this study supported by ELIXIRxNextGenIt-1st NOA Call, we adopted an integrative multi-omics approach to investigate how phytochemicals derived from BN superfoods influence epigenetic and transcriptional processes associated with inflammation and oxidative stresses. Specifically, we analyzed transcriptomic profiles, DNA methylation patterns, in breast cancer cell model SKBR3 treated with SPs, MGs, and BLs extracts, compared to untreated controls. Preliminary findings reveal distinct gene expression and methylation signatures associated with the developmental stage of BN leaves, suggesting differential modulation of key genetic pathways involved in inflammation, oxidative metabolism, and cell survival. Further studies are warranted to elucidate the precise regulatory mechanisms, as well as the impact of phytochemical composition, bioavailability, and synergistic effects, to better translate these findings into dietary recommendations and functional food applications [2].

MATERIAL AND METHODS

Extraction and Analysis of Sprouts (SP), Microgreens (MG), and Baby Leaf (BL) composition

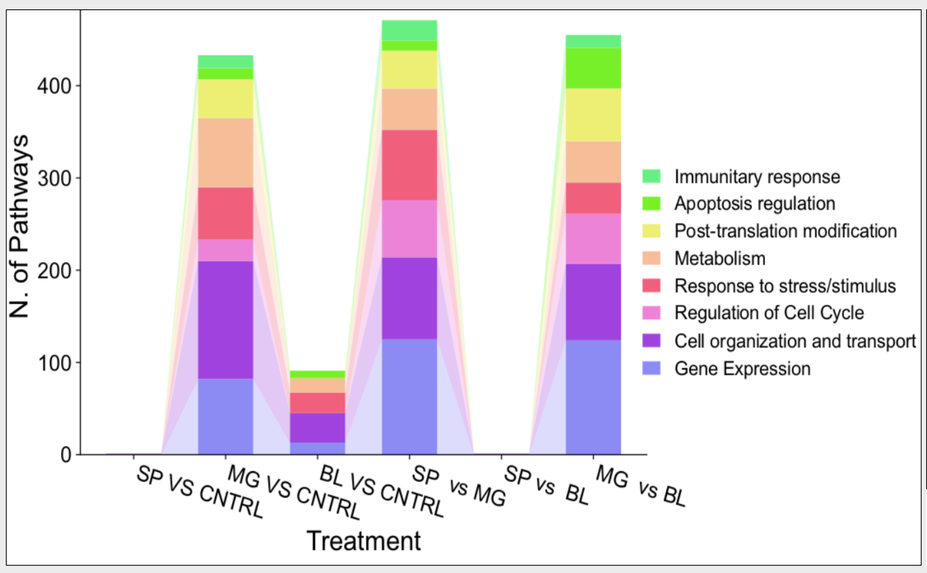


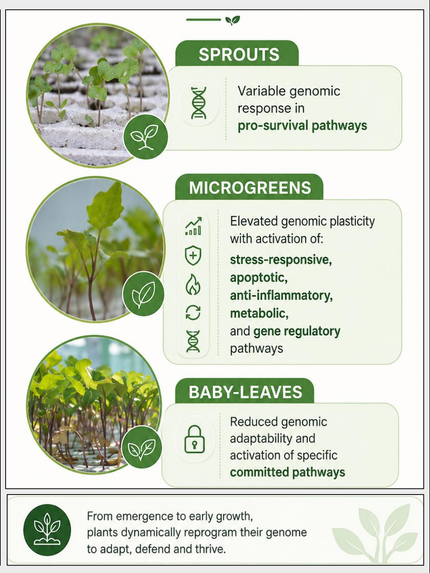
Epigenetic and Transcriptional Impact of Broccoli Development Stages on SKBR3 HER2+ Breast Cancer Cells



RESULTS

Stage-Specific *B. oleracea* Extracts Shape SKBR3 Transcriptional Profiles

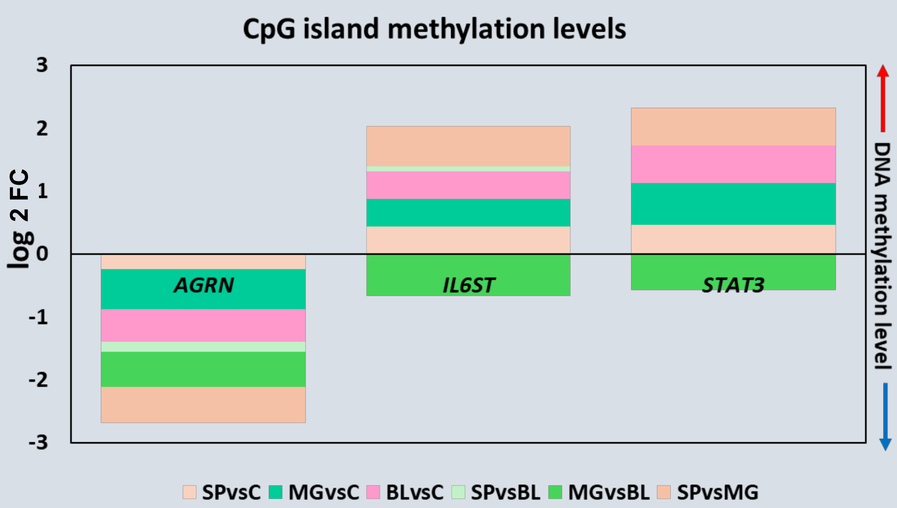




Gene Ontology (GO) pathway of all DEGs identified in RNAseq from exposing SKBR3 cells with SP, MG and BL extracts

Comparative analysis of the number of metabolic and signaling pathways significantly affected by each treatment. The treatments include SP versus CNTRL, MG vs CNTRL, BL vs CNTRL, and pairwise comparisons among plant growth stages (SP vs MG, SP vs BL, MG vs BL). GO analysis performed by GO Resource (https://geneontology.org/) on complete biological processes, with multiple-testing correction applied using the Fisher–Bonferroni method.

Epigenetic remodeling of the *AGRN–IL6ST–STAT3* axis in SKBR3 cells



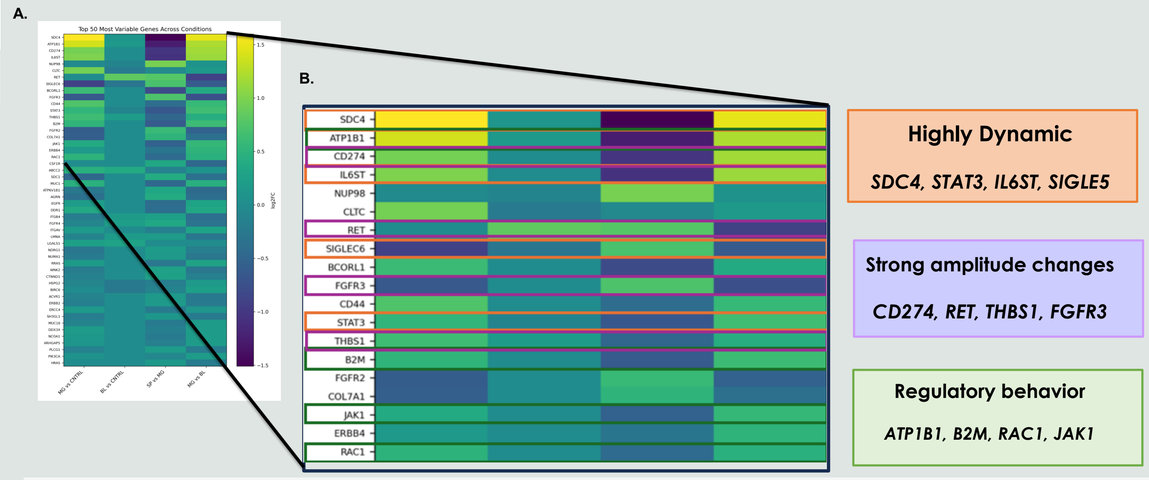
Increased ECM signaling/mechanotransduction (***AGRN*** activation ↑)

Reduced gp130-dependent cytokine signaling (***IL6ST*** silencing ↓)

Reduced STAT3-mediated transcriptional activity (***STAT3*** silencing ↓)

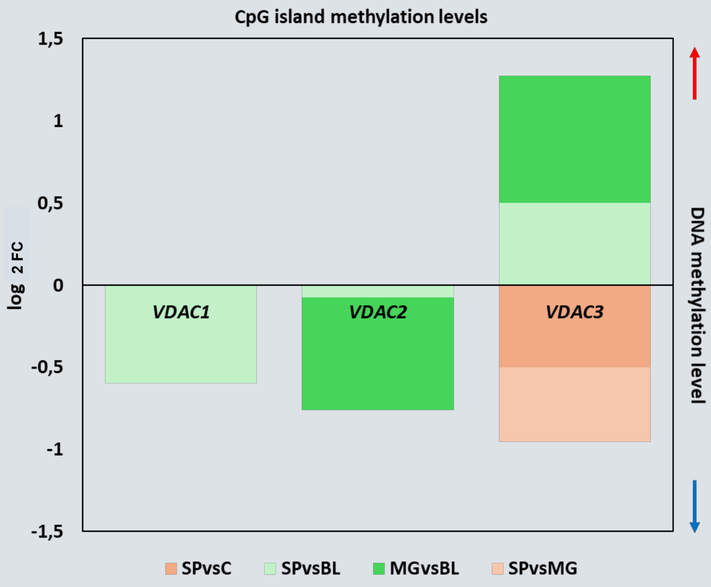
The ***AGRN–IL6ST–STAT3*** axis is a key pro-tumorigenic signaling pathway involved in cell survival and inflammatory responses.
The observed methylation pattern in SKBR3 cells suggests selective epigenetic remodeling characterized by preservation of pro-survival functions alongside attenuation of *STAT3*-mediated oncogenic signaling.
AGRN hypomethylation may indicate enhanced extracellular matrix (ECM) signaling, whereas hypermethylation of IL6ST and *STAT3* may reflect suppression of the *IL-6/JAK/STAT3* pathway [4], potentially reducing inflammatory and stress-responsive transcriptional activity.

Focused pathway analysis on DEGs from a referenced* list of tumor SKBR3 hallmarks



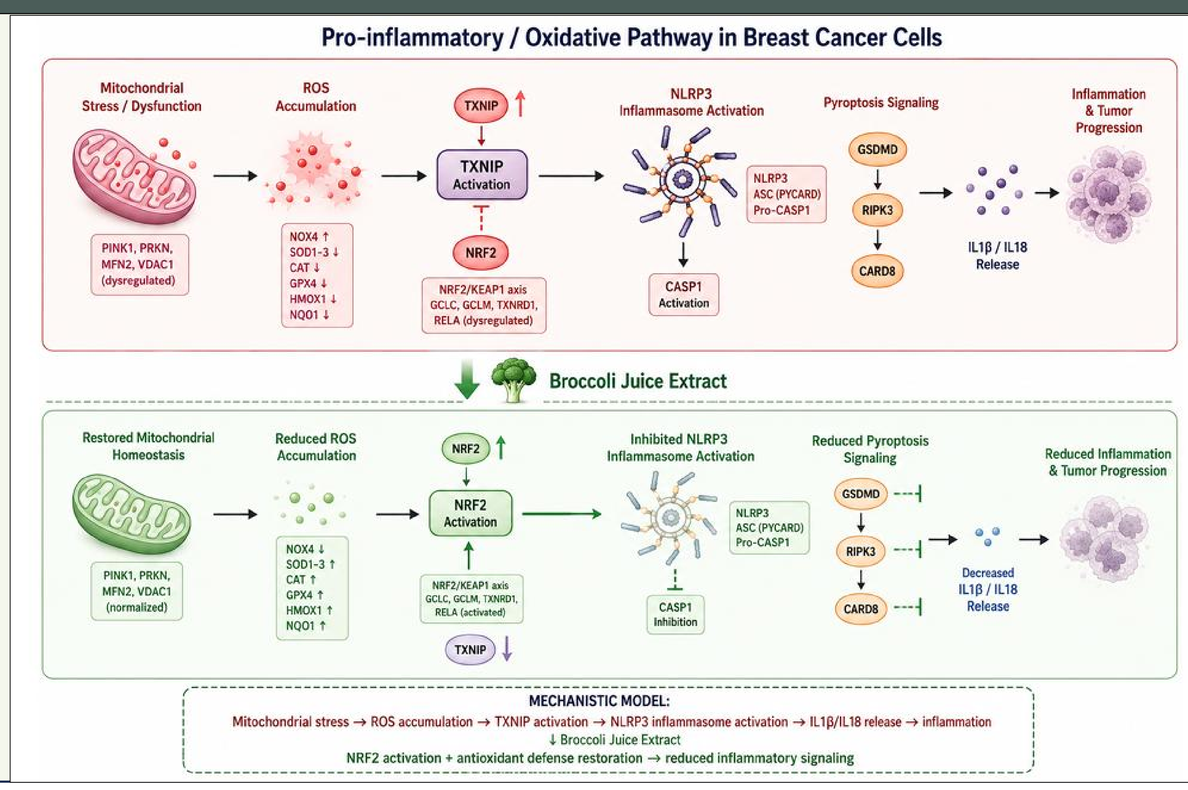
A) Heatmap showing the 50 most variable genes across the four experimental conditions most representative of the overall experimental setting. RNA-seq expression profiles were compared among MG vs CNTRL, BL vs CNTRL, SP vs MG, and MG vs BL. The color scale represents relative gene expression levels, highlighting distinct transcriptional patterns associated with each developmental stage.
B) Focus on 20 genes grouped into categories of highly dynamic expression (***SDC4, STAT3, IL6ST, SIGLE5***), variable response (***CD274, RET, THBS1, FGFR3***), and regulatory behavior (***ATP1B1, B2M, RAC1, JAK1***) across the four experimental conditions. Genes included in the complete heatmap were selected from a panel of 138 marker genes identified in SKBR3 cells, as reported by Lazar IM et al, (2022) [3]*.

DNA methylation analysis of promoter *VDAC* genes in SKBR3 cells treated with SP, MG and BL



VDAC3 methylation appears to be modulated by the developmental stage of Black Broccoli.
Lower methylation levels observed in SP and MG, compared with BL, suggest enhanced epigenetic regulation of *VDAC3* in extracts enriched with antioxidant phytochemicals.
This finding is consistent with the proposed role of *VDAC3* in antioxidant defense and cellular survival pathways [5].
Early developmental stages of Black Broccoli are characterized by higher levels of antioxidant and anti-inflammatory phytochemicals that support plant growth and development and may also contribute to increased cytoprotective and pro-survival effects in tumor cells.

Conclusions



The data presented in this poster are part of a manuscript currently under submission.

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