INVESTIGATION OF THE ROLE OF NFAT1 IN MELANOMA

Ana Paula Fernandes Paz dos Santos (1), Deborah Alves de Lima (1), João Paulo de Biaso Viola (1), Mariana Boroni (1), Patricia Abrão Possik (1).
(1) Brazillian National Cancer Institute (INCA), Rio de Janeiro, Brazil

ABSTRACT

Background: The nuclear factor of activated T cells 1 (NFAT1) is a transcription factor important for various cellular functions, which has been extensively characterized in lymphocytes. NFAT1 is upregulated in metastatic melanoma, and has been shown to play a role in differentiation, apoptosis, proliferation and metastasis. However, the mechanisms through which NFAT1 contributes to the malignant phenotype of melanoma are yet to be elucidated. By exploring publicly available gene expression datasets, we have recently identified *BCL2A1* and *UCN2* mRNAs as up regulated in melanoma cell lines with high *NFAT1* expression. The aim of this study is to dissect the role of NFAT1 and identify functionally relevant transcriptional targets in melanoma. **Methods and Results:** Here we show, by quantitative (q) RT-PCR, that melanoma cells express different levels of *NFAT1* and that *BCL2A1* and *UCN2* are predominantly expressed in NFAT1^{high} melanoma cells. Knockdown of *NFAT1* (shRNA) in NFAT1^{high} cells (A375) was confirmed by western blot. qRT-PCR further revealed down regulation of *BCL2A1* in NFAT1-silenced cells. Treatment of A375 cells with ionomycin induced partial NFAT1 dephosphorylation, which was almost completely abolished by cyclosporine treatment. **Conclusion:** Our preliminary functional experiments point to a role of BCL2A1 as a potential transcriptional target of NFAT1. Finally, we also show that NFAT1 is partially activated in melanoma cells, and that phosphorylation is controlled by calcium levels.

PRELIMINARY DATA

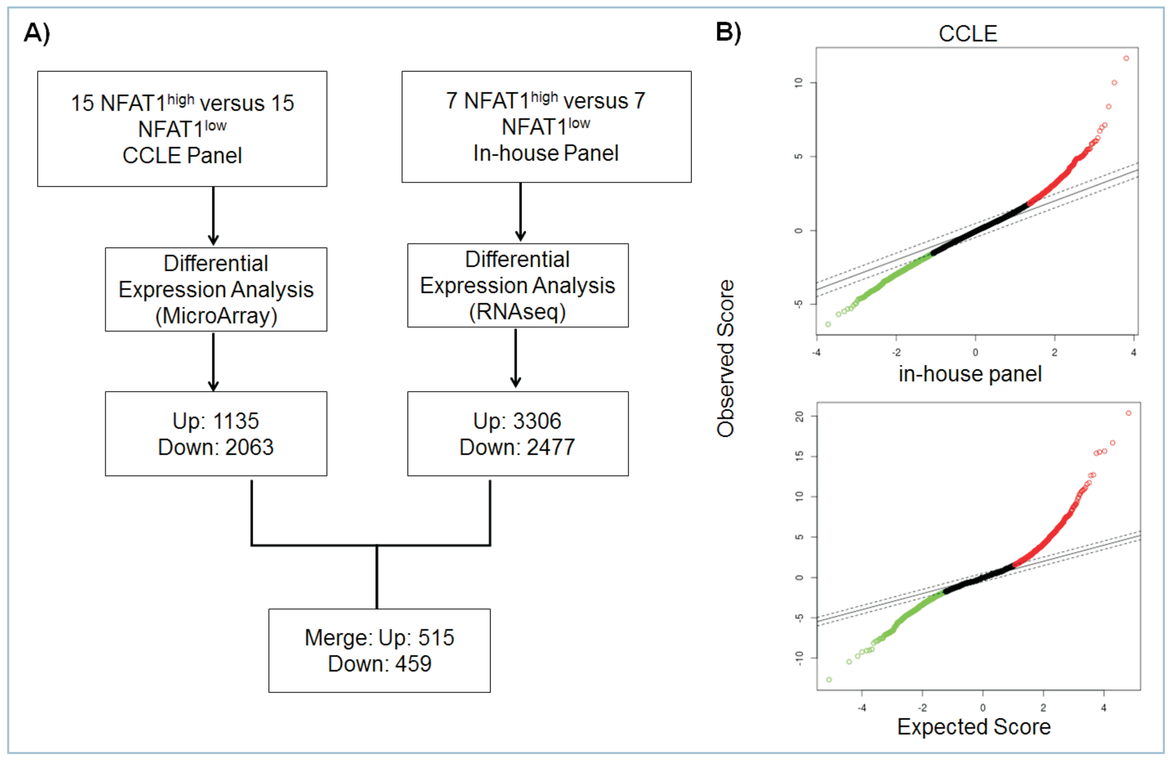

Figure 1: Identification of genes potentially regulated by NFAT1. A) Gene expression data from melanoma cell lines deposited in the CCLE (Cancer Cell Line Encyclopedia) and available in our lab (in-house) was used to identify genes differentially expressed between NFAT1^{high} and NFAT1^{low} melanoma cell lines. **B)** Q-Q plot illustrating expected and observed scores for genes expressed in NFAT1^{high} and NFAT1^{low} melanoma cell lines. Dotted lines define genes with no significant difference between expected and observed scores (black dots). Genes downregulated in NFAT1^{high} cells are illustrated by green dots, whereas genes upregulated in NFAT1^{high} cells are illustrated by red dots.

RESULTS

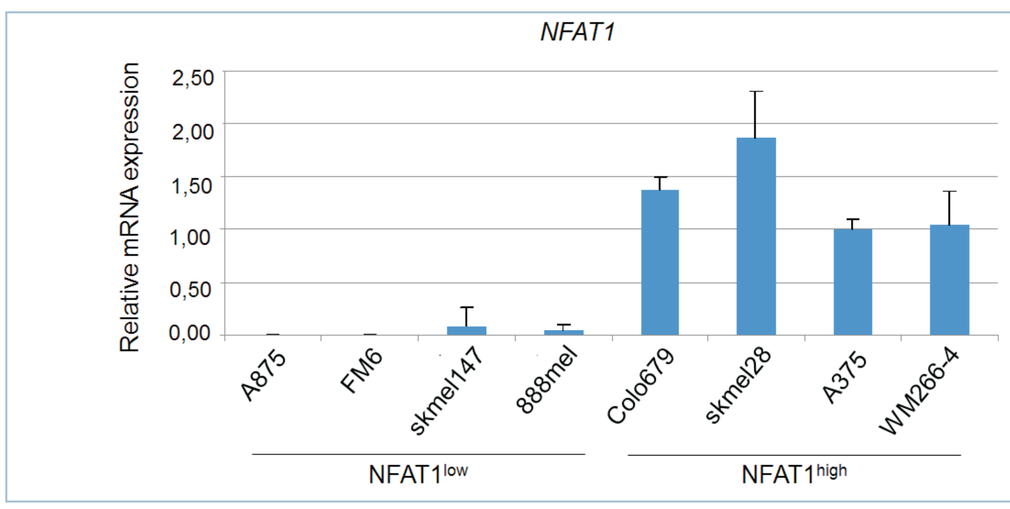

Figure 2: Melanoma cell lines express different levels of *NFAT1*. qRT-PCR of *NFAT1* expression among eight melanoma cell lines. *BACT* was used as endogenous control. Bar graphs represent fold change in *NFAT1* expression compared to A375 (set to 1). Cell lines were classified into NFAT1^{high} and NFAT1l^{ow} according to results. Error bars represent SD from 3 technical replicates.

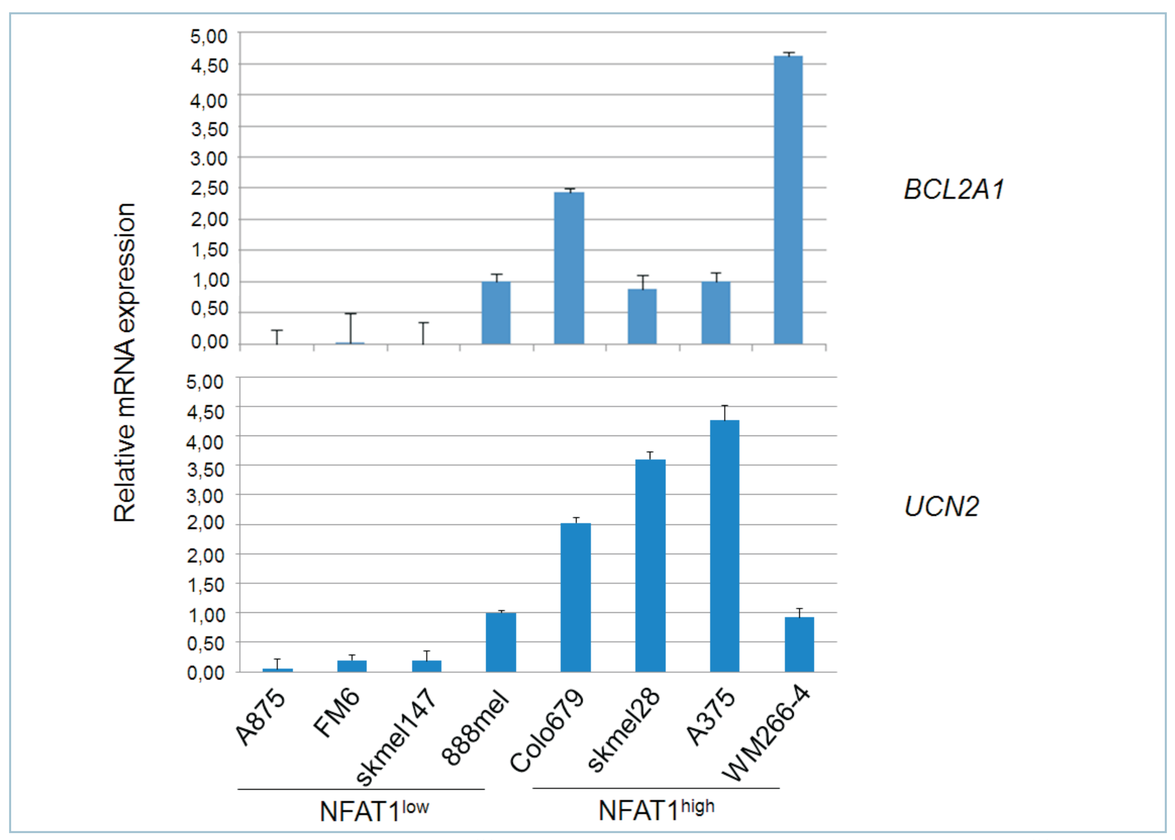

Figure 3: *BCL2A1* and *UCN2* are upregulated in NFAT1^{high} melanoma cell lines. qRT-PCR of *BCL2A1* and *UCN2* in NFAT1^{high} and NFAT1^{low} melanoma cell lines. *BACT* was used as endogenous control. Bar graphs represent fold change in gene expression compared to A375 (set to 1). Error bars represent SD from 3 technical replicates.

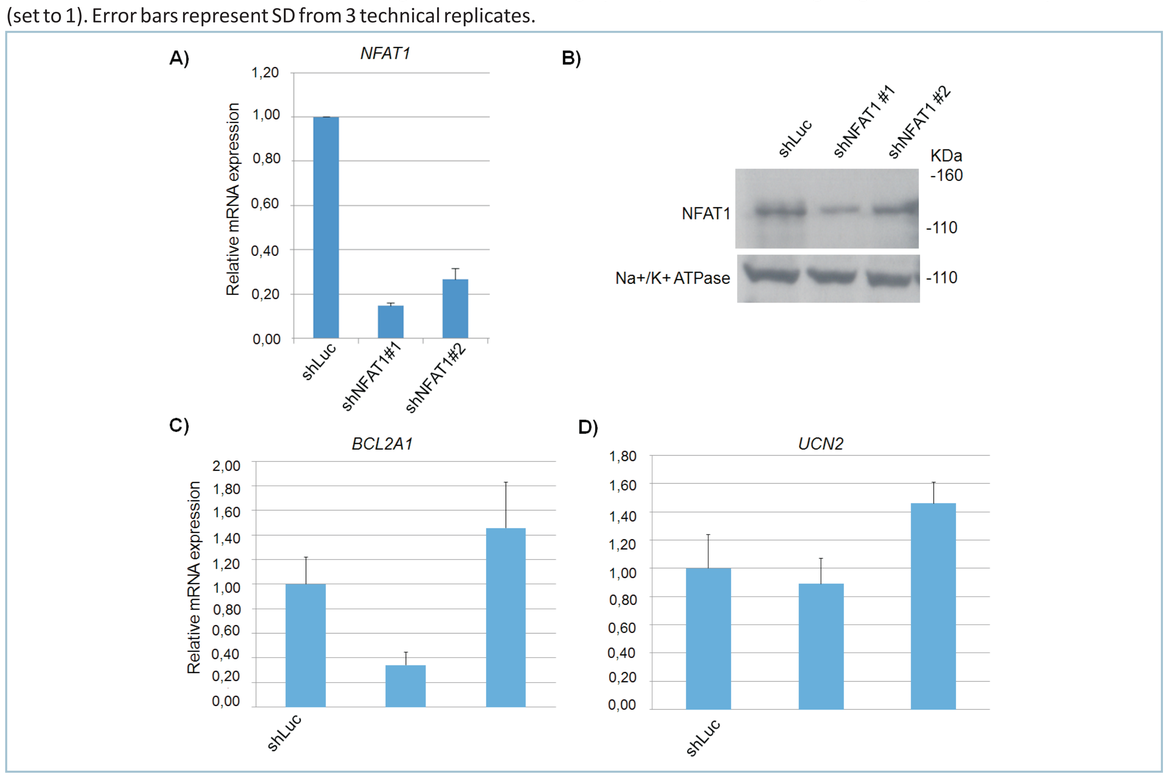

Figure 4: Effect of *NFAT1* knockdown in the expression of *BCL2A1* and *UCN2*. A375 cells were transduced with pLKO.1-Puro carrying shLuciferase (shLuc, control), shNFAT1#1 or shNFAT1#2 and selected with puromycin. RNA and protein extracts were collected 14 days after transduction. **A)** qRT-PCR of *NFAT1*. **B)** Western Blot for NFAT1. Na+/K+ ATPase was used as loading control. **C) and D)** qRT-PCR of *BCL2A1* and *UCN2*. For **A, C** and **D**, *BACT* was used as endogenous control. Bar graphs represent fold change compared to shLuc (set to 1). Error bars represent SD from 3 technical replicates

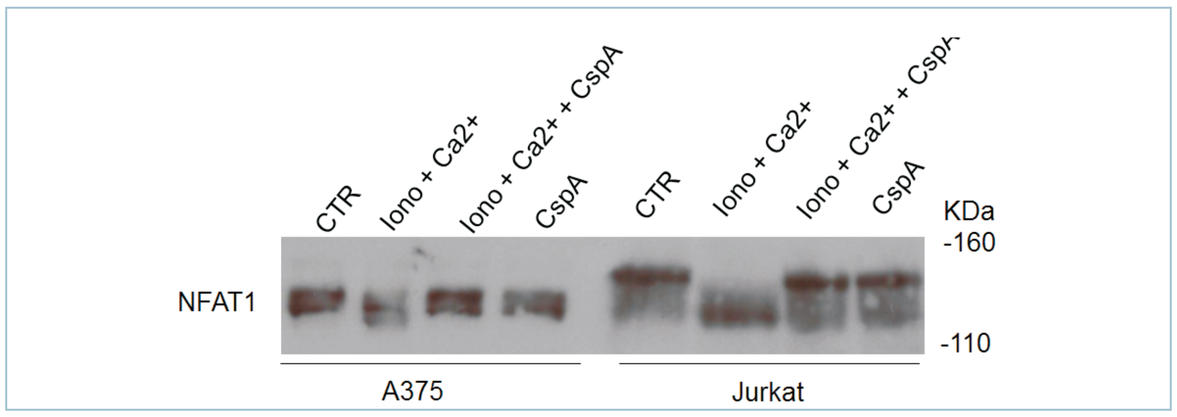

Figure 5: Comparison of changes in NFAT1 phosphorylation under different treatments in A375 and Jurkat cells. A375 (melanoma) and Jurkat (T CD4^{+} lymphocytes) cells were treated with Cyclosporine A (Csp A, 5 M) and/or Calcium Chloride (Ca2+ , 2mM) for 15 min, followed by Ionomycin (Iono 2 M) for 10 min. Protein extracts were collected for Western Blot. Treatment of Jurkat cells were used as a control to illustrate phosphorylated (CTR, Iono/Ca2+/CspA, CspA) and dephosphorylated (Iono/Ca2+) forms of NFAT1. Similar treatments in A375 cells show two distinct bands that differ in molecular weight to the expected phosphorylated and dephosphorylated forms of NFAT1

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Projeto Gráfico: Setor de Edição e Informação Técnico-Científica / INCA

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