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Highlighting a crucial role for EGR2 mutations in CLL pathogenesis and BTKi resistance

Alessia Morabito[1], Jessica Bordini[2], Chiara Lenzi[1], Michela Frenquelli[2], Lydia Scarfò[2], Alessandro Campanella[1,2], Paolo Ghia[1,2]

1 Università Vita Salute San Raffaele, Milan, Italy

2 B cell neoplasia Unit, IRCCS Ospedale San Raffaele, Milan, Italy

INTRODUCTION

- **Recurrent mutations in the EGR2 gene** (E356K, H384N, D411H) were identified in 8% of advanced-stage patients with Chronic Lymphocytic Leukemia (CLL)[1].
- Clinical data suggest that EGR2 mutations are associated with a **rapidly progressive disease course and are also frequent in BTKi-resistant patients**[2].
- However, the functional role of mutated EGR2 in the leukemogenic process remains to be elucidated.
- We hypothesize that **EGR2 mutations may change the affinity to the DNA**, causing an alteration at the transcriptional and epigenetic level, thereby **modulating relevant intracellular pathways that contribute to CLL aggressiveness and dissemination.**

OBJECTIVES

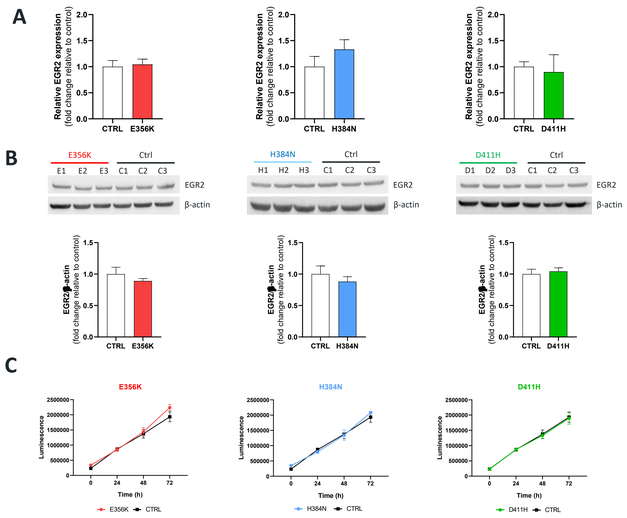
The aim of the project is to study the functional role of EGR2 missense mutations in CLL development and BTKi resistance

CONCLUSION

- Our findings helped shed light on the molecular and functional consequences of EGR2 mutations, showing **enhanced migratory capacity and altered expression of EGR2 target genes.**
- In particular, we demonstrated a **cross-talk with the NOTCH-1 pathway leading to its activation**, a well-known mechanism acting in the pathogenesis of the disease.
- We were also able to confirm the **association of EGR2 mutations and BTKi resistance in CLL**, thus warranting further exploration to identify druggable molecules to reinstall drug sensitivity.

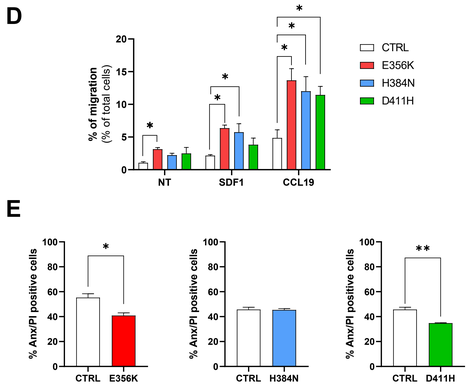
RESULTS

1. EGR2-mutated cell lines exhibit **similar EGR2 expression**, both at mRNA **(A)** and protein level **(B)**, and **similar proliferation rate (C)** compared to WT cells



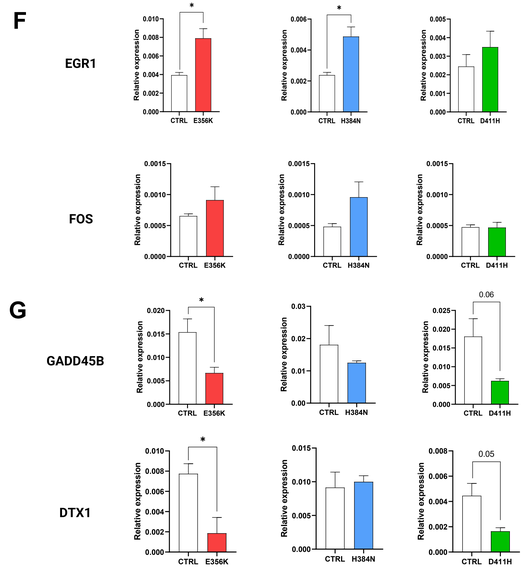
(A) Real-time PCR analysis for the expression of EGR2 mRNA in the E356K-, H384N-, D411H-EGR2 mutated cell lines; **(B) Western blot analysis for the expression of EGR2 protein** (upper panels: representative images, lower bar graphs: densitometry summary of two independent experiments); **(C) Cell Titer Glo assay to measure the growth** of mutated cell lines

2. EGR2-mutated cell lines show an **increased migration capacity (D)**, both in presence or absence of chemotactic stimuli, and are **less sensitive to ibrutinib-induced apoptosis (E)**



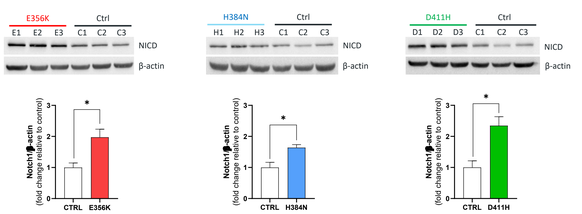
(D) Transwell migration assay of EGR2 mutated cell lines in the presence or absence of chemotactic stimuli (**SDF-1 and CCL19**); **(E) Annexin V-PI staining** of EGR2 mutated cell lines **treated with Ibrutinib 10 μM** for 72 hours

3. EGR2 mutations **alter the expression of EGR2-target genes**, with **some genes that are upregulated** in the presence of the mutation **(F)** while **others are downregulated (G)**. Interestingly, DTX1 is a regulator of the NOTCH1 pathway, a known driver mechanism of the disease.



(F-G) Real-time PCR analysis for the expression of EGR2 target genes: EGR1, FOS, GADD45B and DTX1 in the E356K-, H384N-, D411H-EGR2 mutated cell lines

4. Moreover, EGR2-mutated cell lines show an **increased expression of NOTCH1 intracellular domain (H)**, indicating a potential **activation of the NOTCH1 pathway.**



(G) Western blot analysis for the expression of NOTCH1 intracellular domain (NCID), which is the active form of NOTCH1

REFERENCES

1. Young, E et al. EGR2 mutations define a new clinically aggressive subgroup of chronic lymphocytic leukemia. Leukemia 2017
2. Bonfiglio S et al. BTK and PLCG2 remain unmutated in one-third of patients with CLL relapsing on ibrutinib. Blood Adv. 2023

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