Identification of Tipifarnib Sensitivity Biomarkers in T-Cell Tumor cell lines

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INTRODUCTION

T-cell lymphoma (TCL) is a very aggressive and heterogeneous group of hematological malignancies with poor prognosis and inadequate response to current therapies that represent about 10% of all non-Hodgkin's lymphoma. The standard first-line treatments have resulted in unsatisfactory patient outcomes and unfortunately, targeted treatments are still at a preliminary phase. The development of robust tools for biomarker-driven risk stratification and therapeutic decision making guided by these biomarkers is necessary in order to improve personalized therapy. The RAS/MAPK pathway is crucial for TCR signaling of T-cells and it is deregulated in TCL. We aimed to determine the therapeutic value of the farnesyl transferase inhibitor (FTI) tipifarnib in TCL cell lines. Tipifarnib blocks the localization of some RAS proteins to the intracellular membrane, thereby inhibiting their activation. Tipifarnib is a potent and specific FTI with a prominent anti-proliferative effect.

OBJECTIVE

This study tested tipifarnib in TCL cell lines for in vitro sensitivity and for genomic and inmunohistochemical biomarker discovery with a focus on TCR pathway inhibition as a rational therapeutic approach in T-Cell Lymphomas with molecularly-target therapeutic options. To that end, effects on cell viability, apoptosis, cell cycle and gene expression were evaluated.

METHODS

We tested tipifarnib in 22 TCL cell lines for *in vitro* sensitivity and for biomarker discovery, both genomic and immunohistochemical. We selected cell lines with available genomic data from COSMIC, CCLE or generated by our group. The MAPK, NFAT, NFKB and JAK/STAT pathways were tested by immunohistochemistry analysis under basal conditions. The range of drug concentrations to perform IC_{50} analysis was established between 0-10,000 nM (ten points), using CellTiter-Glo® Luminescent Cell Viability Assay kit (Promega, Madison, WI, USA) following manufacturer´s instructions at 0h, 48h and 96h. All experiments were done in sextuplet and all numerical data were expressed as the average of the values ± the standard error of the mean. IC_{50} analyses were performed with GraphPad Prism v5. Clinically-relevant drug sensitivity was defined as IC_{50} <100 nM at 96h (defined as sensitive). RNA-seq was performed under basal culture conditions in 22 cell lines and in 4 sensitive cell lines with tipifarnib and DMSO (vehicle) in three biological replicates at 72h. Differential gene expression (DE) analysis was carried out under these two conditions. The pathways analysis was analyzed using Gene Set Enrichment Analysis (GSEA). Induction of apoptosis and cell viability were tested by flow cytometry in 4 sensitive cell lines.

CONCLUSIONS

- This study suggests that tipifarnib could be a potential therapeutic option in T-cell lymphomas/leukemias, given that the majority of lines were sensitive to tipifarnib at clinically-achievable concentrations.
- Tipifarnib is able to decrease cell viability, increased apoptosis and block the progression of the cell cycle in the G1 phase, and prevent reaching the cell replication phase (phase G2).
- Analyzing pathways involved in T cell development, we detected that tipifarnib decreased MYC targets and mTORC1 and MAPK signalling.
- **p-ERK** (IHC) and the mutational state of ***NOTCH1*** could serve as potential biomarkers of sensitivity to tipifarnib.
- **RelB** (IHC) could serve as a potential biomarker of tipifarnib resistance.

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RESULTS

59.1% (n=13) of cell lines were sensitive to tipifarnib at concentrations which are readily achievable in the clinic (i.e. IC_{50} <100nM at 96h). RAS, RAS-guanine nucleotide exchange factors (GEFs) and RAS-GTPase activating proteins (GAPs) genes were mutated in 45.5%, 50% and 27.3%, of cell lines respectively. We found *TP53 and DNMT3* mutated in 80% and 33.3% in sensitive cell lines respectively, and in 63.6% and 0% in resistant cell lines. The mutational state of *NOTCH1* was associated with tipifarnib sensitivity, 66.7% in sensitive cell lines versus 9.1% in resistant cell lines (p=0.005). The activation ERK (MAPK pathway) was significantly associated with drug sensitivity (p=0.046). Conversely, RelB (NFκB pathway) was associated with drug resistance (p=0.007). *GSEA* analysis showed that tipifarnib downregulated expression of genes associated with cell cycle progression, protein localization to membranes, metabolism, and ribosome and mitochondrial activity. The downregulation of cell cycle was validated by flow cytometry assays. Tipifarnib was able to decrease cellular viability, increase apoptosis and block cell cycle progression in G1 phase and prevent them from reaching the cellular replication phase (G2). Differential gene expression (DE) was carried out in presence and absence of tipifarnib. We were able to detect more than 300 differentially expressed genes, 24 of which changed their expression in the same way in the three cell lines. GSEA of molecular pathways regulated by tipifarnib identified downregulation of MYC targets and MTOR and MAPK pathways. MYC is a target of *NOTCH1*, mutated in sensitive cases and associated to tipifarnib sensitivity.

Figure 1

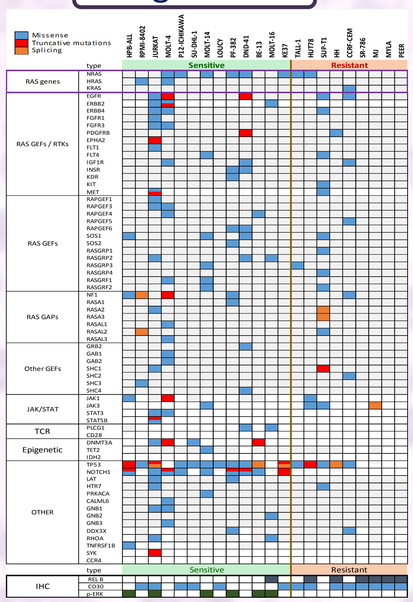

Figure 1. Mutational landscape of RAS/MAPK, JAK/STAT, TCR pathways and T cell lymphoma related genes in TCL cell lines and the IHC markers. Cell lines were grouped into highly sensitive (in green) and less sensitive (in red) in the superior part of the table. Missense mutations are marked in blue, truncative in red and splicing mutations in orange in the upper part of the table. In the lower part referring to immunohistochemistry, dark blue, light blue and green correspond to positive labeling for TP53, CD30 and p-ERK, respectively.

Figure 2

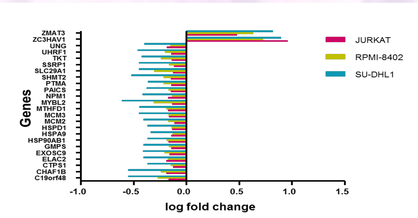

Figure 2. Common DE genes in JURKAT (pink), RPMI-8402 (yellow) and SU-DHL-1 (blue) downregulated (left part of the graph) and upregulated (right part of the graph) with tipifarnib p value (p adj< 0.05).

Figure 3

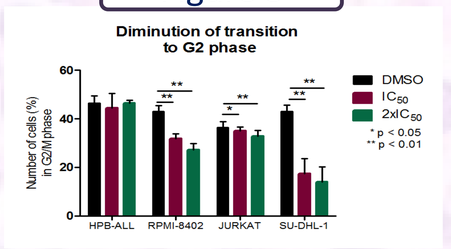

Figure 3. Cell cycle blockade in G1 phase in Tipifarnib-sensitive cell lines (% relative to DMSO). Cell lines were incubated for 96h at 1xIC_{50} (in purple) and 2xIC_{50} (in green) values of tipifarnib and DMSO as a vehicle. Each experiment was doing in triplicate.

Figure 4

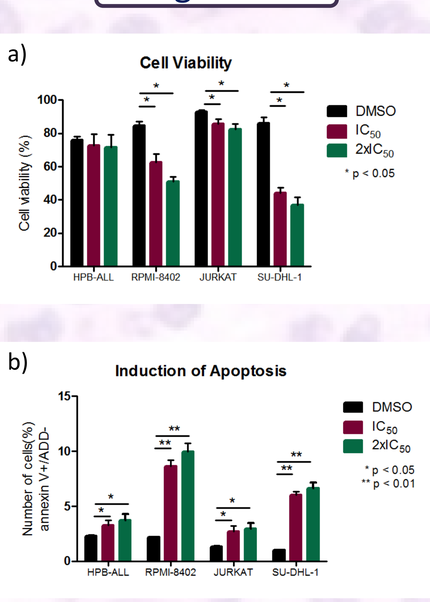

Figure 4. (a) Cell viability and **(b)** induction of apoptosis induction in tipifarnib-sensitive cell lines. Cell lines were incubated for 96h at 1xIC_{50} (in purple) and 2xIC_{50} (in green) values of tipifarnib, and DMSO (in black) as a vehicle. Each experiment was doing in triplicate. Error bars is shown.

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