Novel approaches for targeted therapy in CTCL

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Introduction

Cutaneous T-cell lymphoma (CTCL) is a heterogeneous group of diseases characterized by the clonal expansion of malignant T-cells in the skin1. The two predominant clinical forms of CTCL are Mycosis Fungoides (MF) and Sézary Syndrome (SS). Tumor-stage MF has an unfavorable prognosis with a 10-year survival of approximately 40%, while SS is even more aggressive, with a median survival of around two years2. Despite the molecular pathogenesis of CTCL is still basically unknown, some data including gene expression profiling studies have shown that increased signaling from the T-cell receptor (TCR) can be considered a driving force of CTCL and that the neoplastic T-cells can acquire Treg and Th17 phenotypes3. Using a targeted ultrasequencing analysis focused on 524 genes with known biological relevance in normal T-cells, we have studied the mutational status of paired (non tumoral vs. tumoral) genomic DNA from 11 CTCL patients. As part of our results we found activating mutations currently affecting PLCG1 (3/11 patients and also 20% in an independent series analysis). Mutant PLCG1 proteins showed enhanced activity towards NFAT activation, when compared to wild type proteins both in CTCL cell lines and patients. On the other hand the IL-6ST–JAK/STAT pathway genes like IL-6ST, JAK1 and JAK3 were also found mutated in 3/11 patients4, suggesting that this pathway can acquire Treg and Th17 phenotypes3. Therefore, CTCL cells might be targeted by T-cell receptor (TCR) signals from the TCR can be considered a driving force of CTCL and that the neoplastic T-cells are prone to develop in patients treated with targeted therapies. Our results indicate that targeted inhibition of these pathways using already clinically tested drugs can control proliferative, survival and phenotypic activities in normal T-cells, we have decided to explore the biological mechanisms including the regulation of the expression of T-cells pathways using already clinically tested drugs can control proliferative, survival and phenotypic activities in normal T-cells, we have decided to explore the biological implications of the phenotype disruption in patients treated with targeted therapies.

Materials and Methods

Cutaneous T-cell lymphoma cell lines used were HH (MF) and MJ (MF) obtained from ATCC (Rockville, MD, US ) and My-La (MF) and HUT-78 (SS) obtained from ECACC (Salisbury, UK). IgG2a isotype controls conjugated with PE and APC. The cells were acquired by flow cytometer (FACS Canto-II, Becton Dickinson). The appropriate amount of inhibitor, as indicated in the figures, at 0h, 24h and 48h following manufacturer's instructions. Cell lysates were performed using CaliTiter-Glo® Luminiscence Cell Viability Assay kit (Promega, Madison, WI, USA) with the appropriate amount of inhibitor, as indicated in the figures. All cell proliferation analyses were performed using CaliTiter-Glo® Luminiscence Cell Viability Assay kit (Promega, Madison, WI, USA) with the appropriate amount of inhibitor, as indicated in the figures. All cell proliferation analyses were performed using CaliTiter-Glo® Luminiscence Cell Viability Assay kit (Promega, Madison, WI, USA) with the appropriate amount of inhibitor, as indicated in the figures. All cell proliferation analyses were performed using CaliTiter-Glo® Luminiscence Cell Viability Assay kit (Promega, Madison, WI, USA) with the appropriate amount of inhibitor, as indicated in the figures. All cell proliferation analyses were performed using CaliTiter-Glo® Luminiscence Cell Viability Assay kit (Promega, Madison, WI, USA) with the appropriate amount of inhibitor, as indicated in the figures.

Conclusions

• Tacrolimus and Ruxolitinib produced a dose dependent inhibition of My-La, HUT-78, HH and MJ cell proliferation.
• Tacrolimus but not Ruxolitinib markedly decreased cell survival at 24 hours.
• Combined use of Tacrolimus and Ruxolitinib produced a significant greater inhibition of CTCL cell proliferation than each inhibitor alone.
• CTCL cells can simultaneously express phenotypic markers for Treg and Th17 lineages.
• Targeted inhibition of PLCG1 and JAK downstream signaling can disrupt the phenotypic status of CTCL cells.

Perspective

• Can we activate TCR and JAK downstream signaling to a more aggressive disease?
• Can we develop new therapeutic strategies for CTCL based on patient mutational profiling?
• Can we take clinical advantage of the phenotype disruption in patients treated with targeted therapies?

References


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