Immunomodulatory actions of lanthionine synthetase C-like protein 2-based drugs

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Abstract

Current inflammatory bowel disease (IBD) treatments are associated with significant side-effects. We identified lanthionine synthetase component C-like protein 2 (LANCL2) as a target for abscisic acid, a naturally occurring compound with potent anti-inflammatory effects. The goal of this study was to determine the role of LANCL2 as a therapeutic target for developing novel anti-inflammatory drugs. Structure-based virtual screening was performed using a compound library from National Cancer Institute Diversity Set II. To validate the anti-inflammatory efficacy of the top-ranking compound 61610, a series of *in vitro* and pre-clinical efficacy studies were performed using a mouse model of dextran sodium sulfate-induced colitis. Our findings showed that oral administration of 61610 (20 mg/kg/day) ameliorated experimental colitis by down-modulating colonic inflammatory gene expression and favoring regulatory T cell responses. We also investigated the cell specificity and molecular targets underlying the anti-inflammatory mechanism of 61610. Our *in vivo* findings indicate that anti-inflammatory efficacy of 61610 depends on macrophage expression of peroxisome proliferator-activated receptor γ, a receptor downstream of LANCL2. In summary, we used an integrated drug discovery platform consisting of molecular modeling approaches followed by experimental validation to confirm LANCL2 as a novel therapeutic target against IBD and demonstrated that 61610 is a novel anti-inflammatory drug.

Introduction

Lanthionine synthetase component C-like protein 2 (LANCL2) is a member of the eukaryotic lanthionine synthetase component C-Like protein family involved in signal transduction and insulin sensitization. LANCL2 is a target for the binding and signaling of abscisic acid (ABA), a plant hormone with anti-diabetic and anti-inflammatory effects by activating peroxisome proliferator activated receptor γ. Our objective is to determine the role of LANCL2 as a potential therapeutic target for developing novel drugs against inflammatory diseases.

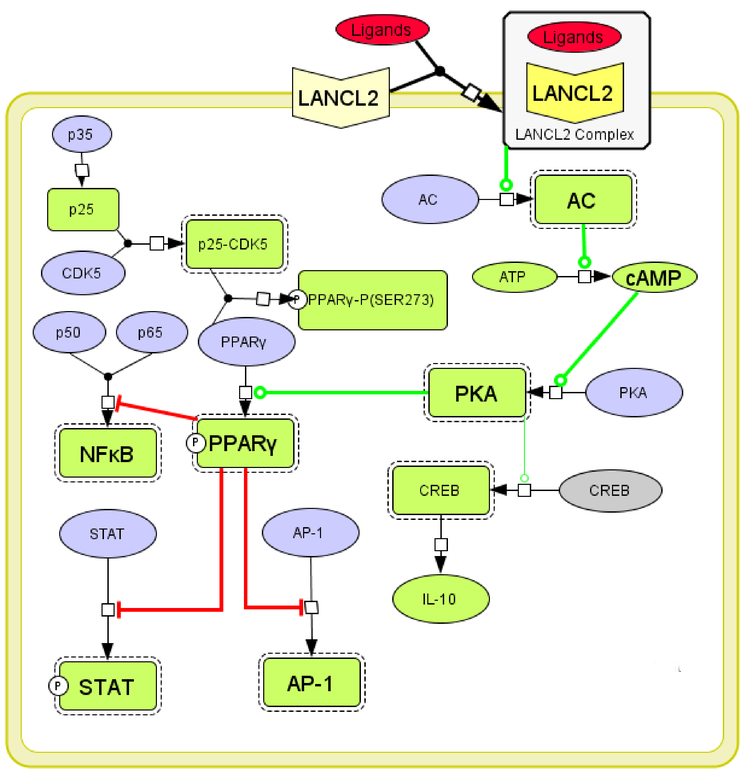

Figure 1. Proposed anti-inflammatory mechanism of drugs targeting lanthionine synthetase component C-like protein 2 (LANCL2). AC: adenylate cyclase; cAMP: cyclic adenosine monophosphate; PKA: protein kinase A; PPAR γ: peroxisome proliferator-activated receptor γ; NFκB: nuclear factor kappa-light-chain-enhancer of activated B; CDK5: cycline dependent kinase 5; STAT: signal transducers and activators of transcription; AP-1: activator protein 1; CREB: cAMP response element-binding; IL-10: interleukin-10; black line: state transition and heterodimer association; green line: catalysis; red line: inhibition.

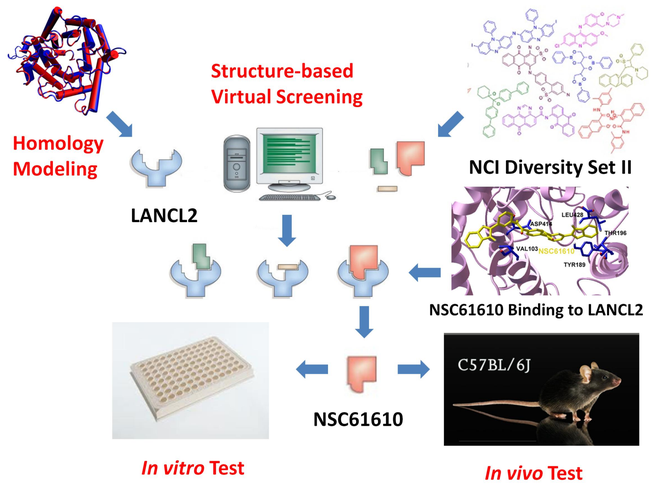

Figure 2. Overview of studies. The structure of lanthionine synthetase component C-like protein 2 (LANCL2) was predicted by homology modeling. National Cancer Institute (NCI) Diversity Set II was selected as virtual library of compounds. LANCL2 structure-based virtual screening was performed. Compounds were ranked according to their estimated free energy of binding. 61610 was the top ranked compound with highest possibility of binding to LANCL2. The efficacy of 61610 was tested by a series of *in vitro* and pre-clinical efficacy studies.

Homology Modeling

Since the experimental structure of LANCL2 is not available, we performed homology modeling to determine the structure of LANCL2 using the crystal structure of LANCL1 as a template. **Sequence Identity of LANCL1 and LANCL2 = 54% > 35% => Homologous**

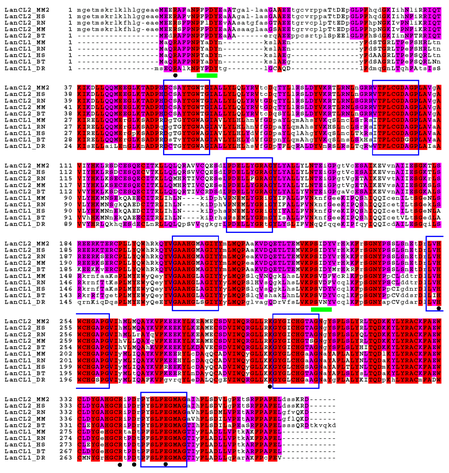

Figure 3. Multiple sequence alignment of selected LanC proteins. Completely conserved residues in the listed sequences are highlighted with a red background. Identical residues are highlighted with a magenta background. Different residues are shown in lower–case letters. Seven conserved GxxG motifs and corresponding loop bulges are outlined by blue boxes. Canonical SH3-binding motifs are underlined with green lines. Positions of GSH-binding residues in LANCL1 are denoted by black dots.

Structural-based Virtual Screening

The docking of compounds available in the NCI Diversity Set II (N=1,364 compounds) into the LANCL2 computational model was performed with AutoDock Vina. Compounds were ranked according to their estimated free energy of binding. 61610 is the top-ranking compound with the lowest free energy of binding (-11.1 kcal/mol).

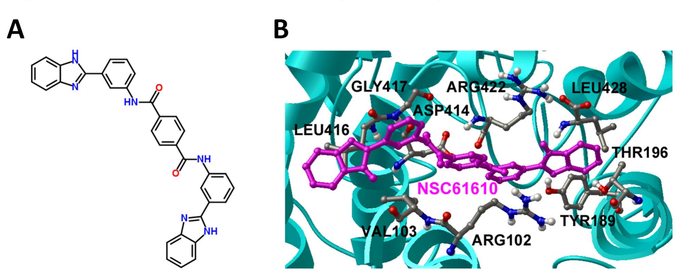

Figure 4. Lanthionine synthetase C-like protein 2 and 61610 *in silico*. (A) 2-D structure of 61610. (B) Representative binding mode of the most stable docked orientation of 61610 with LANCL2. The LANCL2 model is shown in ribbon mode. 61610 pose is colored in magenta. Amino acid residues surrounding 61610 are labeled and depicted by stick-and-ball models.

In vitro Test

In vitro studies are aimed to test the efficacy of 611610 on peroxisome proliferator activated receptor γ activation and to investigate the corresponding mechanism.

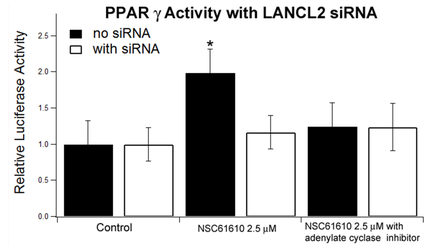

Figure 5. Effect of LANCL2 disruption and cAMP inhibition on peroxisome proliferator activated receptor γ (PPAR γ) activation in RAW 264.7 macrophages. Cells were cotransfected with a pTK.PPRE3x luciferase reporter plasmid driven by the PPRE-containing Acyl-CoA oxidase promoter with or without LANCL2 siRNA. Then, cells were treated with vehicle (DMSO) or 61610 (2.5 μM), the adenylate cyclase-specific inhibitor 2'5'-dideoxyadenosine (10 μM). Luciferase activity was normalized to pRL activity in the cell extracts and relative luciferase activity was calculated a ratio of the activity in the treatment wells to control wells. Data are represented as mean ± standard error. Points with an asterisk indicate that a treatment is significantly different from its control (*P<0.05*).

In vivo Test

In vivo studies were performed to test the anti-inflammatory efficacy of 61610 and to investigate the corresponding mechanism in experimental inflammatory bowel disease.

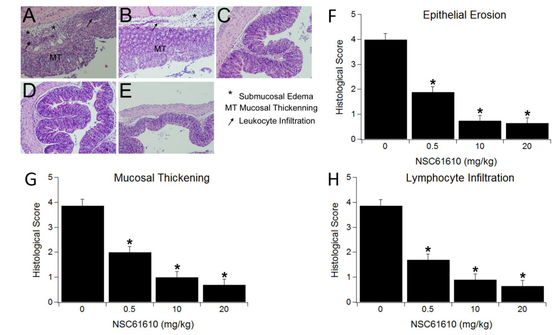

Figure 6. Oral treatment with 61610 ameliorates inflammatory lesions in mice with inflammatory bowel disease. Mice were challenged with 2.5% dextran sodium sulfate in the drinking water for 7 days. Representative photomicrographs from the control (A-B) and 61610 treatment (C-E) groups are illustrated. Colonic specimens underwent blinded histological examination and were scored based on epithelial erosion (F), mucosal wall thickening (G), and leukocyte infiltration (H). Data are represented as mean ± standard error (n=10). Bars with an asterisk indicate that a treatment is significantly different from its control (*P<0.05*).

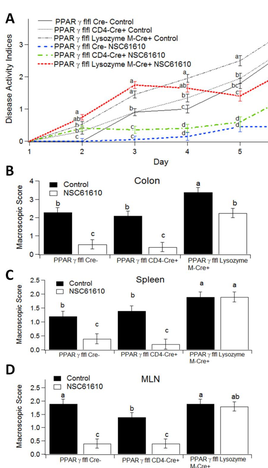

Figure 7. Anti-inflammatory efficacy of 61610 is dependent on PPAR γ expression in macrophages. Mice were challenged with 2.5% dextran sodium sulfate in the drinking water. Panel A illustrates the effect of oral 61610 treatment on disease severity. Panels B-D illustrate the effect of oral 61610 on macroscopic lesions in the colon (B), spleen (C), and mesenteric lymph nodes (MLN) (D). Data are represented as mean ± standard error (n=10). Bars with an asterisk indicate that a treatment is significantly different from its control (*P<0.05*). Wild-type mice: PPAR γ fl/fl, Cre-; macrophage-specific PPAR γ null mice: PPAR γ fl/fl, lysozyme M-Cre+; T cell-specific PPAR γ null mice: PPAR γ fl/fl; CD4-Cre+.

Conclusion

We developed an integrated drug discovery pipeline consisting of molecular modeling approaches followed by experimental validation and we proposed an alternative mechanism to activate PPAR γ. Our results confirm that LANCL2 is a novel therapeutic target for IBD and 61610 is a potential novel anti-inflammatory drug.

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