DISCOVERY OF SMALL MOLECULES FOR IL-17 MEDIATED INFLAMMATORY DISEASES

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Introduction

Inflammation is an adaptive response of the body to harmful stimuli. Chronic inflammation is the classic sign of autoimmune diseases and is associated with the malfunction of tissue. Such diseases have a great impact in the quality of life and the outcomes of patients, since they occur at early age. Although treatments have improved significantly with the introduction of biologics, novel small molecule approaches may enable further development of therapeutics[1].

Lately, interest has been sparked in interleukin-17 (IL-17), which binds to surface receptors with dimer structure to activate inflammation, as a novel pharmacological target for inflammatory autoimmune diseases[2]. Therapeutic antibodies selectively targeting IL-17A have been approved for clinical use in psoriasis, a condition characterized by abnormal accumulation of skin cells (psoriatic plaques) due to overactivation of the immune system[3,4]. Recently, a class of macrocyclic compounds was discovered, indicating that the IL-17 druggability with molecules might be feasible[5].

Objective

We aim to identify chemical tools to study the IL-17 mediated inflammatory mechanisms in psoriasis. Our specific objective is to develop a experimental strategy to identify molecules that act as pharmacological modulators.

Methods

We produced human recombinant IL-17A in mammalian cells. IL-17A was cloned into pcDNA3.1(+) vector containing a C-terminal His_6 tag. The plasmid was transfected in HEK 293 cells and supernatants, where IL-17A is secreted, were collected after 48 hours. Subsequently, protein was purified by affinity chromatography on Ni-Sepharose Fast Flow column with ÄKTA™ start chromatography system. We confirmed IL-17A expression by means of western blotting.

In order to identify novel ligands for IL-17A, we used DMR (Dynamic Mass Redistribution) technology. We tested the protein immobilization at different concentrations and pH in 384 well microplates. EnSpire-LFB high sensitivity plates were activated with 400 mM EDC + 100 mM Sulfo-NHS buffer 30 min at room temperature. Immobilization was performed overnight at 4°C and baseline was read the next day after equilibration inside the EnSpire® Multimode Plate Reader.

In silico selection of candidate binders from our chemical library was performed for the 9 crystalline structures of IL-17A available in Protein Data Bank. Docking was performed with Autodock Vina software.

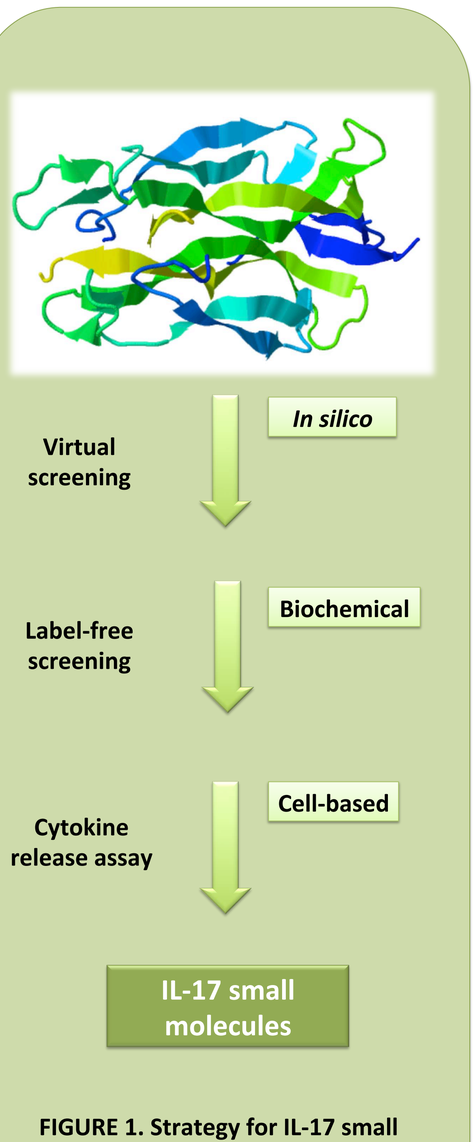

FIGURE 1. Strategy for IL-17 small molecule discovery.

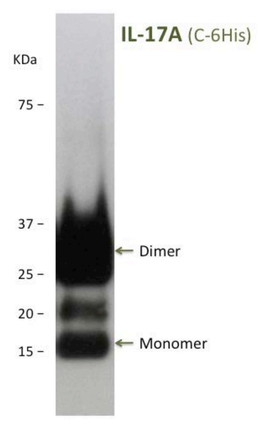

FIGURE 2. IL-17A can be produced in mammalian cells.
Purified supernantant of HEK 293 transfected cells. IL-17A is identified by means of western blot with an anti-IL17A rabbit antibody (Sigma-Aldrich HPA052258). Both dimer and monomer states are detected. Monomer expected mass is 17.5 kDa.

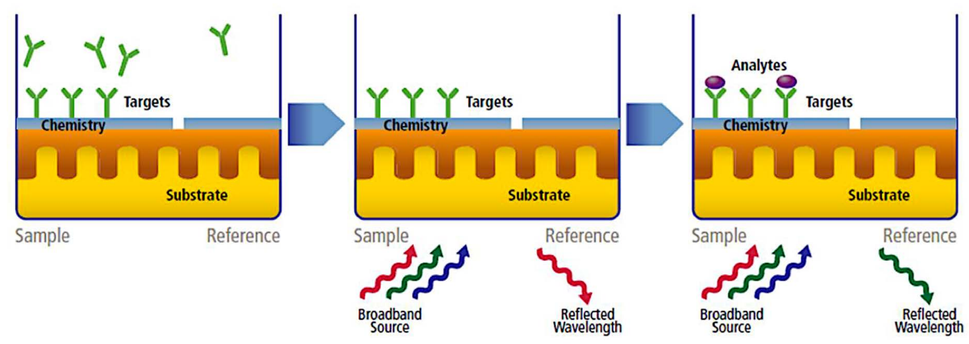

FIGURE 3. Undraggable proteins such as IL-17 can be tagged by using label-free technologies. DMR principle.
First, target is immobilized on the microplate amine-coupling surface. After an overnight incubation and wash of the microplate, baseline is read. Finally, addition of compounds and final reading are performed.

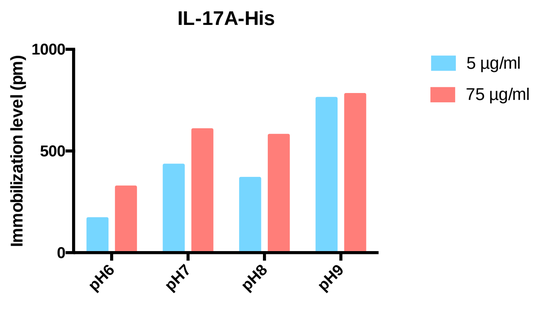

FIGURE 4. IL-17A is a soluble protein difficult to target by label-free binding assays.
IL-17A was immobilized by amino coupling at different concentrations and pH. Immobilization levels were suboptimal, indicating that it is not a reliable method for the screening of potential binders, probably due to the physicochemical characteristics of the target.

Ligand	Target	AE
CBG007144	4HSA	-11.9
	5HI5	-12.3
	5HI3	-11.7
CBG012320	4HSA	-11.3
	5HI5	-13
	5HI3	-12.4
	4HR9	-10.5
CBG013062	5HI3	-11.9
	4HSA	-11.6
CBG013140	5HI5	-12.7
	5HI3	-11.9
CBG013151	5HI3	-12.1
	5HI5	-12.4
CBG019512	5HI5	-11.8
	5HI3	-11.9
CBG019847	5HI3	-11.9
	4HR9	-10.3
CBG019904	5HI3	-12
	5HI5	-11.8
CBG019927	4HSA	-11.3
	5HI5	-11.8

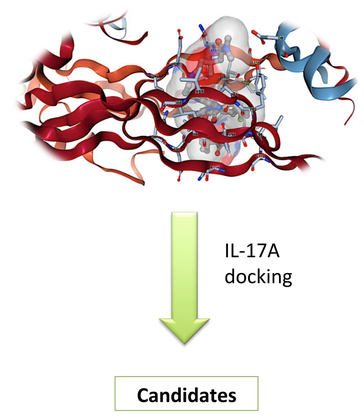

FIGURE 5. *In silico* selection of IL-17A candidate binders.
Virtual screening resulted in the identification of candidates that could bind IL-17A in its binding pocket. Top 10 lower affinity energies against at least two targets are shown.

Conclusion

We developed a strategy to identify novel IL-17A ligands. A protocol for the production of human recombinant IL-17A in mammalian cells was set up. We identified small molecules that bind IL-17A by means of a virtual screening. We tested a biochemical label-free assay (DMR technology) for IL-17A, although it was below the ideal standards. Further technological improvements of the label-free assay may provide opportunities for the discovery of novel IL-17A ligands.

References

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Acknowledgements

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