News Release

Title

Systematic characterization of seed overlap microRNA cotargeting associated with lupus pathogenesis

Key Points

- We report pathogenic impacts of combinatorial miRNA regulation on inflammation in systemic lupus erythematosus (SLE).
- miRNA expression profiling of SLE mice model revealed the downregulation of two miRNAs, miR-128 and miR-148a, in plasmacytoid dendritic cells (pDCs). miR-128 and miR-148a target KLF4 via extensively overlapped target sites ("seed overlap" miRNA cotargeting) and negatively regulate inflammatory cytokine production.
- "Seed overlap" miRNA cotargeting increases susceptibility to multiple miRNAs, while "neighborhood" miRNA cotargeting cooperatively enhances target repression.
- The integrative bioinformatics analysis uncovered two major classes of highly conserved sites of broadly conserved miRNAs in mammals and their unique relationships with RNA repression, miRNA cotargeting, and haplo-insufficiency of target genes.

Summary

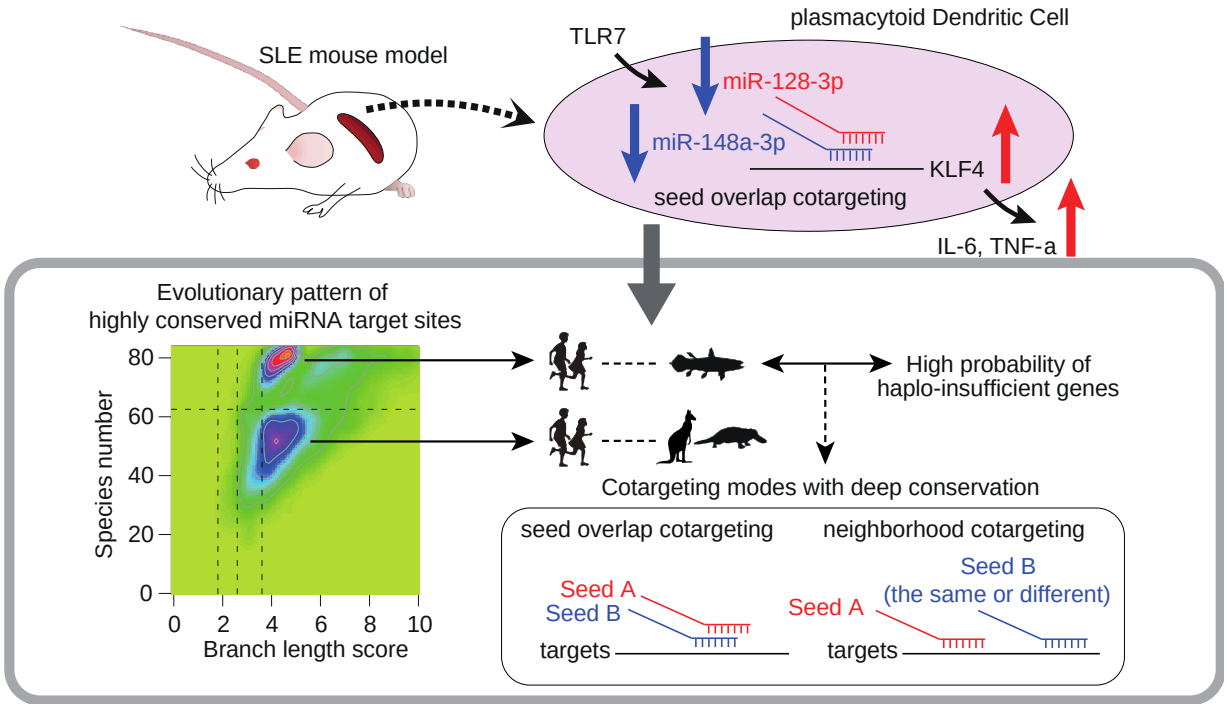
According to research published online on November 11, 2022, in BMC Biology, a group of researchers, headed by Prof. Hiroshi I. Suzuki, Department of Molecular Oncology, and Lecturer Noritoshi Kato and Researcher Hiroki Kitai, Department of Nephrology, Nagoya University Graduate School of Medicine, have reported systematic characterization of seed overlap microRNA cotargeting associated with lupus pathogenesis.

MicroRNAs (miRNAs) are small non-coding RNAs approximately 22 nucleotides (nt) in length that bind to the 3′ untranslated regions (3′ UTRs) of target mRNAs and repress them. Since target repression via single sites is typically modest, combinatorial gene regulation by multiple microRNAs is widespread, and closely spaced target sites often act cooperatively to achieve stronger repression ("neighborhood" miRNA cotargeting).

In this study, the research group revealed that a unique mode of combinatorial miRNA-mediated target regulation, called "seed overlap" miRNA cotargeting, has important roles in the pathogenesis of systemic lupus erythematosus (SLE). miRNA expression profiling of SLE mice model revealed the downregulation of two miRNAs, miR-128 and miR-148a, in plasmacytoid dendritic cells (pDCs). miR-128 and miR-148a target KLF4 via extensively overlapped target sites ("seed overlap" miRNA cotargeting) and negatively regulate inflammatory cytokine production.

Further, the researchers conducted the integrative bioinformatics analysis to analyze various features of "seed overlap" miRNA cotargeting. Systematic characterization revealed that extensive "seed overlap" is a prevalent feature among broadly conserved miRNAs. Highly conserved target sites of broadly conserved miRNAs are largely divided into two classes—those conserved among eutherian mammals and from human to Coelacanth, and the latter, including KLF4-cotargeting sites, has a stronger association with both "seed overlap" and "neighborhood" miRNA cotargeting. Furthermore, a deeply conserved miRNA target class has a higher probability of haplo-insufficient genes.

This study collectively suggests the complexity of distinct modes of miRNA cotargeting and importance of their perturbations in human diseases.



Research Background

MicroRNAs (miRNAs) are small non-coding RNAs approximately 22 nucleotides (nt) in length that bind to the 3′ untranslated regions (3′ UTRs) of target mRNAs and repress them (Fig. 1, left). Since target repression via single sites is typically modest, combinatorial gene regulation by multiple microRNAs is widespread, and closely spaced target sites often act cooperatively to achieve stronger repression (Fig. 1, right, "neighborhood" miRNA cotargeting). While miRNA cotarget sites are suggested to be more conserved and implicated in developmental control, the pathological significance of miRNA cotargeting remains elusive.

Systemic lupus erythematosus (SLE) is an autoimmune disease, in which the deposition of immune complexes (IC) occurs in multiple organs due to the production of autoantibodies. Recently, it has been shown that plasmacytoid dendritic cells (pDCs) have critical roles in SLE by secreting type I IFN, IL-6, and TNF-α. Although alteration of multiple miRNAs in various immune cells has been implicated in SLE, alterations of miRNAs in pDCs have not been explored in depth.

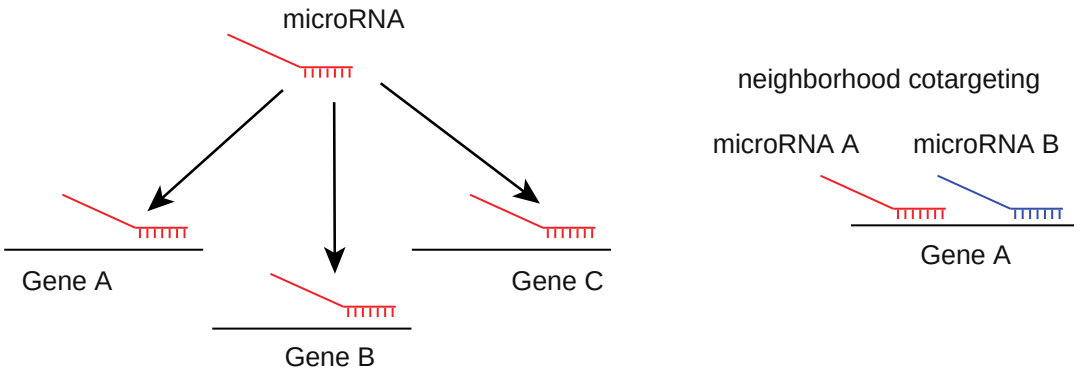


Fig. 1. Gene regulation by miRNAs and "neighborhood" miRNA cotargeting.

Research Results

(1) Downregulation of two miRNAs, miR-128 and miR-148a, in pDCs of SLE mouse model.

This study first investigated the genome-wide miRNA expression profiling in the SLE mouse model induced by TLR7 agonist imiquimod (IMQ). The analysis identified the downregulation of two miRNAs, miR-128 and miR-148a, in pDCs (Fig 2, left). Functional analyses demonstrated that miR-128 and miR-148a additively target KLF4 via extensively overlapping target sites ("seed overlap" miRNA cotargeting) and suppress the inflammatory responses (Fig 2, right).

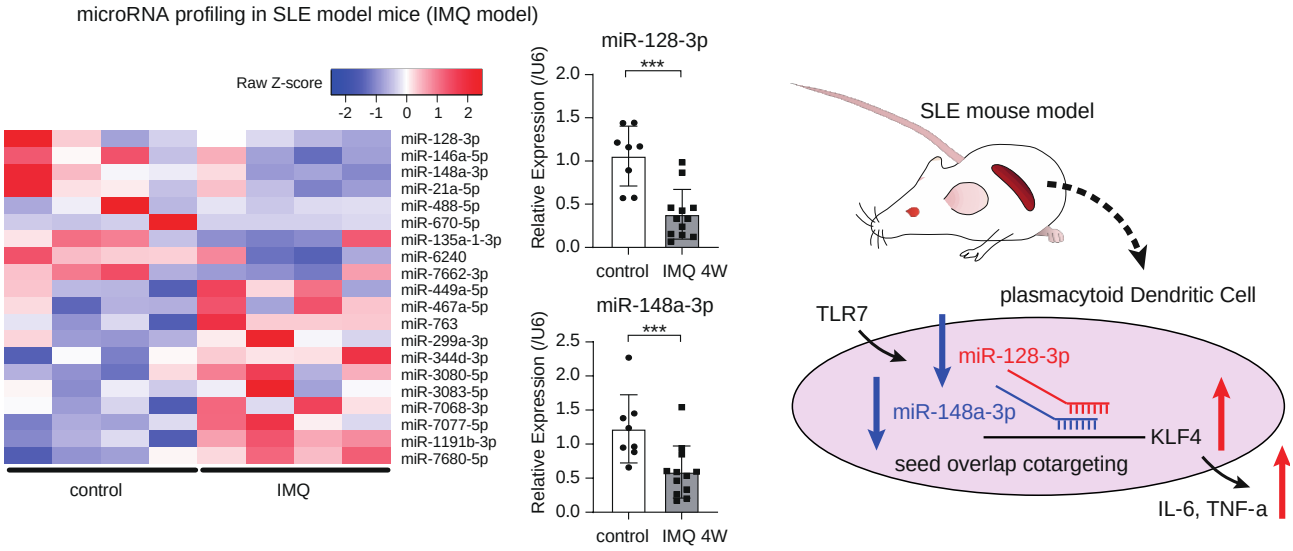


Fig. 2. Downregulation of two miRNAs, miR-128 and miR-148a, in pDCs of SLE mouse model.

(2) Systematic characterization of "seed overlap" miRNA cotargeting.

In contrast to closely spaced two target sites, "seed overlap" cotarget sites cannot be bound by two miRNAs simultaneously. Consistent with this, RNA-seq analysis, which investigated

transcriptome-wide responses of "seed overlap" cotarget sites, have demonstrated that "conserved overlap" sites increase susceptibility to overall downregulation by two distinct miRNAs through additive recruitment of two miRNAs to these sites.

During these analyses, the researchers found that the "conserved overlap site of KLF4 3′ UTR is deeply conserved across most species between human and *Coelacanth* (Fig. 3). Based on these observations, the researchers expanded these findings by integratively analyzing the seed overlap patterns of all miRNAs and conservation patterns of "seed overlap" target sites (Fig. 4).

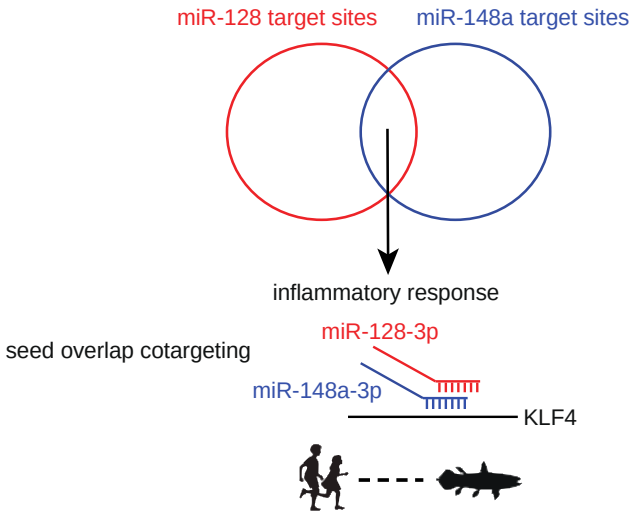


Fig. 3. "Seed overlap" miRNA cotargeting of KLF4 3'UTR by two miRNAs, miR-128 and miR-148a.

Systematic characterization revealed that extensive "seed overlap" is a prevalent feature among broadly conserved miRNAs. Intriguingly, highly conserved target sites of broadly conserved miRNAs are largely divided into two classes—those conserved among eutherian mammals and from human to *Coelacanth*, and the latter, including KLF4-cotargeting sites, has a stronger association with both "seed overlap" and "neighborhood" miRNA cotargeting (Fig. 4). Furthermore, a deeply conserved miRNA target class has a higher probability of haplo-insufficient genes.

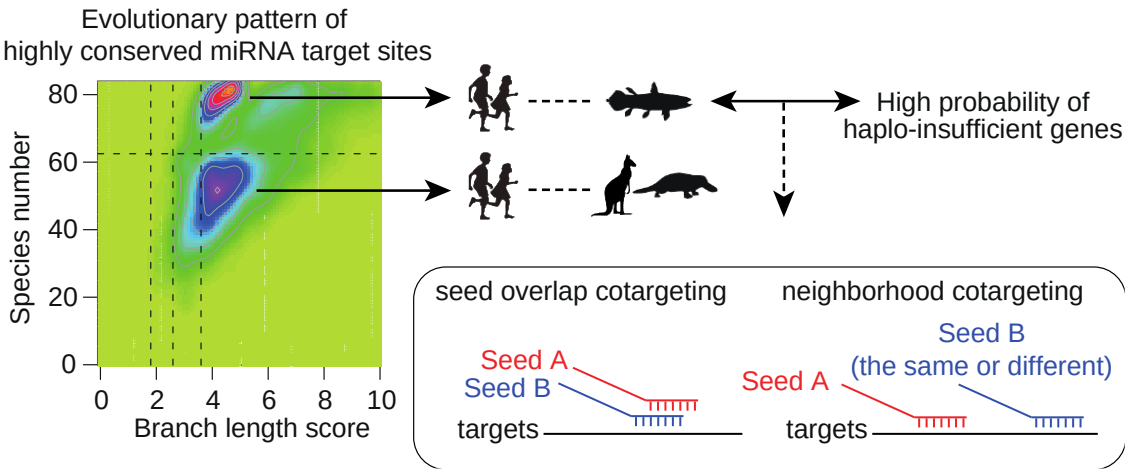


Fig. 4. Evolutionary features of miRNA target sites and the relationships with "neighborhood" and "seed overlap" miRNA cotargeting.

Research Summary and Future Perspective

In this study, the research group report pathogenic impacts of "seed overlap" miRNA cotargeting in SLE. Integrative analyses further demonstrated that "seed overlap" miRNA cotargeting is a prevalent feature of both deeply conserved miRNAs and their target sites, and importantly uncovered two major conservation classes of target sites, those conserved among eutherian mammals and between human and *Coelacanth*. The latter has a stronger association with both "seed overlap" and "neighborhood" miRNA cotargeting and implicates higher dosage sensitivity. These findings highlight importance of perturbed miRNA cotargeting in human pathology and unique evolutionary aspects of miRNA cotargeting and miRNA target site conservation.

Publication

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