GENETICS AND GENODERMATOSES

BLAU PATIENT-DERIVED IPS CELLS REVEAL GAIN-OF-FUNCTION MUTATION OF NOD2 SELECTIVELY IMPAIRS ITS LIGAND SPECIFIC IMMUNE RESPONSES

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Introduction: NOD2 is crucial for innate immune response and mainly expressed in hematopoietic lineage cells, especially in monocytic cells. On the activation by it ligands, muramyl dipeptide (MDP), NOD2 leads to activation of NF-κB pathway, causing upregulation of pro-inflammatory cytokines. Mutations of NOD2 have been associated with Blau syndrome, but details regarding the mutant NOD2 leads to auto-inflammation and granuloma formation are still unclear.

Objective: To clarify the mechanisms associated mutant NOD2 leads to auto-inflammation and granuloma formation in Blau syndrome, we investigated the relation between mutation of NOD2 and the immune response in iPS cell model.

Methods: The iPS cells with R334W mutation in NOD2 have been established from a Blau patient and we corrected the mutation of the iPS cells into wild type (WT) by using a CRISPR-Cas9 system. These isogenic iPS cells were differentiated into monocytic cell lineages, then transfected with the lentiviral vectors and maintained in StemPro-34 medium with M-CSF and GM-CSF. With these iPS cell groups, we investigate the expression of NOD2, NF-κB activation and secretions of pro-inflammatory cytokines.

Results: Both in iPS-derived monocytes with wildtype and those with R334W, IFNγ equally induced the upregulation of NOD2. According with NOD2 expression, even without MDP simulation, cytokine production was found only in those with mutant NOD2, suggesting that Blau-associated NOD2 is gain-of-function mutation. Interestingly, after stimulation with MDP, the cells with R334W secreted less amounts of cytokines than wildtype cells regardless of NOD2 expression levels by IFNγ treatment. On the other hand, both the cell groups showed the comparative immune response to TNFα and LPS treatment, unrelated to NOD2 mutation.

Conclusion: These results suggest that Blau-associated mutant NOD2 may selectively impair MDP-NOD2 dependent pro-inflammatory signaling that do not share with TNF and

TLR4 in NF-κB pathway.