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Proteasome disability syndrome: an analysis of the pathogenesis of a new autoinflammatory syndrome, Nakajo-Nishimura syndrome

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Disclosure; Nothing to Disclose

A very rare inflammatory and wasting disease (Nakajo in 1939)
- Autosomal recessive inheritance
- High fever
- Skin eruptions
- Myositis
- Long-clubbed fingers
- Partial lipodystrophy
- Calcification of basal ganglia

Nakajo-Nishimura syndrome (MIM 256040)

Identification of the responsible gene
SNP microarray-based homozygosity mapping

Whole genome search
One candidate region (1.1Mbp)
Chromosome locus: 6p21.31-32
Only one mutation in a coding region with amino acid change

PSMB8 (602G>T)

β5i subunit of proteasome (G201V)

Proteasome

The 26S proteasome complex is a highly conserved protein degradation machine.
The 20S catalytic core is composed of 7-membered rings of α and β subunits. Each of different subunits occupies defined position.

α ring (α1-7) β ring (β1-7) γ ring (γ1-7) δ ring (δ1-7)

The 26S proteasome is involved in a diverse array of biological processes, including cell-cycle progression, DNA repair, apoptosis, immune response, signal transduction, transcription, metabolism, protein quality control and developmental programs.

Structural modeling of mutated protein

The mutant residue was close to the catalytic threonine residue.

Conformational changes affected the surface contact of β5i with the adjacent β4 subunit.

This suggested that mutated protein interfere the proteasome assembly.

Immunoproteasome

- The three proteolytic active β-subunits of the standard proteasome can be replaced in the immunoproteasome by IFN-γ, TNF-α, or LPS.
- The inducible proteasomal subunits are homologs of the catalytic active subunits β1, β2, and β5, which are replaced by β1i, β2i, and β5i.
- The immunoproteasome has increased chymotrypsin-like and trypsin-like activities, which are favorable for the production of antigenic peptides that bind to the groove of MHC class I molecules.

Mutated protein
Non-mutated protein

The mutant residue was close to the catalytic threonine residue.

Conformational changes affected the surface contact of β5i with the adjacent β4 subunit.

This suggested that mutated protein interfere the proteasome assembly.
Assembly of the 20S immunoproteasome is severely impaired in the NNS patients

Immunoblot analysis
Extracts from lymphoblastoid cell lines derived from patients after the glycerol gradient centrifugation

Accumulation of the immature 20S immunoproteasome before dimerization (presence of proforms of β1i, β2i, and hUmp1, and insufficient forms of β5i)

Assembly of the 20S immunoproteasome is severely impaired in the NNS patients

Influence of the β5i mutation on the proteasome activity
(Extracts from each fraction were assayed for three different peptidase activities.)

Chymotrypsin-like (β5i) Trypsin-like (β1i) Caspase-like (β2i)

40.3% (Het) 7.6% (NNS)
62.2% (Het) 36.3% (NNS)
56.9% (Het) 47.0% (NNS)

Decrease in the catalytic activity of β5i
Impaired assembly of the 20S immunoproteasome

Profile of cytokine and chemokine levels in serum and supernatant of cultured fibroblasts

Sera (multiplex bead-based ELISA) Supernatant (cultured fibroblast)

IL-6 (pg/ml)
HC        RA        NNS
IP-10

* p< 0.05, ** p< 0.01, *** p< 0.001

Accumulation of ubiquitinated proteins

Lymphoblastoid cell line Skin biopsy

CD68 Ubiquitin Merge

NNS WB Anti-Ub Fasciitis

The ubiquitin signals were strongly detected in the lymphoblastoid cell line and in the infiltrated CD68 positive cells in the NNS patient.

NF-κB activity in fibroblasts from a NNS patient

EMSA

No differences in the amount of the p65/p50 heterodimer were observed in nuclear extracts from NNS fibroblasts and healthy control fibroblasts.

Increased levels of p-p38 in the nuclear extracts from fibroblast and PBL derived from NNS patients
We detected a mutation in the PSMB8 gene, which encodes the LMP7 protein or the β5i subunit of the immunoproteasome (IP) in patients with Nakajo-Nishimura syndrome (NNS). The results of the amino acid substitution (G201V) disturbed the maturation of the IP, and abolished the all three peptidase activity of proteasome, and finally resulted in the accumulation of ubiquitinated proteins in cells derived from the NNS patients. Nuclear phosphorylated p38 and the secretion of IL-6 are increased in patient cells both in vitro and in vivo. This is the case where proteasome disability caused definitive phenotypes in mammals. Our results show that the ubiquitin-proteasome system plays an important regulatory role in the inflammation process.

Summary

1) We detected a mutation in the PSMB8 gene, which encodes the LMP7 protein or the β5i subunit of the immunoproteasome (IP) in patients with Nakajo-Nishimura syndrome (NNS).

2) The results of the amino acid substitution (G201V) disturbed the maturation of the IP, and abolished the all three peptidase activity of proteasome, and finally resulted in the accumulation of ubiquitinated proteins in cells derived from the NNS patients.

3) Nuclear phosphorylated p38 and the secretion of IL-6 are increased in patient cells both in vitro and in vivo.

4) This is the case where proteasome disability caused definitive phenotypes in mammals.

5) Our results show that the ubiquitin-proteasome system plays an important regulatory role in the inflammation process.