Karolinska Institutet

Department of Medicine

The TRIM21/Ro52 E3 ligase and its antibodies in autoimmune disease

PhD dissertation

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ABSTRACT

Patients with the systemic autoimmune diseases systemic lupus erythematosus (SLE) and Sjögren's syndrome (SS) often have autoantibodies against Ro/SSA (composed of the TRIM21/Ro52 and Ro60 antigens) and La/SSB. The biological function of the TRIM21/Ro52 protein itself has remained unknown until its recent description as a tripartite-motif (TRIM) family member and an E3 ligase involved in ubiquitination. Congenital heart block (CHB) is a passively acquired autoimmune condition that may develop in fetuses of anti-Ro/La positive women following transfer of maternal autoantibodies across the placenta and disruption of the fetal atrioventricular (AV) conduction system. Although anti-Ro, and especially anti-TRIM21/Ro52 antibodies, have been associated with development of CHB, the risk for CHB in an anti-Ro positive pregnancy is only 1-2%. In addition, a recurrence rate of 12-20% despite persistence of maternal antibodies indicates that additional factors are required for the establishment of heart block.

The aims of this thesis were 1) to contribute to the elucidation of the biological function of the TRIM21/Ro52 protein, especially regarding its role in autoimmunity, and 2) to characterize the involvement of anti-TRIM21/Ro52 antibodies in CHB pathogenesis and identify risk factors other than maternal autoantibodies for the development of heart block.

Using in vitro and in vivo studies, TRIM21/Ro52 was shown to be an IFN-inducible protein expressed in immune cells, mainly localized in the cell cytoplasm but able to translocate into the nucleus upon inflammatory stimuli such as IFNα. Ubiquitination assays demonstrated that TRIM21/Ro52 is a RING-dependent E3 ligase that can interact with different E2s both in the cytoplasm and in the nucleus, and that its E3 enzymatic activity is inhibited by anti-RING antibodies present in serum of patients with SLE or SS. Importantly, TRIM21/Ro52 was shown to ubiquitinate several interferon regulatory factors (IRFs), which are transcription factors activated downstream of TLR/IFN signaling, and disruption of the *Trim21* locus in vivo led to increased production of pro-inflammatory cytokines and development of systemic autoimmunity following tissue injury/infection.

Anti-TRIM21/Ro52 antibodies recognizing the p200 part (amino-acids 200-239) of the protein were specifically implicated in the pathogenesis of CHB by demonstrating that they induced AV block in rodents following transfer during gestation, while antibodies targeting other domains of TRIM21/Ro52 did not. Using a rat immunization model of heart block, maternal MHC genes were shown to regulate the generation of pathogenic anti-TRIM21/Ro52 antibodies, while a different MHC haplotype was linked to susceptibility to disease in the offspring. In addition, maternal age and seasonal timing of pregnancy were identified as risk factors for the development of CHB in anti-Ro/La antibody positive pregnancies in a Swedish cohort of families with individuals affected with CHB.

In summary, the TRIM21/Ro52 autoantigen is a negative regulator of IFN/TLR responses via ubiquitination of several IRFs and as such may play an important role in the pathogenesis of SLE and SS. Anti-TRIM21/Ro52 p200 antibodies can initiate development of fetal heart block and factors such as fetal genetic susceptibility, maternal age and seasonal timing of pregnancy may promote the establishment of CHB.

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