Role and Mechanism of RNASET2 in the Pathogenesis of Vitiligo

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Vitiligo is an acquired pigmentary disorder characterized by the development of depigmented lesions in a variable distribution, owing to the loss or destruction of functioning melanocytes (Guerra *et al.*, 2010). Complex interactions between genetic, immunological, biochemical, and environmental factors are likely to be related to the development of vitiligo (Malhotra and Dytoc, 2013). Despite having identified multiple risk factors of vitiligo, the molecular mechanisms of its pathogenesis remain obscure.

In a recent genome-wide association study for generalized vitiligo in the Chinese Han population, the gene encoding ribonuclease (RNASE) T2 at 6q27 was identified as a disease risk factor (Quan *et al.*, 2010); however, the functional roles of RNASET2 in vitiligo pathogenesis have yet to be determined. RNASET2 is the only human member of the Rh/T2/S family of acidic hydrolases (Campomenosi *et al.*, 2006). The ribonucleases are ubiquitous, conserved enzymes with a broad spectrum of normal physiological functions. Studies concerning RNASET2 first focused on its antitumorigenic characteristics. A decreased expression of RNASET2 was found in different types of tumor tissues (e.g., ovarian cancer, melanoma cancer, lymphoma, and so on), and both *in vitro* and *in vivo* studies showed that RNASET2 could inhibit the proliferation of several tumor cells, indicating the possible role of RNASET2 as a tumor suppressor gene (Acquati *et al.*, 2001; Monti *et al.*, 2008; Acquati *et al.*, 2013). Notably, the hyperexpression of RNASET2 at the tumor sites was accompanied by abundant infiltration of monocyte/macrophages. In mice models pretreated with the macrophage-depleting agent clodronate, the tumor-suppressing activity of RNASET2 turned out to be largely impaired (Acquati *et al.*, 2011; Acquati *et al.*, 2013), yet inactivating its RNase activity did not affect its tumor-suppressive properties (Smirnoff *et al.*, 2006). Taken together, we propose that RNASET2 may exert its antitumor effects by promoting immune response against tumors. On the other hand, RNASET2 has also recently been shown to take part in the stress response. RNASET2 is typically secreted from the cell or localized to internal compartments under normal conditions (Thompson and Parker, 2009). However, in the presence of oxidative stress, RNASET2 proteins would enter the cytoplasm. For example, the RNASET2 ortholog in *Saccharomyces cerevisiae*, Rny1, was released from the vacuole into the cytosol during oxidative stress and promoted cell death during oxidative stress independently of its catalytic activity (Thompson and Parker, 2009).

Although the precise etiology of vitiligo remains uncertain, most researchers agree that autoimmune and oxidative stress greatly contribute to precipitating the disease (Laddha *et al.*, 2013). Vitiligo lesions are characterized by an infiltration of $CD4^+$ helper T cells and $CD8^+$ cytotoxic T lymphocytes (Steitz *et al.*, 2005; van den Boorn *et al.*, 2009). Anti-melanocyte antibodies are detected in the sera of vitiligo patients (Kemp *et al.*, 2007; Kemp *et al.*, 2011; Sandoval-Cruz *et al.*, 2011). Certain known stressors including ultraviolet (UV) irradiation, mechanical injuries, and inflammation can cause depigmentation in previously normal skin or invoke a Koebner phenomenon in about half of the patients (van Geel *et al.*, 2012). Therefore, it is of great interest to identify the molecular connection between oxidative stress and autoimmune response. As RNASET2 has the dual functions in both autoimmune and oxidative stress responses, we asked how to define the involvement of RNASET2 in the pathogenesis of vitiligo? According to the ''danger theory'' by Matzinger (2002), when a cell is stressed, even in the absence of any foreign substance, it emits molecules as ''alarm signals'' to activate immune cells and initiate an immune response. This theory establishes a synergistic interaction of oxidative stress with immune response and provides new insights. A lot of those ''alarm signals'' have been determined, such as heat-shock proteins (Vabulas *et al.*, 2002) and high-mobility group box 1 (Park *et al.*, 2006). To investigate the pathogenesis of vitiligo, we hypothesize that RNASET2 might act as an ''alarm signal'' in the development of vitiligo. The risk factors of vitiligo, such as peroxides, UV exposure, injuries, or inflammation, will cause damage to the melanocytes or keratinocytes. And those cells under stress conditions will release RNASET2 as ''alarm signals''

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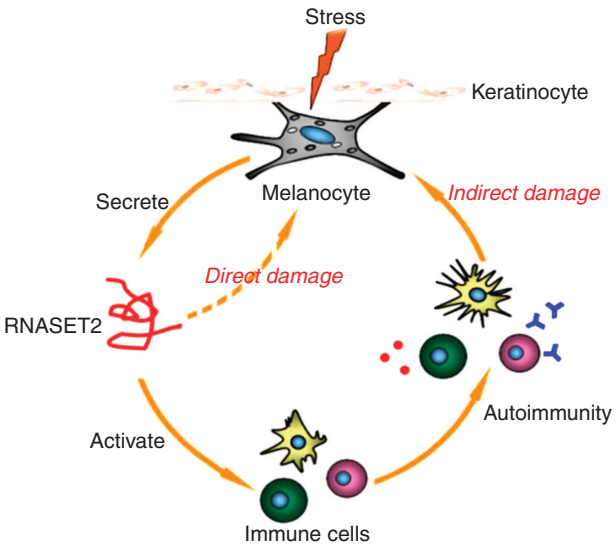

Figure 1. "RNASET2 alarm signal" hypothesis in vitiligo.

into the immediate environment to alarm the immune system. On one hand, RNASET2 might directly inhibit the growth of melanocytes. On the other hand, RNASET2 might act as an endogenous ligand to activate antigen-presenting cells, for example, dendritic cells, thus initiating an immune response against melanocytes (Figure 1).

In order to test our hypothesis, we investigated the expression of RNASET2 in vitiligo lesions as well as its molecular functions involving apoptosis-related signaling proteins and pathways. We found an overexpression of RNASET2 in epidermis of vitiligo patients compared with the healthy controls. *In vitro* analyses indicated that the overexpression of RNASET2 was inducible in cultured primary human melanocytes and keratinocytes by stress conditions such as UV irradiation, hydrogen peroxide, and inflammatory factors. *In silico* analysis of the RNASET2 amino-acid sequence identified a putative binding motif for the tumor necrosis factor receptor-associated factor 2 (TRAF2), which has recently been identified as a threshold determinant factor of apoptosis through its ubiquitin ligase activities acting upon caspase-8. This finding becomes particularly intriguing when taking into account that biopsies of vitiligo lesions have revealed that apoptosis, rather than cell death, was the main cause for the melanocyte loss. Therefore, we further analyzed the interaction of TRAF2 and RNASET2. We demonstrated that RNASET2 indeed led to enhanced cell apoptosis via the TRAF2 caspases pathway through the physical interaction of RNASET2 and TRAF2. Thus, RNASET2 may contribute to the development of vitiligo by inhibiting TRAF2 expression and lead directly to apoptosis of melanocytes (Wang *et al.*, 2014).

Apart from that, autoimmunity is another key factor involving in the pathogenesis of vitiligo. Although as yet there is no study about the immunological functions of RNASET2, it was recently shown that omega-1, an RNaseT2 family member secreted from the eggs of *Schistosoma mansoni*, could induce Th2 polarization by priming dendritic cells (Everts *et al.*, 2012). It will be our goal to determine whether or not human RNASET2 has similar effects and is therefore involved in the pathogenesis of vitiligo. Future studies concerning the immunological functions of RNASET2 and its role linking oxidative stress and immune response may allow the development of novel therapies for vitiligo.

CONFLICT OF INTEREST

The authors state no conflict of interest.

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Disclaimer

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