Clinical Implications

- T<sub>FH</sub> cells drive GC formation and have been implicated in maintenance of homeostatic conditions, have a protective role in cancer, and are central players in immune-based pathologies such as autoimmune diseases and allergies.
- The efficacy of vaccines is largely dependent on the development of a potent antibody response. T<sub>FH</sub> cells are obligatory for the generation of long-lived plasma cells in germinal centers and hence antibody response.
- Understanding the molecular mechanisms involved in generating and maintaining T<sub>FH</sub> cells would potentiate novel therapeutic regimens to either subtype or enhance the number of T<sub>FH</sub> cells as necessary.

understand whether initial priming with DCs modifies the epigenome making it accessible or inaccessible to further modifications. A recent study suggested that encapsulation of antigen into the core of the polymersomes in the nanoparticle, rather than being immobilized on the surface, may better augment CD4<sup>+</sup> T-cell activation expanding the frequency of T<sub>FH</sub> cells (Rincon-Restrepo et al., 2017). Therefore, for clinical translation, it may also be useful to understand how different nanoparticles differentially activate and mobilize epidermal and dermal DCs, contributing to humoral immunity.

CONFLICT OF INTEREST
The authors state no conflict of interest.

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REFERENCES
Crotty S. T follicular helper cell differentiation, function, and roles in disease. Immunity 2014;41:529–42.

Itoho AA, McSorley SJ, Reinhardt RL, Eft MD, Igulli E, Rudensky AY, et al. Distinct dendritic cell populations sequentially present antigen to CD4<sup>+</sup> T cells and stimulate different aspects of cellular biodistribution to control CD4<sup>+</sup> vs CD8<sup>+</sup> T cell responses. Biomaterials 2017;132:48–58.

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Metabo-miR: miR-211 Regulates Mitochondrial Energy Metabolism in Vitiligo

Vladimir S. Spiegelman<sup>1</sup> and Irina A. Elcheva<sup>1</sup>

The study by Sahoo et al. established miR-211 as a critical regulator of cellular metabolism in vitiligo cells. miR-211, which is expressed from the transient receptor potential melastatin 1 intronic region, regulates oxidative phosphorylation and mitochondrial energy metabolism in vitiligo. Loss of miR-211 in melanocytes was shown to alter expression patterns of newly identified target genes, and those that regulate respiratory functions in melanocytes are among them. This study highlights the importance of miR-211 for the melanocyte biology and development of vitiligo.


<sup>1</sup>Division of Pediatric Hematology/Oncology, Department of Pediatrics, Pennsylvania State University, College of Medicine, Hershey, Pennsylvania, USA

Correspondence: Vladimir S. Spiegelman, Pennsylvania State University, College of Medicine, Milton S. Hershey Medical Center, Department of Pediatrics, Division of Pediatric Hematology/Oncology, PO Box 850, MC H085, 7830E, 500 University Drive, Hershey, Pennsylvania 17033-0850, USA; E-mail: vspiegelman@pennstatehealth.psu.edu

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A reflection in the mirror is one of the first things that most of us see every morning starting a new day. Regardless of whether we like or dislike our own looks, a certain part of our physical appearance is genetically determined, and develops and changes as we age. Experiencing an adverse body transformation that we cannot control may lead to emotional distress or depression. Vitiligo is a noncontiguous skin disorder characterized by a loss of pigment producing skin cells, melanocytes, causing progressive skin depigmentation (Le Poole et al., 1993). In cases of nonsegmental or generalized vitiligo, patches of lighter skin usually appear symmetrically on both sides of the body. Segmental vitiligo is much more rare and appears on one side of the body. People of all races and ages can develop vitiligo, though for those whose family members have had vitiligo the risk is higher (Taieb and Picardo, 2009). What at first appears as a cosmetic issue associated with skin discoloration is in fact a complex disease, the molecular roots of which are still poorly understood. Genetic, immunologic, and environmental factors contribute to the development of vitiligo with a predominant role attributed to the immunologic component (Schallreuter et al., 2008).

**miR-211 is a key regulator of cellular metabolism: loss of miR-211 negatively affects oxidative phosphorylation and mitochondrial energy metabolism in vitiligo cells.**

Other critical factors in melanocyte degeneration are increased sensitivity to oxidative stress due to impaired cellular antioxidant and other metabolic abnormalities (Maresca et al., 1997). Elevated levels of reactive oxygen species (ROS) such as peroxynitrite and hydrogen peroxide coincide with the low levels of antioxidant and other metabolic enzymes such as catalase, glutathione peroxidase, glucose-6-phosphate dehydrogenase, and superoxide dismutase that are detected in patients with vitiligo. Structural defects in mitochondria and impaired mitochondrial activity were detected in the epidermis of vitiligo skin biopsies as well, leading to increased levels of ROS. ROS accumulation causes DNA damage, and cellular toxicity along with increasing the production of proinflammatory and antimelanogenic cytokines, stimulating autoimmune responses described above (Laddha et al., 2013). Thus, systemic redox defects and autoimmune responses work together in the development of vitiligo.

To uncover the molecular signature of vitiligo, Sahoo et al (2017) performed global transcriptomic analysis, global lipidomics, and metabolomics analysis of the immortalized cell line PIG3V, derived from the perilesional skin of a patient with vitiligo, compared with the primary human epidermal melanocyte-light cell line HEM-1 (Sahoo et al., 2017). Hierarchical clustering analysis of RNA-seq data revealed that signaling pathways involved in the synthesis of melanin, immune response, and cell cycle were among the most differentially expressed between normal and vitiligo cell lines. Melanin production, as well as expression of major pigmentation pathway genes such as KIT, TYR, PMEL, and transient receptor potential melastatin 1, was significantly downregulated in vitiligo cells. Interestingly, the intronic sequence of transient receptor potential melastatin 1, a calcium permeable cation channel expressed in melanocytes, encodes miRNA-211 which is known to play an important role in melanocyte homeostasis. As expected, expression of miR-211 was almost undetectable in PIG3V cells. To confirm that the loss of miRNA-211 is associated with human vitiligo, the authors examined expression of miRNA-211 in biopsy samples of vitiligo lesions compared with a normal skin. miRNA-211 was significantly downregulated in 10 of 11 biopsies from patients with vitiligo. Moreover, loss of miRNA-211 expression correlated with the activity of the disease in nonlesional, perilesional, and lesional skin.

miRNA-211 is highly expressed in primary melanocytes where it influences various cellular processes and positively regulates pigmentation by targeting transforming growth factor beta receptor 2. Loss of miRNA-211 is implicated in the stress response and melanogenesis. Recently this research group determined that miRNA-211 acts as a metabolic switch in nonpigmented melanoma cells (Mazar et al., 2016). These data suggest that miRNA-211 plays a critical role in the pathophysiology of human vitiligo, and can be positioned at the apex of a normal melanocyte gene network. The authors hypothesized that among genes overexpressed in the absence of miRNA-211 in human vitiligo cells, at least some are predicted targets of this miRNA, and that identification of these targets will shed light on the molecular mechanisms by which loss of miRNA-211 promotes vitiligo.

The transcriptional coactivator PPARGC1/PGC1-α, known as a master regulator of mitochondrial biogenesis, was among differentially expressed targets identified in this study with the elevated expression levels of PPARGC1/PGC1-α in vitiligo PIG3V cells compared with HEM-1. Utilizing a PPARGC1/PGC1-α 3’UTR reporter construct, Sahoo et al. demonstrated its direct regulation by miRNA-211. Furthermore, transient expression of miR-211 in PIG3V vitiligo cells led to reciprocal downregulation of PPARGC1/PGC1-α levels. Expression levels of PPARGC1/PGC1-α were significantly upregulated in 7 of 11 patient samples with vitiligo compared with controls. Other identified targets, including RRMs2 and TAOK1, which also contain miR-211 binding sites at their 3’ UTRs, were upregulated in a vast majority (10/11) of biopsy samples.

Given that PPARGC1/PGC1-α is central to the metabolic control and particularly to mitochondrial biogenesis, adaptive thermogenesis, and gluconeogenesis, the authors investigated the respiratory functions and intactness of mitochondrial complexes in vitiligo cells. One could predict that upregulation of PPARGC1/PGC1-α would lead to an increased capacity to utilize oxygen in vitiligo cells. Surprisingly, oxygen consumption rates in PIG3V cells were significantly lower than in normal melanocytes. Although oxidative phosphorylation was active in both cell lines, the capacity to respond to respiratory stress was dramatically
lower in vitiligo cells. Glycolysis, measured by the extracellular acidification rate, was also lower in PIG3V cells compared with HEM-1 cells, suggesting that glycolysis does not substitute regular energy metabolism in these cells, but that there must be an intrinsic respiratory defect in vitiligo cells. Indeed, Sahoo et al. determined that mitochondrial oxidative phosphorylation complexes I, II, and IV were significantly decreased in PIG3V cells compared with HEM-1 cells, indicating that the impaired respiratory stress response in PIG3V cells can be attributed to mitochondrial complex dysfunction. In addition, the authors showed that the vitiligo cell line PIG3V produces more ROS than normal melanocytes. They hypothesized that overexpression of PPARGC1/PGC1-α can be at least partially responsible for overproduction of ROS in vitiligo cells, and demonstrated that knockdown of PPARGC1/PGC1-α led to a significant reduction of intracellular ROS.

Sahoo et al. have also compared lipid and metabolic profiles of vitiligo PIG3V cells and HEM-1 cells. PIG3V cells contained significantly higher levels of cardiolipin and a key substrate for its synthesis—phosphatidylglycerol. In contrast, the levels of phosphatidic acid and diacylglycerol were decreased in PIG3V cells and were associated with the accumulation of triacylglycerol, suggesting alterations in fatty acid synthesis and energy storage in PIG3V cells. Significant changes in such cell membrane components as phosphatidylcholine, sphingomyelin, and phosphatidylserine were also observed in vitiligo cells. Metabolomic analysis revealed decreased levels of succinate, malate, and alpha-ketoglutarate suggesting alterations in the mitochondrial tricarboxylic acid cycle in vitiligo cells. Analysis of amino acids revealed increases in alanine, arginine, glycine, and methionine and decreases in citrulline and ornithine levels in PIG3V cells. Whereas this report provides important observations on the changes of lipid and metabolic profiles in vitiligo cells, the role of miR-211 in these changes needs further investigation.

Overall the study by Sahoo et al. established miR-211 as a critical regulator of cellular metabolism in vitiligo cells. Loss of miR-211 negatively affects oxidative phosphorylation and mitochondrial energy metabolism. Importantly, several novel downstream miR-211 targets were identified. miRNAs are small in length, but powerful regulators of mRNA stability and thus gene expression. Downregulation of transient receptor potential melastatin 1 leads not only to a change of biochemical events where this protein is involved, but results in the downregulation of miR-211, which is expressed from the transient receptor potential melastatin 1 intrinsic region. Loss of miR-211 in turn alternates expression patterns of numerous target genes, and those that regulate respiratory functions in melanocytes are among them. This study highlights the importance of miR-211 for melanocyte biology and development of vitiligo. Finding molecular markers that link together autoimmunity and respiratory mechanisms in vitiligo cells is of great importance, because more evidence appears for the combined impact of oxidative stress-mediated toxicity and autoimmunity in pathogenesis of this disease. It would be interesting to see if miR-211 is downregulated in other skin diseases that involve autoimmune responses and directly targets genes involved in autoimmune pathogenesis of vitiligo, and if the levels of miR-211 are altered in segmental vitiligo, where the role of immune system in disease development is not as obvious as in nonsegmental vitiligo. miRNAs usually affect cellular phenotypes through their pleiotropic effects on multiple target mRNAs, and it is not unreasonable to suspect that miR-211 effects on metabolism in vitiligo are not restricted to downregulation of PGCG-α. This group has recently reported that ectopic expression of miR-211 in nonpigmented melanoma cells results in an increase in mitochondrial number and oxygen consumption through miR-211-dependent post-transcriptional downregulation of pyruvate dehydrogenase kinase 4 (Mazar et al., 2016). Endoplasmic reticulum stress has also been reported to affect mitochondrial metabolism and production of ROS (reviewed in Tabas and Ron, 2011), and to be involved in vitiligo pathogenesis (reviewed in Manga et al., 2016). Interestingly, miR-211 was shown to transcriptionally downregulate expression of CHOP—a pro-apoptotic protein that can cause mitochondrial damage (Chitnis et al., 2012).

Studies of regulatory pathways between expression of miR-211, its downstream targets, and external signals could uncover mechanisms involved in the pathogenesis of vitiligo. The promising results for restoring respiratory mechanisms in vitiligo cells by overexpression of miR-211 provide a rationale for possible future therapeutic applications for this disease.

CONFLICT OF INTEREST

The authors state no conflict of interest.

REFERENCES


