Lupus: The Immunological Impact of Altered DNA Methylation

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Systemic lupus erythematosus (SLE), a chronic autoimmune disease characterized by overproduction of autoantibodies against a series of nuclear antigens, causes widespread tissue damage. Although multiple genes take part in determining the predisposition to SLE, environmental factors, largely reflected by epigenetic alterations, DNA methylation changes in particular, have been demonstrated to be crucial for the pathogenesis of SLE. DNA methylation is a most widely studied epigenetic mark that is stable, inheritable, and reversible. Gene transcription can be repressed through hypermethylation of active promoter regions associated with CpG-rich sequences. In contrast to gene hypermethylation, DNA hypomethylation facilitates transcription factor binding to promoter regions, thereby promoting gene expression.

Accumulating evidence indicates that DNA hypomethylation in $CD4^+$T cells contributes to the onset and development of drug-induced and idiopathic lupus (Lu, 2013; Liu *et al.*, 2013a; Liu *et al.*, 2013b). An array of genes, sensitive to DNA methylation status in their promoter regions, is overexpressed in T cells from lupus patients, and these gene expression levels parallel SLE disease activity. Among the identified targets undergoing demethylation are genes involved in autoreactivity (ITGAL), osmotic lysis and apoptosis (PRF1), and B-cell/T-cell interaction (CD70 and CD40LG). ITGAL (CD11a) has a core role in cellular adhesion and co-stimulatory signaling. The CD11a overexpression in T-cell clones contributes to the development of T-cell autoreactivity *in vitro*, and adoptive transfer of CD11a-overexpressed, autoreactive T cells can induce a lupus-like disease in mice (Richardson *et al.*, 1994; Yung *et al.*, 1996). Our team has demonstrated the increased expression of CD11a in T cells from patients with active lupus due to the hypomethylation of specific sequences flanking the promoter of *ITGAL*, the gene encoding CD11a (Lu *et al.*, 2002). The hypomethylated $CD4^+$T cells were also found in patients with subacute cutaneous lupus erythematosus (SCLE), a less severe form of lupus, with inversely elevated CD11a mRNA (Luo *et al.*, 2008). Perforin, encoded by the *PRF1* gene, was observed to be overexpressed in $CD4^+$T cells from patients with active lupus as well as SCLE (Zhang *et al.*, 2013). The overexpression is related to demethylation of the perforin promoter region (Lu *et al.*, 2003; Luo *et al.*, 2009). Another example of functional consequence of relevant DNA hypomethylation in T cells from SLE patients is the overexpression of CD70, a cellular ligand expressed by T cells for the TNF receptor family member CD27 on B cells, resulting in autologous B-cell stimulation and IgG production (Lu *et al.*, 2005). Similar to CD70, CD40 ligand (CD40L) is also a B cell–costimulatory molecule but is encoded on the X chromosome. Epigenetic regulation, particularly DNA methylation, has a key role in CD40L expression in women. We previously reported that *CD40L* gene is unmethylated in normal men, while one copy of this X-chromosome gene is methylated and the other is unmethylated in normal women. Compared with normal controls, CD40L is hypomethylated and overexpressed in $CD4^+$T cells from female but not male lupus patients. With 5-azaC treatment, the expression of CD40L doubled in $CD4^+$T cells from normal women but not from men. Taken together, our studies demonstrate that overexpression of CD40L contributes to the pathogenesis of SLE in females. This is partially a result of demethylated regulatory sequences on the inactive X chromosome in T cells (Lu *et al.*, 2007; Zhang *et al.*, 2013). Besides those well-characterized methylation-sensitive genes mentioned above, other genes such as *PP2Aca* (Sunahori *et al.*, 2011), *HRES-1* (Fali *et al.*, 2013), the *KIR* gene family (Liu *et al.*, 2009), *LINE-1* (Sukapan *et al.*, 2014), and some cytokines (Mi and Zeng, 2008) are also emerging as instigators of SLE due to abnormal methylation patterns.

Apart from those classic methylation-sensitive autoimmunity-related genes in lupus $CD4^+$T cells, the genome-wide methylation pattern has also been explored recently, providing us a more full-scale picture of the abnormal methylation patterns in SLE. Absher *et al.* (2013) performed genome-wide DNA methylation analysis with Illumina Methylation 450 microarrays to assess DNA methylation status in $CD4^+$ T cells, $CD19^+$ B cells, and $CD14^+$ monocytes from SLE patients. They

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Table 1. Mechanisms underlying DNA demethylation in lupus T cells

Mechanisms	Examples	Expression level	Contribution to lupus pathogenesis	Refs
microRNAs	miRNA-21, miRNA-126, miRNA-148a	↑	Downregulate DNMT1 expression leading to elevated hypomethylation in CD4+T cells from lupus patients	(Pan *et al.*, 2010; Zhao *et al.*, 2011)
Transcription factors	RFX1	↓	Recruit less DNMT1 to the promoter regions of specific genes, such as *CD11a* and *CD70*	(Zhao *et al.*, 2010)
Pathway signaling	MAPK pathway	↓	Reduce DNMT1 expression in lupus T cells	(Deng *et al.*, 2001; Deng *et al.*, 2003)
Global demethylator	Gadd45α	↑	Act as a global demethylator in CD4+T cells from patients with SLE	(Li *et al.*, 2010)
Other DNA modifications	DNA hydroxymethylation	↑	Demethylation intermediate	(Zhang *et al.*, 2013)

Abbreviations: ERK, extracellular signal-regulated kinase; Gadd45α, growth arrest and DNA damage-induced 45α; MAPK, mitogen-activated protein kinase; RFX1, regulatory factor X 1.

found that 166 CpGs in B cells, 97 CpGs in monocytes, and 1,033 CpGs in T cells showed significantly altered DNA methylation status, and that genes involved in interferon signaling (type I) were persistently hypomethylated. Genes involved in cell division and MAPK signaling in $CD4^+$ T cells also showed methylation changes but with a lower amplitude shift (Absher *et al.*, 2013). Coit *et al.* (2013) also performed a genome-wide DNA methylation study to decipher the DNA methylome in naive $CD4^+$ T cells in lupus. This study quantified DNA methylation for over 485,000 methylation sites across the genome, and 86 differentially methylated CG sites in 47 genes were identified and replicated. Significant hypomethylation in interferon-regulated genes was observed in naive $CD4^+$ T cells from lupus patients, but the hypomethylation was not associated with lupus activity . However, the results of these two studies suggest DNA methylation as a mechanism for type-I interferon hyper-responsiveness in lupus T cells.

Certain miRNAs (Pan *et al.*, 2010; Zhao *et al.*, 2011), such as miRNA-126, regulatory factor X 1 (RFX1) (Zhao *et al.*, 2010), defective ERK pathway signaling (Deng *et al.*, 2003), the growth arrest and DNA damage-induced 45α (Gadd45α) protein (Li *et al.*, 2010), and DNA hydroxymethylation (Zhang *et al.*, 2013) have been proposed as potential mechanisms leading to DNA demethylation in lupus (Table 1). In the case of DNA hydroxymethylation, 5-methylcytosine (5-mC) can be converted to 5-hydroxymethylcytosine (5-hmC) by the ten-eleven translocation (TET) family proteins, and 5-hmC can be further oxidized into 5-formylcytosine (5fC) and 5-cyboxycytosine (5caC) to achieve active DNA demethylation (Sun *et al.*, 2014). High levels of TET1, TET2, and 5 hmC were observed in $CD4^+$T cells from SLE patients compared with healthy controls (Zhang *et al.*, 2013).

A comprehensive understanding of DNA demethylation contributing to SLE may lead to the development of new therapeutic agents and strategies that target the dysregulated genes or correct the altered methylation patterns.

CONFLICT OF INTEREST

The author states no conflict of interest.

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