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Commentary

Circular RNAS: novel biomarkers of disease activity in systemic lupus erythematosus?

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Circular RNAs (circRNAs) are a class of non-coding RNAs that regulate gene expression by acting as competitive endogenous RNAs (ceRNAs) and modulating gene transcription. Several studies support the implication of circRNAs in a variety of human diseases, but research on the role of circRNAs in systemic lupus erythematosus (SLE) is lacking. In a study recently published in Clinical Science (2018), Zhang et al. identified hsa_circ_0012919 as a potential biomarker of disease activity in SLE patients. The authors observed different circRNA expression between SLE patients and healthy controls, an association with clinical variables and with the abnormal DNA methylation present in SLE $CD4^+$ T cells. Finally, Zhang et al. demonstrated that hsa_circ_0012919 acts as a miRNA sponge for miR-125a-3p, regulating the gene expression of targets RANTES and KLF13 that are involved in the physiology and pathophysiology of acute and chronic inflammatory processes. These findings support the role of circRNAs in the pathophysiology of SLE.

Systemic lupus erythematosus (SLE) is a chronic disease caused by autoimmune tissue damage in the majority of organs. The physiopathology of SLE is complex, resulting from an interplay of multiple factors including environmental, genetic, hormonal, and immunoregulatory ones [1]. However, many aspects in the pathogenesis of the disease remain poorly understood. SLE diagnosis is challenging, given the heterogeneity both in clinical presentation and in disease activity measurement over the course of disease [2,3]. Research has focussed on finding disease activity markers, generally using activity indices such as SLEDAI, BILAG or complement, and dsDNA levels. These tools are not entirely accurate in assessing disease activity, however, underlining the need to find other more sensitive biomarkers of activity and locate more specific therapeutic targets. Nevertheless, to date none of the available disease activity indices could be considered the gold standard [4–9]. Identifying useful biomarkers for diagnosis and disease activity in cells and fluids is a challenge in current research. In an attempt to reach greater understanding of the pathogenesis, many recent studies have focussed on the role of epigenetic factors such as miRNAs and cirRNAs.

Circular RNAs (circRNAs) are a class of small non-coding RNAs that form a covalently closed continuous loop [10]. Recent findings have shown that they are very stable, abundant and have specific tissue/developmental expression [11–13]. At the physiological level, endogenous circRNAs can bind to miRNAs, acting as miRNA 'sponges', and consequently repressing their function [11,12]. Emerging evidence indicates that circRNAs could be involved in RNA interference pathways, suggesting that the more stable circRNAs and mRNAs compete for binding to common miRNAs in the cytoplasm and consequently interfere in gene expression [14]; these RNA sponges are then named competitive endogenous RNA (ceRNA). Given that circRNAs affect miRNA target regulation, research on these circRNAs and their miRNA partners has been able to shed light on gene regulation during the progress of certain diseases [15–18]. Although circRNAs are a new focus of research in many human diseases, little is known about their role in autoimmune diseases. A previous study showed that circ_0005402 and circ_0035560 are

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down-regulated in multiple sclerosis patients and could serve as biomarkers for the disease [19]. Ouyang et al. identified increased levels of four cirRNAs in peripheral blood mononuclear cells of patients with rheumatoid arthritis and demonstrated their role as potential diagnostic biomarkers [20]. Furthermore, Luo et al. recently found that has_circ_0044235 in peripheral blood was significantly decreased in patients with rheumatoid arthritis [21,22].

In a recent study published in Clinical Science, Zhang et al. filled a gap in the knowledge by providing an analysis of circRNA profiles and their association with SLE-related genes in $CD4^+$ T cells of SLE patients, acting as a miRNA sponge [23]. In addition, this work investigated the involvement of circRNAs in regulating methylation status of CD70 and CD11a. The authors compared the expression profile of circRNAs in the $CD4^+$ T cells of SLE patients and matched control subjects by microarray analysis. They obtained 12 up-regulated and two down-regulated circRNAs, but the potential candidate selected was has_circ_0012919 for its differential expression between active and inactive patients and healthy controls, and for its association with clinical variables. These results are in concordance with concurrent studies by Li et al. [24], which demonstrated decreased hsa_circ_0045272 expression in SLE, and Li et al. which also found that hsa_circ_400011, hsa_circ_102584, and hsa_circ_101471 are overexpressed and hsa_circ_100226 is underexpressed in SLE patients [25]. Ouyang et al. reported that plasma circRNA_002453 was significantly elevated in patients with lupus nephritis compared with SLE patients without nephritis, patients with rheumatoid arthritis and healthy controls [26]. However, they did not find any association with clinical variables.

The second part of Zhang et al.'s study identified the association with expression of SLE-related genes, such as DNMT1, involved in DNA methylation [27] and CD70 and CD11a, involved in the expansion, differentiation and activation of $CD4^+$ T cells [28]. The authors observed an inverse correlation with DNMT1 and direct correlation with CD70 and CD11a. Additionally, isolated $CD4^+$ T cells from SLE patients transfected with siRNA targetted against hsa_circ_0049224, reversing low DNMT1 expression and reducing CD70 and CD11a levels. Furthermore, the DNA methylation grade of CD70 and CD11a was inversely associated with hsa_circ_0049224 and these DNA methylation levels had an obvious increase after transfection. These results indicate that hsa_circ_0049224 could be associated with the abnormal DNA methylation present in SLE $CD4^+$ T cells. A previous work also showed that circRNA down-regulation in SLE T cells may contribute to the enhanced apoptosis observed in SLE [24]. In the most intriguing discovery by Zhang et al., luciferase assay showed that endogenous hsa_circ_0049224 works as a miRNA sponge of miR-125a-3p, regulating the gene expression of targets RANTES and KLF13. RANTES is a chemokine known for enhanced levels and detrimental effects in inflammatory disorders such as SLE [29], whose induction in activated T cells was controlled by the transcription factor KLF13 [30].

The findings reported by Zhang et al. provide new insights into the role of hsa_circ_0012919 in SLE, suggesting that hsa_circ_0012919 could be used as a potential biomarker for SLE and the ceRNA for miR-125a-3p. However, a more extensive *in silico* analysis of circRNAs and mRNA or microRNA targets could be a better approach to identify novel pathways in SLE pathogenesis. A recent study by Li et al. constructs a complex circRNA target network between circRNA, mRNA, and miRNAs in children with SLE, providing new insight into the mechanisms of SLE pathogenesis [31].

On the other hand, these studies have two methodological flaws that undermine their conclusions. One limitation is the relative small sample size analyzed for SLE patients and healthy controls, which needs to be greatly increased to improve statistical validity. This will require establishing collaborative study groups to share biological samples from hundreds of subjects. Another potential shortcoming is the lack of detailed patient selection criteria and clinical characteristics description. SLE is a complex autoimmune disease with a highly varied clinical presentation, so the pathogenesis mechanisms involved are different according to organ damage (kidney, skin, etc). In this report, all patients were analyzed together, while they should be studied according to stricter lupus criteria; likewise, associations with activity markers (C3 and C4, dsDNA) should have been analyzed.

The role of circRNAs in systemic lupus erythematosus has been in the spotlight in recent months [32]. Taken together these studies expand our knowledge of the complexity of gene regulation and in the case of Zhang and colleagues add the new layer of epigenetic mechanism. Moreover, they also highlight the possibility that manipulation of miRNAs by circRNAs in SLE treatment may hold antiautoimmune potential. Future use could be made in SLE patient treatment of their roles as miRNAs sponges whose overexpression may promote SLE pathogenesis. However, further studies are needed to identify circRNAs' target network in SLE pathogenesis and confirm its role. When New circRNAs are characterized and their mechanism of action on miRNAs better determined, their utility as biomarkers will be improved.

Competing Interests

The authors declare that there are no competing interests associated with the manuscript.

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Abbreviations

ceRNA, competing endogenous RNA; circRNA, circular RNA; SLE, systemic lupus erythematosus.

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