Epigenetic profiling of twins identify repression of KLF4 as a novel pathomechanism in systemic sclerosis

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Systemic sclerosis (SSc) is an idiopathic autoimmune connective tissue disease with vascular abnormalities, inflammation and fibrosis. The fibrosis affects the skin as well as several internal organs. An incomplete understanding of the pathways that govern activation of the cells involved in disease pathogenesis has hampered new therapies. In this issue of Annals of the Rheumatic Diseases, Malaab et al (REF to be inserted by ARD) employ a unique cohort of 15 twins discordant for the diagnosis of SSc to demonstrate a key role of epigenetics including methylation, alterations of microRNA and ultimately dysregulation of Kruppel-like factor 4 (KLF4) that impacts on fibrosis. This sheds light on underlying epigenetic mechanisms and exposes a new therapeutic target.

Epigenetics is the study of heritable changes in gene expression not mediated by changes in the DNA sequence itself. Three main mechanisms mediate epigenetic changes: non-coding RNAs, DNA methylation and histone modifications.1 DNA and the cytosine base of DNA can be modified by the addition of a methyl group by a family of three DNA methyltransferases enzymes on the fifth carbon of cytosine. This modification enables binding of DNA methylation proteins such as methyl binding domain proteins, which in turn recruit histone modifying proteins and other epigenetic modulators that ultimately represses gene expression. Non-coding RNAs are defined as a long or short set arbitrarily defined by length. In the case of microRNAs, they regulate gene expression by binding the 3'UTR of mRNA that leads to gene repression. Long non-coding RNAs are usually much longer and regulate gene expression, both positively and negatively, through only partially unknown mechanisms. Finally, alteration in the histone tails of chromatin can also regulate gene expression via many different modifications with complementary functions. Histone acetylation by histone acetyltransferase enzymes has been studied most to date. Other modifications besides acetylation include methylation, ubiquitination, sumoylation and lactylation. All of these modifications can alter chromatin dynamics to promote or repress gene expression and can thus poised cells towards a certain state of activation. These epigenetic modifications are, however, reversible, and the epigenome undergoes continuous modifications to fine-tuning cellular dynamics. Recent evidence has highlighted epigenetic alterations as key-drivers in the pathogenesis of multiple diseases including SSc.2–4 Epigenetic alterations may also contribute to other features of SSc such as inflammation, autoimmunity and vascular disturbances.

The authors used a cohort of 15 twins discordant for the diagnosis of SSc for an integrated analysis of genome wide methylation patterns and gene expression. Using this combined 'omics' approach, they identified several differentially expressed genes regulated at the level of DNA methylation between controls and patients.

Among those were several homeobox genes including HOXB3 and HOXB8. HOX genes are a family of evolutionarily conserved genes encoding transcription factors with central roles in stem cell differentiation, in the formation of organ symmetry and in topographic memory. First evidence also implicates HOX genes in wound healing and fibrosis,5,6 thereby suggesting that dysregulated HOX gene expression may have the potential to contribute to the aberrant activation of fibroblasts in SSc. The authors provide evidence that HOX genes repress the expression of microRNA10a/10b. Mir10a is located upstream of HOXB4 on chromosome 17, while mir10b is located upstream of HOXD4 on chromosome 2. Analysis of SSc patients dermal fibroblast levels of both microRNAs revealed reduced levels of these microRNAs. Reduction of these microRNAs would thus lead to derepression of its cognate targets. Indeed, the microRNA10a/10b targets TFAP2A and TBX5 were indeed upregulated in this twin cohort. Silencing of mir10a and mir10b in normal dermal fibroblasts led to upregulated miRNAs for TFAP2A and TBX5. The functional role of this signalling cascade for fibroblast activation was further highlighted by rescue experiments in fibroblasts derived from patients with SSc. Restoring normal levels of miR10a and 10b in SSc fibroblasts normalised the expression of TFAP2A and TBX5 and reduced the aberrant expression of type I collagen.

The authors went on to examine embryonic stem cell transcription factors including SRY-box 2 (SOX2) and KLF4. They found enhanced SOX2 and decreased KLF4 expression particularly in early stages of SSc. A downregulation of KLF4 was also observed in hepatic stellate cells in human fibrotic liver and carbon tetrachloride induced liver fibrosis,7 suggesting that impaired KLF4 expression may be a general feature of fibrotic diseases. The downregulation of KLF4 might in part be mediated by TGFβ, which has been shown to inhibit KLF4 expression in a kidney epithelial cell line.8 KLF4 is a conserved zinc finger transcription factor with pleomorphic functions. It is best known as one of four transcription factors that are required to induce fibroblasts into pluripotent stem cells so-called Yamanaka factors.

Malaab et al13 demonstrated that small interfering RNA mediated depletion of KLF4 increased ACTA2 (encoding α-Smooth muscle actin), CTGF and WNT4A. Conversely, adenosine overexpression of KLF4 led to reduced collagen, fibronectin and CTGF protein levels suggesting that KLF4 regulates antifibrotic transcriptional programmes in fibroblasts. These findings are consistent with studies in isolated kidney cells, in which KLF4 repressed the expression of key fibrosis markers including α-smooth muscle actin.15 Finally, the authors demonstrated that KLF4 conditional KO mice spontaneously develop skin fibrosis with upregulation of multiple profibrotic transcripts. The authors also noted a prominent activation of WNT signalling with accumulation of β-catenin, the central integrator of canonical WNT signalling. This is particularly intriguing, as WNT signalling has emerged as a core pathway of fibrosis in SSc.14–17

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Editorial
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Consistent with the findings of Malaab et al., a recent study identified significantly reduced KLF4 in idiopathic pulmonary fibrosis samples, a disease that shares excessive fibroblast activation and fibrotic tissue remodelling with SSc. Overexpression of KLF4 in mice with bleomycin-induced pulmonary fibrosis model significantly retarded fibrosis compared with wildtype mice. Interestingly, siRNA interference of KLF4 potentiated Dishevelled 2 (Dvl2) expression. Dvl2 is a member of the WNT family that integrates and promotes wingless-related Integration site (WNT) signalling by stabilising the key effector molecule β-catenin, thereby preventing its degradation and promoting canonical WNT signalling.

Accordingly, the downregulation of KLF4 in SSc, eventually via TGFβ-dependent mechanisms, may upregulate Dvl2 expression to promote stabilisation and nuclear translocation of β-catenin with subsequent activation of WNT-induced proliferotic programmes. What are the possible ways to use this information for clinical use? No approaches to induce KLF4 expression or activity directly are available to date. However, this study identifies two microRNAs that are regulating homeobox proteins that could regulate KLF4 amounts. One possible way to restore KLF4 levels would be to elevate the diminished levels of miR10a/b. This could be achieved using microRNA mimics. These are chemically modified microRNAs with increased stability to endogenous RNAses in vivo. This approach would ideally enhance the expression of KLF4. However, microRNAs do not regulate individual genes, but rather sets of different genes and miR10a/b mimics may thus modulate other, potentially homoepoietic pathways as well as KLF4.

Alternatively, the WNT pathway as a downstream mediator de-repressed by the downregulation of KLF4 offers therapeutic potential. After long being considered as ‘undruggable’, several inhibitors of WNT signalling have been developed during the last years. These include among others, inhibitors of porcupine. Porcupine is an acetyltransferase that palmitolyates WNT proteins, which is required for secretion of WNT ligands. In that regard, a porcupine inhibitor was used in different preclinical models of skin fibrosis and in clinically relevant doses was found to be effective. Other inhibitors such as tankyrase inhibitors such as XAV-939 have also shown beneficial effects in models of SSc. These data all suggest targeting WNT signalling could offer therapeutic potential in a disease that currently has no therapy that modifies the fibrosis.

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