Mitochondrial Ca^{2+} uptake directs fibrosis.

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Abstract: During development, disease or in response to changes in local environmental and/or nutrient supply, cellular metabolism is substantially remodeled. Reduced mitochondrial Ca^{2+} uptake was recently reported to induce metabolic remodeling, which through stimulating alterations in the epigenome causes changes in gene expression associated with fibroblast to myofibroblast differentiation.

Metabolic switching via altered mitochondrial Ca^{2+} accumulation. Ca^{2+} uptake into the mitochondria is a driver of tricarboxylic acid (TCA) cycle activity [1]. Mitochondrial Ca^{2+} accumulation during physiological Ca^{2+} signaling events allows dynamic coupling between cell activity, metabolic demands and supply. Ca^{2+} uptake into the mitochondria occurs via the mitochondrial uniporter channel (MCU), which exists in a complex with a number of regulatory proteins [2]. One such protein is mitochondrial Ca^{2+} uptake protein 1 (MICU1), which influences the threshold and cooperativity for Ca^{2+} uptake. Indeed, MICU to MCU stoichiometry was recently reported to underlie the different sensitivities of Ca^{2+} uptake of cells from different tissues [3]. For example, cardiomyocytes have a low MICU to MCU ratio, which favors Ca^{2+} uptake at lower levels with low cooperativity, while liver cells possess a high MICU to MCU ratio that is responsible for their higher threshold and greater cooperativity for Ca^{2+} uptake. The MICU to MCU ratio may thus tune mitochondrial Ca^{2+} uptake and its relationship with metabolism.

A functional role for mitochondrial Ca^{2+} uptake and metabolism in fibroblast to myofibroblast differentiation has recently been described [4] (Figure 1). This phenotypic switch is characterized by a contractile phenotype and increased production of extra cellular matrix (ECM). While fibroblast differentiation plays an important role in wound healing and repair, overt fibrosis is deleterious, contributing to the pathological features of diseases in tissues such as the heart and liver. Fibroblast differentiation is induced by pro-fibrotic mediators such as TGF- β , endothelin and AngII, and involves increases in cytosolic $[\text{Ca}^{2+}]$. Demonstrating the additional importance of mitochondrial Ca^{2+} uptake in this process, Lombardi et al showed that genetic deletion of MCU promoted spontaneous differentiation of fibroblasts to myofibroblasts. Moreover, a dramatic increase in the ratio of MICU1 to MCU and increased threshold for mitochondrial Ca^{2+} uptake was observed during TGF- β -induced fibroblast differentiation. Not surprisingly, TCA activity as well as abundance of many of its intermediates was reduced. Increased glycolysis was observed, which via overexpression of phosphofructokinase 2, was shown to be sufficient to induce fibroblast to myofibroblast differentiation.

Metabolic control of the epigenome: An increase of the TCA cycle intermediate α ketoglutarate (α KG; also termed 2-oxoglutarate) arising via glutaminolysis was detected in myofibroblasts (Figure 1). α KG has important roles independent of the TCA cycle, acting

together with Fe^{2+} , oxygen and ascorbate as a co-factor for enzymes involved in regulation of the epigenome. It also regulates the activity of prolyl hydroxylases involved in hypoxia inducible factor signaling [5]. The epigenome represents a panoply of mechanisms selectively controls gene expression across the genome [6]. The epigenome is also dynamic and via its remodeling underlies reprogramming of cell transcriptomes and phenotypic transformation during differentiation and disease. Epigenetic regulation of the genome is mediated by covalent modifications of histone proteins and DNA as well as via lncRNAs (long non-coding RNAs) and microRNAs. Histone proteins are the building blocks of the nucleosome, around which the DNA is wrapped. They are subject to a multitude of modifications including acetylation and methylation, which serve to affect interactions with other regulatory proteins or compaction of chromatin, and through these mechanisms, result in activation or repression of gene expression [6].

Amongst the enzymes regulated by αKG are Jumonji C (JmjC)-domain-containing histone demethylases (often termed lysine demethylases (KDMs)) and the Ten-Eleven Translocase (TET) family of DNA demethylases [7]. These enzymes catalyse the removal of methyl groups from histone proteins and from DNA respectively, thereby inducing alterations in transcription. JmjC-domain-containing histone demethylases can demethylate multiple lysine and arginine residues on histones H3 and H4. By converting 5-methylcytosine (5-mC) to 5-hydroxymethylcytosine (5-hmC) and subsequent metabolites, TET enzymes participate in the demethylation of DNA [6]. In the study by Lombardi et al, whereas no significant alterations in DNA methylation during fibroblast myofibroblast differentiation were detected, a loss of the repressive H3K27me2 mark was observed. In control fibroblasts, this mark was enriched on the periostin (*Postn*) and PDGF receptor (*Pdgfra*) promoters, which encode transcripts shown to be increased in myofibroblasts. These epigenetic marks flanked recognition sequences for transcription factors with roles in myofibroblast differentiation, including serum response factor, SMAD3, nuclear factor of activated T cells (NFAT) and myocyte enhancer factor 2 (MEF2).

Conclusion and perspectives: Given the prevalence of fibrosis in disease, the relationship between mitochondrial Ca^{2+} remodeling, the epigenome and cell differentiation has significant clinical implications. The success of small molecule inhibition of the histone demethylase responsible for demethylating H3K27me2/3 (KDM6) prevented TGF- β - or MCU deletion-induced fibroblast to myofibroblast transition *in vitro* reinforces this notion. Further, the enhanced fibrosis in a mouse model of myocardial infarction following MCU deletion demonstrates the activity of this pathway *in vivo*. While KDM inhibition was effective in prevention of myofibroblast transition, inhibition of disease progression or reversal are the most clinically relevant situations. Future studies are therefore necessary to test whether epigenetic therapy or maintenance of MCU activity represent strategies to reverse deleterious fibroblast remodeling [8]. The pathway described by Lombardi et al, may also hold true in other cell types that undergo long term remodeling of their phenotype that also show a shift in their metabolism to glycolysis and alteration in mitochondrial Ca^{2+} handling. For example, in stem cell differentiation, in cardiomyocytes during failure, and in oncogenic transformation [7, 9]. Indeed, hypertrophic cardiomyocytes show decreased histone methylation across re-expressed fetal genes [10]. Via enhanced activity of the TCA cycle and active intermediates, acute increases in mitochondrial Ca^{2+} uptake may also influence the epigenome [1]. For example, acetyl-Co-A is an important substrate involved in histone acetylation, NAD^+/NADH ratio can influence the activity of sirtuin family of NAD-dependent histone deacetylases and succinate inhibits the activity of the same enzymes activated by αKG [7].

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Competing interests:

None to declare

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Fibroblast to myofibroblast differentiation links Ca^{2+} signaling and epigenetic reprogramming. Profibrotic mediators such as $\text{TGF}\beta$ and AngII trigger a reduction in mitochondrial Ca^{2+} (mt Ca^{2+} uptake) uptake through a mechanism involving an upregulation of MICU1 and its raising of the threshold for mitochondrial Ca^{2+} uniporter activation (MCU) (1). The reduction in mitochondrial Ca^{2+} decreases TCA cycle activity (2). To meet metabolic demands, glycolysis is enhanced (3). Despite reduced TCA cycle activity, αKG , an intermediate of the TCA cycle is increased via catabolism of glutamine and glutamate by glutaminolysis (4). αKG plays a crucial role in the epigenetic reprogramming of fibroblasts to myofibroblasts, serving as a co-factor of JmJC-KDMs enzymes. Activated KDMs promote H3K27me2 demethylation at myofibroblasts specific genes resulting in induction of the myofibroblast gene program (5). During fibroblast to myofibroblast differentiation, the molecules and molecular pathways marked with green are upregulated and those in red, downregulated.