Muscles cannot break a NuRDy heart

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The recent article from Gómez-del Arco *et al* (2016) in *Cell Metabolism* reveals the essential role of chromodomain-helicase-DNA-binding protein 4 (CHD4) in the control of alternative gene expression in cell types that share seemingly redundant, yet distinct, biological properties, such as the contractile activities of striated cardiac or skeletal muscles. An altered expression of genes for alternative sarcomeric and metabolic programs occurred upon genetic inactivation of *Chd4* in skeletal or cardiac cells, leading to formation of “hybrid” striated muscles and pathological outcomes.

See also: P Gómez-del Arco *et al* (May 2016)

Despite harboring identical genetic information, the vast majority of the cells in multicellular organisms activate selective patterns of gene expression to differentiate into specialized cell types. In eukaryotes, a central mechanism for cell type-specific gene regulation is achieved through the concerted action of tissue-specific transcriptional activators, chromatin- and DNA-modifying complexes that leads to unique patterns of epigenetic modifications required for selective gene expression. The individual contribution of distinct epigenetic regulators to the specification of the cell types that comprise different tissues and organs during mammalian development has begun to be appreciated in the last decade. However, a puzzling issue that remains to be resolved relates to the control of genes that encode proteins implicated in shared, yet distinct, biological properties of certain cell types, as in the case of the contractile activity that is common to striated muscles—for example, cardiac and

skeletal fibers. Indeed, these cells express tissue-specific proteins that build upon a common sarcomeric framework to meet distinct functional requirements for cardiac or skeletal muscle activities. The identity of components of the epigenetic program that controls such a distinctive pattern of gene expression is currently unclear.

In their recent article, Gómez-del Arco *et al* (2016) found that chromodomain-helicase-DNA-binding protein 4 (CHD4)-containing nucleosome remodeling and deacetylase (NuRD) complex plays a crucial role in maintaining the functional identity of cardiac and skeletal muscles, by regulating the proper expression of contractile and metabolic genes.

The authors investigated the role of CHD4 in heart development and homeostasis, by conditionally ablating *Chd4* in cardiomyocytes during development or in adulthood. Genetic deletion of *Chd4* in cardiomyocytes during mouse embryonic development, using *Tnnt2*^{Cre/+}; *Chd4*^{flox} or *Nkx2-5*^{Cre/+}; *Chd4*^{flox} models, delayed heart development by reducing cardiomyocyte proliferation in the myocardium of the ventricular compact wall. Ablation of *Chd4* in adult differentiated cardiomyocytes, using *Corin*^{Cre/Cre}; *Chd4*^{flox} or *Myh6*^{Cre/+}; *Chd4*^{flox} mice, showed severe defects in heart homeostasis leading to cardiac fibrosis. Interestingly, gene expression analysis performed in *Chd4*-deficient cardiomyocytes from embryos and adult hearts revealed an aberrant expression of genes encoding proteins of the skeletal muscle lineage, among others, with a significant enrichment in myofibrillar and sarcomeric genes implicated in skeletal muscle contractile activity. Furthermore, the loss of CHD4 in adult cardiomyocytes also induced the

downregulation of genes implicated in cardiac-specific mitochondrial activity. Overall, this evidence indicates that CHD4 plays an essential role in the repression of alternative striated gene expression and in the activation of mitochondrial genes in cardiomyocytes.

The authors further extended their analysis to address the reciprocal nature of this control, by investigating the consequences of *Chd4* deletion in skeletal myofibers using *Mck*^{Cre/+}; *Chd4*^{flox} mice. *Chd4*-deficient skeletal muscles displayed aberrant gene expression that resulted in altered skeletal muscle homeostasis. Of note, a subset of the genes upregulated upon *Chd4* ablation encoded cardiac lineage-specific proteins. Moreover, *Chd4* deletion in skeletal myofibers also resulted in the downregulation of a subset of genes involved in mitochondrial processes, leading to disrupted mitochondrial activity and signs of muscle wasting.

Collectively, these data point to CHD4 as an essential component of the epigenetic machinery that regulates contractile and metabolic programs in distinct types of striated muscles.

To investigate the mechanism by which CHD4 regulates gene expression in cardiac and skeletal muscles, Gómez-del Arco *et al* (2016) used ChIP-qPCR and detected CHD4 binding to the promoter regions of a subset of target genes that were de-repressed in *Chd4*-mutant hearts and *Chd4*-mutant skeletal muscles. Importantly, other components of the NuRD repressive complex—the methyl-CpG-binding domain proteins MBD2 and MBD3, and to a lesser extent the histone deacetylase HDAC1—were also detected on a subset of these promoters. Consistently, promoter binding of CHD4/NuRD coincided with DNA methylation of CpG sites, which is

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Figure 1. CHD4 coordinates gene expression in striated muscles to prevent formation of “hybrid” pathological contractile tissues.

Heart (left) and skeletal myofibers (right) express cardiac-specific genes (orange rectangle) and skeletal muscle-specific genes (purple rectangle), respectively, in a mutually exclusive way. This tissue-specific pattern of gene expression depends on the integrity of the chromodomain-helicase-DNA-binding protein (Chd4)-containing NuRD complex that represses alternative transcription of skeletal muscle genes in heart and vice versa. In the absence of CHD4, de-repression of target genes causes inappropriate expression of alternative genes that interfere with tissue function and leads to pathological outcomes.

in agreement with a previously annotated association between NuRD occupancy of repressed genes and DNA methylation at their promoters (Ramírez *et al*, 2012; de Dieuleveult *et al*, 2016). Thus, promoter recruitment of CHD4/NuRD controls the cellular identity of striated muscles by repressing the transcription of alternative genes.

Interestingly, the de-repression of skeletal muscle-specific contractile genes in cardiomyocytes upon *Chd4* ablation during development is reminiscent of the outcome observed by Delgado-Olguín *et al* (2012) following the loss of EZH2 methyltransferase, the catalytic subunit of the polycomb repressive complex 2 (PRC2), in cardiac progenitors. The aberrant expression of a common subset of skeletal muscle genes, enriched in sarcomeric protein-coding genes (such as *Pvalb*, *Acta1*, *Tnni1*, *Tnnt1*, *Myom3*, *Tnnt3*), observed in cardiomyocytes upon either *Chd4* or *Ezh2* ablation (Delgado-Olguín *et al*, 2012; Gómez-del Arco *et al*, 2016), suggests a potential cooperation between NuRD and PRC2 in repressing skeletal muscle contractile program during cardiac development and homeostasis. Reciprocally, a significant subset of cardiac genes (including *Adam11*, *Bcl9*, *Mybphl*, *Myh6*, *Myl3*, *Mylk3*, *Pbx3*, *Slc*, *Syt7*, and *Tesc*), that were found repressed by CHD4 in skeletal myofibers (Gómez-del Arco *et al*, 2016), display high levels of H3K27me3 repressive histone mark (which is catalyzed by EZH2) on their promoters in terminally differentiated C2C12 myotubes (Asp *et al*,

2011), indicating that NuRD and PRC2 might also cooperate to repress cardiac gene expression in skeletal muscles. Functional interactions between NuRD and PRC2 have been reported to regulate the formation of repressive chromatin in pluripotent cells during development (Laugesen & Helin, 2014), possibly through a model of cooperative activity, whereby NuRD-mediated H3K27 deacetylation may favor recruitment and activity of PRC2 at a subset of developmental genes (Reynolds *et al*, 2012). Overall, these data indicate that CHD4 and PRC2 cooperate to maintain repression of alternative contractile programs in different types of striated muscles.

The activation of skeletal muscle- or cardiac-specific gene transcription observed upon *Chd4* deletion in cardiomyocytes or skeletal myofibers challenges the current notion that alternative lineage gene expression in terminally differentiated cells is permanently silenced by the sequential and synergistic activity of multiple complexes that lead to formation of repressive chromatin and to DNA methylation. Conceivably, reactivation of transcription at the silenced loci should entail a sequence of events that reverse these repressive modifications. Thus, future studies should address whether gene reactivation in the absence of CHD4 is accompanied by changes in histone modifications and DNA methylation at de-repressed genes. Moreover, the expression of skeletal muscle genes in *Chd4*-deficient cardiomyocytes despite the absence of

skeletal muscle regulatory factor (MRF), such as *Myod1*, *Myog*, *Myf5*, and *Myf6*, is particularly intriguing, given that MRFs are known to be required for the activation of muscle gene transcription (Fong & Tapscott, 2013). In this regard, it should be noted that genomewide studies have recently revealed that inhibition of CHD4 activity can lead to the opening of chromatin at 4,688 regions, most of which contain DNA-binding factor-specific motifs with potential regulatory function that would otherwise be inaccessible (Morris *et al*, 2014). Thus, it is possible that transcriptional activation of skeletal muscle genes in *Chd4*-deficient cardiomyocytes could be a result of increased accessibility of skeletal muscle-associated regulatory regions to “opportunistic” transcriptional regulators. A similar example was observed in cardiomyocytes, whereby an aberrant skeletal muscle gene transcription upon EZH2 deletion was driven by de-repression of the *Six1* transcription factor (Delgado-Olguín *et al*, 2012).

Adding an additional layer of complexity, Gómez-del Arco *et al* (2016) found that CHD4/NuRD also directly binds to promoters of a subset of mitochondrial genes that were downregulated in *Chd4*-mutant hearts or skeletal muscles. This finding implies the direct involvement of CHD4 in the activation of gene transcription. This is consistent with recent genomewide studies showing nucleosome co-occupancy by CHD4 together with multiple chromatin-remodeling complexes (including the SWI/SNF catalytic subunit

BRG1) that can either activate or repress transcription through timely and reciprocal mechanisms (Morris *et al*, 2014; de Dieuleveult *et al*, 2016).

How can CHD4 either activate or repress transcription? Previous studies indicate that CHD4 function depends on the cell type, chromatin accessibility, and its binding partners. For instance, CHD4 can be recruited to the chromatin by cell type-specific transcription factors, such as GATA3, and CHD4/GATA3 can form an activator complex with the histone (lysine) acetyltransferase p300 or a repressor complex with the rest of NuRD (Hosokawa *et al*, 2013). Moreover, CHD4 activity has been shown to be dependent on the promoter status. At H3K4me3-marked promoters CHD4 favors transcription, while at bivalent (H3K4me3 and H3K27me3-marked) and/or methylated promoters CHD4 interacts with the rest of NuRD repressing transcription (de Dieuleveult *et al*, 2016).

Collectively, these data point to CHD4 as a central component of the epigenetic machinery that coordinates activation and repression of specialized gene networks that enable skeletal and cardiac muscles to meet specific contractile and metabolic requirements (see scheme in Fig 1). Such a discretionary activity of CHD4 on transcription highlights the functional versatility of CHD4 as a contextual and facultative activator or repressor of gene expression. While the reason for the specific susceptibility of certain genes to de-repression upon loss of just one component of NuRD machinery remains unknown, it is tempting to speculate that CHD4 may have played an

important role in the evolution of the skeletal muscle and cardiac muscle lineages from more primitive precursors. Specialized chromatin-remodeling complexes, such as the CHD4-containing NuRD, might have evolved as dedicated regulators of aberrant gene transcription. Loss-of-function mutations of components of these complexes (as in the case of CHD4) could result in lethal “hybrid” skeletal/cardiac muscles that would have been negatively selected through evolution and might underlie the pathogenesis of some idiopathic myopathies.

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