Introduction

The heart is a remarkable organ that adapts to the changing hemodynamic needs of the organism throughout life by modulating its output. Acute increases in cardiovascular demand are met by an increase in cardiac contractility, whereas chronic increases associated with development, pregnancy, or sustained exercise are accommodated by cardiac growth. Despite this apparent plasticity, cardiovascular diseases are a prime health burden worldwide and are anticipated to increase even further (1). Pathologies such as chronic hypertension and aortic stenosis similarly induce cardiac growth, but while these responses are initially adaptive, they ultimately compromise cardiac output, leading to heart failure and death (2).

Since cardiomyocytes (CMs) in the adult heart are generally

highly similar intronic RNA transcript counts (Pearson's r^2

processes specific to CMs, whereas PCM1$^-$ nuclei were enriched in markers of other cardiac cell types (fibroblasts and endothelial and immune cells), highlighting the purity (>98%) and proper identity of our CM nuclei (Supplemental Figure 2, D–G). Overlaying epigenomic and transcriptomic profiles revealed that genes and regions heavily covered by H3K9me2 or H3K27me3 were transcriptionally silent, as anticipated (Figure 3, A and B, Supplemental Figure 2, H and I) (17). Notably, however, whereas H3K9me2 levels decreased linearly with increasing levels of transcription, H3K27me3 levels were consistently low across the expressed genes (Figure 3, A and B and Supplemental Figure 2, J and K). This suggests that, in adult CMs, H3K9me2 is involved in modulating gene expression levels, while H3K27me3 marks an on/off state.

In line with the more marked epigenetic changes observed upon AB, we also noted that transcriptomic changes were more pronounced in pathological hypertrophy than in physiological hypertrophy (Figure 3, C and D), with 705 and 331 protein-coding genes (3.1% and 1.4%), respectively, being differentially expressed (FDR 10%; Supplemental Tables 1 and 2). A comparison between differentially expressed genes here and those found in studies in which transcriptional changes were assessed in heart tissues confirmed that genes overexpressed in non-

hypertrophy. Surprisingly, the observed loss of *Ehmt1/2* mRNA expression was not evident from our nuclear expression analyses. Indeed, qRT-PCR confirmed that nascent *Ehmt* transcript levels were unaltered in CMs upon AB, and likewise in NRVMs upon ET-1 treatment (Supplemental Figure 6, A and B). This suggested that pathological hypertrophy reduced *Ehmt1/2* mRNA expression posttranscriptionally, possibly via a miR-dependent mechanism (33). To assess whether this effect was through mRNA destabilization via the 3′-UTR of *Ehmt1/2*, we generated luciferase reporter constructs harboring the 3′-UTRs of *Ehmt1* and *Ehmt2* and analyzed their activity in NRVMs treated with ET-1. Consistent with our hypothesis, ET-1 application reduced the luciferase activity of both reporters (Figure 7A). To identify the miR responsible for this effect, we scanned these 3′-UTRs for miR-binding sites (seed sequences) using standard tools (34, 35). Of those identified, miR-217 was predicted to target both UTRs (Supplemental Figure 6C). We functionally confirmed this prediction, as transfection of a miR-217 mimic significantly reduced the activity of luciferase constructs containing either the *Ehmt1* or *Ehmt2* 3′-UTRs in HEK293 cells, whereas other predicted miRs were without consistent effects (Figure 7B and Supplemental Figure 6D). Supporting its role in hypertrophic remodeling and in line with an earlier report suggesting increased expression with AB (36), *Mir217* expression was substantially augmented in vivo upon AB but not exercise, and also in vitro in NRVMs upon treatment with ET-1 but not IGF-1 (Figure 7, C and D). The sufficiency of this increase in *Mir217* for induction of hypertrophy was next tested. miR-217 mimic transfection reduced endogenous *Ehmt* levels in NRVMs and concomitantly triggered a pathological hypertrophic response (Figure 7E and Supplemental Figure 6E). Importantly, transfection of NRVMs with a miR-217 antagomir (miR-217) significantly blunted the ET-1–induced reduction in luciferase activity of both 3′-UTR reporters and of endogenous *Ehmt1/2* mRNA levels (Figure 7F and Supplemental Figure 6F). Furthermore, miR-217 antagomir transfection attenuated the concomitant pathological hypertrophic response (Figure 7G). These effects of miR-217 were mediated by the direct regulation of *Ehmt1/2* expression (and not through other targets of miR-217), as target site blockers that specifically prevent binding of miR-217 to their seed sequence in 3′-UTRs of *Ehmt1/2* also blunted the ET-1–induced hypertrophic response (Supplemental Figure 6, G–J).

To examine the relevance of this pathway to hypertrophy in vivo, we supp(ph[illegible]ev[illegible]ance [illegible]
antagomirs and alevlev-induced hypertrophy. Mice subject -
to AB or sham operation were injected with antagomirs target
a scrambl[illegible]n or [illegible]anc[illegible] This pr[illegible]ev[illegible]ent[illegible]ed the AB-[illegible]ind[illegible]uced red[illegible]
Supplemental Figure [illegible]A and [illegible]B). While no effects were noted in [illegible]
omirs show[illegible]ly reduc[illegible]AB-induced pathological hyper-

also induce CM hypertrophy, the phenotype of the pathological response is substantially different, as it involves, among others, activation of the fetal gene program. What underlies this difference and how the adult cellular identity is bypassed to give rise to hypertrophic remodeling are largely unknown. Here, we identify what we believe to be a novel epigenetic-based mechanism that determines the different transcriptional responses of CMs during the development of physiological and pathological hypertrophy.

With the aim of identifying epigenetic changes that underlie phenotypic differences in the hypertrophic response, we profiled repressive histone marks, comparing, for the first time to our knowledge, hypertrophy induced by pathological and physiological stressors. Hypertrophy was of equivalent magnitude between both stimuli, and in the case of pathological hypertrophy was of a mild phenotype, since our analysis was aimed at gaining mechanistic insights into the development of hypertrophy. We did not study failing hearts, in which the pathological sequelae may be confounding. Performing our analysis on CM nuclei purified from in vivo hypertrophied hearts enabled us to directly record changes in the most relevant cell type and model. We observed that H3K9me2 was substantially and pervasively reduced upon pathological, but not physiological, hypertrophy of CMs in vivo. The reduction in H3K9me2 affected a large fraction of the chromatin (~20% of all genes) and was also evident upon immunofluorescence and immunoblot analyses, underlining the magnitude of the loss. Reduced H3K9me2 affected regions where this mark was acquired during CM maturation, including those encoding fetal gene program markers (*Nppa*, *Nppb*, and *Myh7*) that are prototypical

iological, CM hypertrophy thus highlights that enzymes involved in H3K9me2 metabolism represent appealing targets for the treatment of heart disease, either directly or by inhibiting miR-217.

Methods

AB. Male Sprague Dawley rats (~170 g; Taconic) and C57BL/6J mice (15–18 g; Janvier and Taconic) were anesthetized, and a 3-0 (rats) or 8-0 (mice) silk suture was tightened around the ascending aorta proximal to the brachiocephalic trunk. Sham-operated animals served as controls. For rats, only those having nonfailing hearts with detectable hypertrophy after 6 weeks were retained. For mice, those with a mild stenosis (2.5–3.5 m/s in maximal flow velocity over aortic stenosis measured on the first or second postoperative day as indicated in the Supplemental Methods) and no signs of congestion (left anterior descending artery [LAD] <2.0 mm) were included.

Exercise training. Male Sprague Dawley rats (Taconic) Jweighing approximately 280 g were assigned to interval training on a treadmill (Columbus Instruments) at a 25-degree inclination, with 5 days of acclimatization at a running velocity of 6 m/min for 30, 45, 60, 75, 90, and 120 minutes. The-0 r w -3 n3n The-es. The-6 ilk s4 75, 90, ecity o0 mrn3n e-es.

Ethical Review Body of the Babraham Institute (United Kingdom) and conformed to the Regulations on Animal Experimentation under The Norwegian Animal Welfare Act, approved by the Norwegian Animal Research Authority (FDU applications 3301, 3820, and 5338). Permission for the analysis of human tissue was granted by the Regional Ethics Committee in Stockholm, Sweden.

Author contributions