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Glucocorticoid receptor-mediated *cis*-repression of osteogenic genes requires BRM-SWI/SNF☆

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ABSTRACT

Glucocorticoids are an effective therapy for a variety of severe inflammatory and autoimmune disorders; however, the therapeutic use of glucocorticoids is severely limited by their negative side effects, particularly on osteogenesis. Glucocorticoids regulate transcription by binding to the glucocorticoid receptor (GR), which then binds the promoters of target genes to induce either activation or repression. The gene activation effects of nuclear hormone receptors broadly require the cooperation of the chromatin remodeling complex known as SWI/SNF, which is powered by an ATPase core. The well-studied SWI/SNF ATPase, BRG1, is required for gene activation by a spectrum of nuclear hormone receptors including GR. However, glucocorticoid-induced side effects specifically related to impaired osteogenesis are mostly linked with GR-mediated repression. We have considered whether *cis*-repression of osteogenic genes by GR may be mediated by a distinct subclass of SWI/SNF powered by the alternative ATPase, BRM. BRM does not have an essential role in mammalian development, but plays a repressor role in osteoblast differentiation and favors adipogenic lineage selection over osteoblast commitment, effects that mirror the repressor effects of GR. The studies reported here examine three key GR *cis*-repression gene targets, and show that GR association with these promoters is sharply reduced in BRM deficient cells. Each of these GR-targeted genes act in a different way. *Bglap* encodes osteocalcin, which contributes to normal maturation of osteoblasts from committed pre-osteoblasts. The *Per3* gene product acts in uncommitted mesenchymal stem cells to influence the osteoblast/adipocyte lineage selection point. Fas ligand, encoded by *FasL*, is a means by which osteoblasts can modulate bone degradation by osteoclasts. Repression of each of these genes by glucocorticoid favors bone loss. The essential role of BRM in cooperation with GR at each of these control points offers a novel mechanistic understanding of the role of GR in bone loss.

1. Introduction

Glucocorticoids (GCs) are an effective therapy for a variety of severe inflammatory and autoimmune disorders; however, the use of glucocorticoids at pharmacological doses is severely limited by their negative side effects, particularly on osteogenesis (for recent reviews, see Moutsatsou et al., 2012; Manolagas 2013; Henneicke et al., 2014; Frenkel et al., 2015; Hartmann et al., 2016). Physiological levels of glucocorticoids promote skeletal growth and development, but pharmacological doses can cause osteoporosis, with fractures occurring in 30–50% of patients receiving extended GC therapy (reviewed in Hartmann et al., 2016). Glucocorticoids affect multiple cell types that impinge on bone formation and remodeling, but disruption of GC signaling in a cell-specific manner indicates that direct action of GCs on osteoblasts is the main basis for glucocorticoid-induced osteoporosis (O′Brien et al., 2004; reviewed in Henneicke et al., 2014 and Hartmann et al., 2016).

Glucocorticoids regulate expression of target genes by binding to the glucocorticoid receptor (GR). Upon binding, the GR translocates from the cytoplasm to the nucleus, where it homodimerizes and can bind to glucocorticoid response elements (GREs) in the promoters of target genes. Binding directly to positive GREs leads to upregulation of gene expression, whereas binding to negative GREs (nGREs) leads to transcriptional repression (reviewed in Moutsatou et al., 2012). The glucocorticoid receptor may also influence gene expression indirectly by binding to other DNA-bound transcription factors. By means of these

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effects on gene expression – direct and indirect, positive and negative – the glucocorticoid receptor mediates increased transcription of anti-inflammatory genes and decreased expression of pro-inflammatory genes. The anti-inflammatory effects are believed to arise from indirect repression of transcription factors that promote inflammation, as well as direct activation of anti-inflammatory gene expression.

In contrast, the side effects that specifically relate to impaired differentiation and maturation of osteoblasts are mostly linked with GR-mediated repression (Moutsatsou et al., 2012). In particular, the glucocorticoid receptor has been shown to target nGREs directly in the promoter of the osteogenic gene, *Bglap* (Aslam et al., 1995; Shalhoub et al., 1998), which encodes osteocalcin, a key component of bone extracellular matrix and a physiological marker of osteoblast activity. In addition, pharmacological doses of glucocorticoids favor diversion of mesenchymal stem cells to the adipocyte lineage rather than the osteoblast lineage (reviewed in Henneicke et al., 2014; Hartmann et al., 2016). This includes transactivation effects on pro-adipogenic genes, but glucocorticoid-mediated repression plays an important role as well by targeting inhibitors of adipogenesis. A well-characterized example is the *Per3* gene product, which forms an inhibitory complex with the pro-adipogenic transcription factor PPARγ, and is repressed (directly or indirectly) with glucocorticoid treatment (Costa et al., 2011).

We have considered whether *cis*-repression by GR may be mediated by a specific subclass of the SWI/SNF chromatin-remodeling complex. Mammalian SWI/SNF is recognized as a necessary effector for the general transcription-activating effects of the nuclear hormone receptor family including GR (King et al., 2012). The complex uses the energy of ATP hydrolysis to remodel chromatin to permit or preclude promoter access for various transcription-regulating factors. Mammalian SWI/SNF exists as subclasses, powered by either of two core ATPases. One is the BRM ATPase, which was named as an analog of the *Drosophila* protein, brahma. The second is BRG1 (the product of BRM-related gene-1). Though the two ATPases are closely related, their requirement in development is very different. Mice lacking BRG1 die early in embryogenesis (Bultman et al., 2000), while BRM-null mice are viable and fertile and live full life spans (Reyes et al., 1998). Consequently, BRG1 has been widely studied, and among other findings, appears to be essential for the transcription activation effects of nuclear hormone receptors, including the glucocorticoid receptor (Clapier and Cairns 2009, McKenna et al., 1999). On the other hand, the involvement of SWI/SNF in nuclear hormone receptor mediated repression has not been broadly addressed, but evidence outlined below suggests that the BRM ATPase may play a role in GR-mediated repression.

Despite the limited role of BRM in development, conservation of this alternative ATPase across all higher eukaryotes argues that BRM enacts biologically significant functions. Evidence that BRM has an important influence on lineage fate comes from studies of gene expression specific to osteoblasts and adipocytes. BRM is not essential for development of either lineage, but BRM depletion in mesenchymal stem cell precursors impairs adipogenic gene expression and favors commitment to the osteoblast lineage. This manifests in BRM-null mice as resistance to age-related osteoporosis with reduced bone marrow adiposity (Nguyen et al., 2015). BRM depletion releases repression of key osteoblastic genes, including the osteocalcin gene *Bglap*, whose promoter is occupied directly by BRM-SWI/SNF prior to induction (Flowers et al., 2009). BRM-depleted cells are unable to maintain effective co-occupation of the *Bglap* promoter by repressor factors that include repressor members of the E2F transcription factor family and their binding partner p130, as well as histone deacetylase-1 (HDAC1) (Flowers et al., 2011). There is a striking correlation between the effects of BRM and the biological effects of glucocorticoids on bone, including the evidence that GR and BRM both target the osteocalcin promoter directly to repress transactivation. Moreover, a recent ChIP-seq approach linked BRM with GR-mediated repression, in direct contrast to the general co-activating role of BRG1 (Engel and Yamamoto, 2011). This analysis was performed in tumor cells, but is likely to be more widely applicable. Given this background, we have investigated the possibility that BRM plays a specific role in GR-mediated repression of osteogenesis.

2. Results and discussion

2.1. BRM depletion blocks glucocorticoid-mediated repression of osteocalcin (Bglap) gene expression

The MC3T3-E1 murine calvarial cell model (Kodama et al., 1981; Sudo et al., 1983; Kartsogiannia and Ng, 2004) was used here to assess the role of BRM in glucocorticoid-mediated repression of gene expression. As a chromatin-remodeling complex, BRM-SWI/SNF acts directly by promoter association. The best-characterized gene target of glucocorticoid-mediated repression in osteoblasts is osteocalcin, a key marker of late-stage osteoblast differentiation. Expression of the osteocalcin-encoding gene (*Bglap*) is negatively affected by glucocorticoid treatment *in vitro* (Strömstedt et al., 1991). We showed previously that induction of osteocalcin gene expression is accelerated in BRM-deficient osteoblasts (Flowers et al., 2009), raising the possibility that glucocorticoid-mediated repression of osteocalcin expression may be dependent on BRM. Analysis by quantitative RT-PCR (qRT-PCR) in parental cells shows typical robust induction of osteocalcin expression at days 7 and 14 post-induction with differentiation medium (Fig. 1A). Treatment with dexamethasone blocks induction almost completely in the parental pre-osteoblasts. However, the BRM-depleted cells show accelerated induction of osteocalcin gene expression that is largely resistant to the suppressive effect of dexamethasone. A parallel experiment comparing cells depleted for the BRG1 ATPase shows BRG1 required for normal transactivation of the osteocalcin gene as expected. Notably, repression of the osteocalcin gene by dexamethasone remains effective in the BRG1-depleted cells, emphasizing that repression *via* BRM in cooperation with GR is distinct from the activation effect of BRG1.

2.2. BRM is required for efficient GR promoter access in the regulation of osteocalcin expression

Very little is known about specific gene targets of GR repression in osteoblasts, but DNAse protection assays indicate GR acts on *Bglap* by direct *cis*-repression (Strömstedt et al., 1991; reviewed in Moutsatsou et al., 2012). The osteocalcin gene is also a direct target of BRM during repression (Flowers et al., 2009), but it is not known whether GR targeting depends on BRM. We addressed this question using chromatin immunoprecipitation (ChIP) analysis (Fig. 1B). The results show that GR targets the osteocalcin promoter directly in non-induced MC3T3-E1 pre-osteoblasts when osteocalcin expression is repressed; however, in BRM depleted cells incubated in the same conditions, GR is undetectable at the promoter. The agarose gel images demonstrate the end-point PCR products directly, and quantitative analysis confirms the loss of GR and BRM. As end-point PCR is more informative in this context, this was used in subsequent assays.

In pre-osteoblasts, osteocalcin gene expression is normally repressed, and the action of GR in Fig. 1B is supported by endogenous ligand. The promoter association patterns of GR and BRM were also examined in cells induced to osteoblast differentiation in the presence or absence of exogenous dexamethasone at pharmacological levels (Fig. 1C). By day 14 post-induction in parental cells in the absence of dexamethasone, GR and BRM have both dissociated from the promoter. In the presence of exogenous dexamethasone, GR remains on the promoter at day 14 despite the differentiation-induction signals, and BRM remains present as well, implying that BRM is recruited by GR. In cells depleted of BRM, GR is not detected at the promoter prior to induction, and even exogenously added dexamethasone is not sufficient to establish GR at the promoter.

Because BRM-SWI/SNF acts at the level of transcription, there is a possibility that the failure to detect GR at the promoter in BRM-depleted cells might be caused by loss of expression of the GR-