Transcription factor MafB may play an important role in secondary hyperparathyroidism

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The transcription factor MafB is essential for development of the parathyroid glands, the expression of which persists after morphogenesis and in adult parathyroid glands. However, the function of MafB in adult parathyroid tissue is unclear. To investigate this, we induced chronic kidney disease (CKD) in wild-type and *MafB* heterozygote (*MafB*+/–) mice by feeding them an adenine-supplemented diet, leading to secondary hyperparathyroidism. The elevated serum creatinine and blood urea nitrogen levels in heterozygous and wild-type mice fed the adenine-supplemented diet were similar. Interestingly, secondary hyperparathyroidism, characterized by serum parathyroid hormone elevation and enlargement of parathyroid glands, was suppressed in *MafB*+/– mice fed the adenine-supplemented diet compared to similarly fed wild-type littermates. Quantitative RT-PCR and immunohistochemical analyses showed that the increased expression of parathyroid hormone and cyclin D2 in mice with CKD was suppressed in the parathyroid glands of heterozygous CKD mice. A reporter assay indicated that MafB directly regulated parathyroid hormone and cyclin D2 expression. To exclude an effect of a developmental anomaly in *MafB*+/– mice, we analyzed *MafB* tamoxifen-induced global knockout mice. Hypocalcemia-stimulated parathyroid hormone secretion was significantly impaired in *MafB* knockout mice. RNA-sequencing analysis indicated *PTH, Gata3* and *Gcm2* depletion in the parathyroid glands of MafB knockout mice. Thus, MafB appears to play an important role in secondary hyperparathyroidism by regulation of parathyroid hormone and cyclin D2 expression. Hence, MafB may represent a new therapeutic target in secondary hyperparathyroidism.

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Parathyroid hormone (PTH) regulates serum calcium and phosphate levels. PTH increases the serum calcium concentration by directly inducing calcium release from bone and calcium reabsorption in the kidney. Chronic kidney disease (CKD)–mineral and bone disorder is a growing health care concern associated with secondary hyperparathyroidism, mineral abnormalities, and increased risk of cardiovascular disease. Secondary hyperparathyroidism is a common disorder in patients with CKD, and is characterized by excessive serum PTH levels and parathyroid hyperplasia. In CKD–mineral and bone disorder, the degree of parathyroid hyperplasia determines the severity of secondary hyperparathyroidism and a poor response to treatment.[1,2]

MafB is a basic leucine zipper transcription factor belonging to the large Maf family.[3] Maf family proteins share a conserved basic region and leucine zipper motif that mediates dimer formation and DNA binding to the MARE (Maf recognition element).[4] MafB is essential for the development and differentiation of the parathyroid gland.[5] In MafB-deficient (*MafB*$^{-/-}$) mice, the parathyroid glands do not separate from the thymus during embryological development. Furthermore, PTH expression is severely impaired in the neonatal *MafB*$^{-/-}$ parathyroid gland. However, the role of MafB in adult mice is unclear, because of this developmental anomaly in *MafB*$^{-/-}$ mice.[6,7] Recent *in vitro* analyses revealed that MafB, Gata3, and Gcm2 synergistically enhance *PTH* gene expression.[8] Although these transcription factors are important for parathyroid development, the expression of Gata3, Gcm2, and MafB persists after parathyroid morphogenesis.[5,9–11] Of these 3 key transcription factors driving PTH expression, the *in vivo* functions of Gata3 and Gcm2 have been established, whereas the activity of MafB remains unclear. *Gata3*$^{+/-}$ mice develop hypocalcemia due to inadequate

PTH production and exhibit an earlier onset of mortality when challenged with a low calcium–vitamin D diet.[12] In humans, *GATA3* haploinsufficiency has been reported to result in the congenital hypoparathyroidism–deafness–renal dysplasia syndrome.[13] Hypoparathyroidism–deafness–renal dysplasia syndrome–associated *GATA3* mutants are defective in activating the *PTH* promoter.[8] Mice harboring a conditional deletion of *Gcm2* display hypocalcemia, as well as low or undetectable serum levels of PTH.[14] In humans, hypomorphic and dominant-negative mutations in *GCM2* lead to a decrease in PTH secretion, resulting in hypoparathyroidism.[15] In this study, to clarify the role of MafB in the adult parathyroid gland, we induced CKD in *MafB*$^{+/-}$ mice by feeding them adenine. Orally administered adenine is metabolized to 2,8-dihydroxyadenine, which precipitates and forms tubular crystals leading to kidney injury.[16] Subsequently, we analyzed the role of MafB in secondary hyperparathyroidism. Moreover, we investigated parathyroid gland function in the *MafB* tamoxifen-induced global knockout (KO) mice to reveal the role of MafB in PTH secretion.

RESULTS

MafB is expressed in the adult parathyroid gland

Although MafB is important for parathyroid development, the expression of MafB in the adult parathyroid gland has not been established. Previously, we demonstrated that green fluorescent protein (GFP) expression in the *MafB*-GFP knockin (*MafB*$^{+/-}$) mouse faithfully recapitulates endogenous MafB expression.[6] Calcium-sensing receptor (CaSR), a member of the C family of G protein-coupled receptors, is expressed most abundantly in the parathyroid gland and kidney. To further identify the GFP-positive MafB-expressing cells of the parathyroid glands, we examined double staining of GFP and CaSR in 6-week-old *MafB*$^{+/-}$ mice (Figure 1). The data confirm that MafB is expressed in the adult parathyroid gland.

MafB haploinsufficiency does not affect renal function in adenine-fed CKD mice

To assess the contribution of MafB to secondary hyperparathyroidism, CKD was induced by supplementing chow with adenine. Six-week-old wild-type (WT) and *MafB*$^{+/-}$ mice were fed either adenine-supplemented or normal chow. After 6 weeks, renal impairment and hyperparathyroidism was assessed at 12 weeks of age (Figure 2a). First, we examined renal function in WT and *MafB*$^{+/-}$ mice by measuring blood urea nitrogen and serum creatinine, as an index of renal function. Adenine supplementation led to increased blood urea nitrogen and serum creatinine levels. However, there were no significant differences between blood urea nitrogen and serum creatinine levels in adenine-supplemented WT and *MafB*$^{+/-}$ mice (Figure 2b). We also found that histological damage was similar in WT and *MafB*$^{+/-}$ mice fed adenine-supplemented chow (Figure 2c). We thus confirmed that MafB haploinsufficiency does not affect renal function in mice fed adenine-supplemented chow.

Secondary hyperparathyroidism is suppressed in *MafB*$^{+/-}$ mice fed an adenine-supplemented chow

Next, we evaluated secondary hyperparathyroidism. The level of serum calcium was reduced in both WT and *MafB*$^{+/-}$ mice fed adenine-supplemented chow (Figure 3a). However, the calcium level was reduced to a greater extent in *MafB*$^{+/-}$ mice. The serum phosphate level was increased in both WT and *MafB*$^{+/-}$ mice fed adenine-supplemented chow. However, the increase in serum phosphate was greater in *MafB*$^{+/-}$ mice (Figure 3a). The level of intact PTH was greatly increased in both WT and *MafB*$^{+/-}$ mice fed adenine-supplemented chow. However, this increase was blunted in *MafB*$^{+/-}$ mice (Figure 3a). The level of serum Fgf23 was increased to a similar extent in both WT and *MafB*$^{+/-}$ mice fed adenine-supplemented chow (Figure 3a).

The lower serum calcium and circulating PTH concentrations observed in *MafB*$^{+/-}$ mice relative to WT mice when fed adenine-supplemented chow (Figure 3a) suggest a blunted PTH secretory response in the *MafB*$^{+/-}$ mice. PTH secretion has been reported to correlate with parathyroid gland size,[17] so we next compared the size of the parathyroid glands in WT and *MafB*$^{+/-}$ mice. The size of the parathyroid glands (surface area) in WT and *MafB*$^{+/-}$ mice was similar (Figure 3b), based on measurement of cross-sectional areas following hematoxylin and eosin staining of tissue sections.[12,18] We additionally investigated the mean cell surface area, as an index for cell volume, and the cell profile number of cross-sectional areas. The mean cell surface area was calculated as the surface area/the cell profile number. The surface area, the cell profile number, and the mean cellular surface area of the parathyroid glands were increased in both WT and *MafB*$^{+/-}$ mice fed adenine-supplemented chow (Figure 3c). However, the surface area and the cell profile number increase were blunted somewhat in *MafB*$^{+/-}$ mice, but the mean cell surface area was not (Figure 3c). The enlargement of the parathyroid gland was due to increased cellular proliferation, as demonstrated by an increase in the cell profile number and mean Ki-67 proliferation rate of the parathyroid glands in both WT and *MafB*$^{+/-}$ mice fed adenine-supplemented chow. However, this effect was again blunted in the *MafB*$^{+/-}$ mice (Figure 3d and e). TdT-mediated dUTP nick end labeling analysis failed to detect apoptotic cells in the parathyroids of WT and *MafB*$^{+/-}$ mice fed a control or adenine-supplemented chow (data not shown). These findings indicate that the reduced parathyroid gland enlargement in *MafB*$^{+/-}$ mice fed the adenine-supplemented chow may be caused by a lower rate of cellular proliferation as opposed to an increased rate of cellular apoptosis.

Increased expression of *PTH* and *Ccnd2* is prevented in *MafB*$^{+/-}$ parathyroid/thyroid tissue

Transgenic mice exhibiting parathyroid-targeted overexpression of the cyclin D1, mimicking a gene rearrangement found in human parathyroid tumors, develop a hyperparathyroidism that resembles human primary adenoma.[19] Meanwhile, translocation and/or overexpression of *MAFB*