Mouse Genetics Studies on Molecular Mechanisms Underlying Skeletal Disorders

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Abstract

This paper summarizes our mouse genetics studies on the molecular backgrounds of representative degenerative skeletal disorders: osteoporosis, ossification of the posterior longitudinal ligament of the spine (OPLL), and bone fracture healing. By analyzing deficient mice, PPARγ, a key adipogenesis molecule intrinsic to bone marrow progenitors, was shown to be involved in age-related osteoporosis. Studies on deficient mice and OPLL patients revealed that insulin and insulin-like growth factor-I (IGF-I) are potent bone anabolic factors through the balance of distinct signals of the two adaptor molecules, insulin receptor substrate (IRS)-1 and IRS-2: IRS-1 for maintenance of bone turnover by up-regulating anabolic and catabolic functions of osteoblasts, while IRS-2 for retaining the predominance of the anabolic function over the catabolic function. IRS-1 was also essential for bone fracture healing. These molecules could be therapeutic targets for the skeletal disorders.

Keywords: Mouse genetics studies; Molecular mechanisms; Skeletal disorders

Introduction

Due to the rapidly increasing number of elderly individuals today, degenerative skeletal disorders are now considered a major public health issue causing chronic disability in most developed countries. Among them, osteoporosis is the representative disorder worldwide with strong social impacts [1]. Ossification of the posterior longitudinal ligament of the spine (OPLL) with a prevalence of 2-4% in Asia and somewhat lower in other countries is a disorder that causes severe tetraparesis [2]. Despite significant social demand for more information of the etiology of the disorders, the molecular mechanisms have not yet been fully elucidated. The mouse genetics approach is now considered to be a useful and efficient method due to recent progression of gene manipulating techniques in vivo. This manuscript introduces our recent mouse genetics studies attempting to elucidate the molecular mechanisms underlying the bone and cartilage disorders.

PPARγ as an intracellular molecule regulating age-related osteoporosis

Ageing is one of the major causes of osteoporosis, and the underlying mechanisms include intracellular and extracellular signals of osteoblastic cells. As an intracellular molecule regulating age-related osteoporosis, Runx2, a key transcription factor for osteoblast differentiation, is the most probable candidate, since the expression is reported to be decreased during cellular ageing of osteoblasts in vitro [3]. However, there is no in vivo evidence of its contribution to bone loss with ageing.

Osteoblasts and adipocytes are known to share a common progenitor: multipotential mesenchymal cells in bone marrow, being driven by respective key molecules Runx2 and PPARγ (Figure 1A) [4].

In addition, ageing is associated with a reciprocal decrease of osteogenesis and an increase of adipogenesis in bone marrow [5]. Hence, we investigated the role of PPARγ in bone metabolism by analyzing heterozygous PPARγ-deficient (PPARγ+/−) mice [6,7], since the homozygous deficient (PPARγ−/−) mice were embryonically lethal [8]. Although they showed no abnormality in major organs, PPARγ+/−
mice exhibited higher bone mass than the wild-type (WT) littermates, especially at older ages (Figure 1B), indicating the involvement of the PPAR, signal in the pathophysiology of age-related osteoporosis [6,7]. Ex vivo cultures of bone marrow cells showed that PPAR, haploinsufficiency caused not only a decrease in the number of adipocytes, but also an increase of osteoblasts (Figure 1C), indicating that PPARγ signal in marrow progenitors functions as a potent suppressor of commitment to osteoblastic lineage. Clinical involvement of PPARγ in bone loss was confirmed by an association study between the human gene polymorphism and bone density in Japanese postmenopausal women [9].

**IGF-I / IRS signal regulating age-related osteoporosis**

There are several cytokines/growth factors that function as extracellular signals regulating age-related osteoporosis [10], and among them IGF-I is the most probable candidate since the serum level is positively correlated with bone density of aged populations [11]. Patients with Laron syndrome caused by IGF-I deficiency also exhibit severe osteoporosis [12], indicating that IGF-I is a potent bone anabolic factor clinically.

IGF-I as well as insulin initiate cellular responses by binding to their respective cell-surface receptors, and then activate essential adaptor molecule insulin receptor substrate (IRS) followed by downstream signalling pathways like phosphatidylinositol-3 kinase (PI3K)/Akt and mitogen-activated protein kinases (MAPKs) [13]. Among the IRS family, we found that IRS-1 and IRS-2 are expressed in bone.14,15 Our further studies on mice lacking IRS-1 (IRS-1−/−) or IRS-2 (IRS-2−/−) revealed that these mice exhibited severe osteopenia with distinct mechanisms: IRS-1−/− mice showed a low bone turnover in which both bone formation and resorption were decreased (Figure 2) [14], whereas IRS-2−/− mice showed an uncoupling status with decreased bone formation and increased bone resorption (Figure 3) [15].

**Insulin signal in OPLL**

Patients with type 1 diabetes with decreased insulin level are often associated with osteoporosis [17], probably via the suppression of IRS-1 and IRS-2 signals above. Besides osteoporosis, the insulin/IGF-I signal may possibly be involved in other skeletal disorders. Although the relationship between osteoporosis and type 2 diabetes is controversial, most OPLL patients have been reported to accompany type 2 diabetes [18]. Our clinical study examining the relationship between the extent of ossification and glucose intolerance in OPLL...
patients revealed that the insulin secretory response, but not the severity of glucose intolerance or diabetes, was associated with the ossification severity (Figure 5) [19,20] confirming the clinical osteoanabolic action of insulin under the pathological condition.

IRS-1 signal in bone fracture healing

To further know the involvement of IRS-1 in bone formation, we compared the healing of bone fracture which was created at the midshaft of mouse tibia and fixed with an intramedullary nail in IRS-1−/− mice (Figure 7A) [21]. After 3 weeks, all 15 WT mice showed bone union, while in IRS-1−/− mice, only four showed bone union but with small fracture callus, and the other 11 mice remained non-union (Figures 7B and 7C). Contrarily, the fracture healing was not suppressed in IRS-2 mice (data not shown). In the ex vivo cultures of WT osteoblasts, cell proliferation stimulated by IGF-I was suppressed strongly by an inhibitor of Akt signal, moderately by an ERK inhibitor, but not by an p38 MAPK inhibitor (Figure 7D). Meanwhile, IRS-1−/− osteoblasts were less responsive to IGF-I and were inhibited only by the ERK inhibitor. These indicate that the osteoanabolic action of IGF-I/IRS-1 is mainly mediated by the Akt pathway.

Taking the clinical and mouse genetics studies together, in OPLL patients with type 2 diabetes the up-regulation of insulin production as a feedback mechanism of the impairment of insulin sensitivity in the target cells may stimulate osteoprogenitor cells in the ligament to induce ossification. Contrarily, in IRS-1−/− and IRS-2−/− mice even the increased insulin may not affect osteoblasts, resulting in osteoporosis (Figure 6).

Figure 5: (A) Correlation between OPLL extent and serum levels of diabetes-related factors in OPLL patients (n=52), (B) OPLL extent of patients with three grades of diabetes severity: non-glucose tolerance (NGT), intermediate glucose resistance (IGR), and diabetes mellitus (DM), Patients with insulogenic index equal to or higher than the average are shown as closed circles and those less than that are shown as open circles.

Figure 6: A schema of mechanisms underlying the ossification in OPLL patients, and osteopenia and impaired fracture healing in IRS-1−/− mice.
attempted to confirm the reproducibility of the mouse findings in humans using human gene polymorphism or clinical biochemical studies [22-27]. Among the molecules we identified in this study, there are some whose suppression ameliorated skeletal disorders under pathological conditions but did not affect physiological conditions, indicating that targeting on these molecules may lead to an ideal treatment without side effects on physiological functions. In fact, trials based on the present findings are being practically planned for clinical application.

References