Promising bone-related targets for rheumatoid arthritis therapy

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Abstract

Rheumatoid arthritis (RA) is a chronic debilitating autoimmune disease that results in inflammation and structural destruction of the joints. A hallmark of RA pathogenesis is an imbalance of the osteoblast–osteoclast axis driven by inflammatory processes, resulting in elevated bone resorption by osteoclasts. Current therapies used to treat this disease have focused on inhibition of synovitis, but such treatments do not adequately repair damaged bone. A key pathway of osteoclast formation involves the receptor activator of NF-κB ligand pathway acting on myeloid progenitor cells. The Wnt pathway has been shown to be important for the differentiation of osteoblasts from mesenchymal lineage precursors, and endogenous Wnt inhibitors such as Dickkopf1 and sclerostin might have important roles in osteoclast dysregulation in RA. Inhibition of the receptor activator of NF-κB ligand pathway, or blockade of Dickkopf1 and sclerostin, might serve to restore the osteoblast–osteoclast balance and repair bone erosion in RA joints. Such treatments, in combination with anti-inflammatory therapies, could stabilize and repair damaged joints and have the potential to be valuable additions to the armory of RA treatments.

Background

In addition to inflammation of the synovium, a major clinical manifestation of rheumatoid arthritis (RA) is the progressive destruction of bone and cartilage structures in the joints of patients, leading to radiographically defined features such as bone erosion and joint space narrowing. Typical pathogenic processes in the synovium of patients with RA include a thickened hyperplastic synovial layer, neoangiogenesis, and ongoing migration of macrophages and autoreactive lymphocytes into the joints resulting from the action of chemokines and proinflammatory cytokines [1]. These inflammatory processes have been intensively studied, and are known to activate bone and cartilage destruction via the action of

tumor necrosis factor (TNF) and receptor activator of NF-κB ligand (RANKL)-mediated signaling, amongst other signaling pathways, on bone resorptive osteoclast cell formation and activation of synovial fibroblasts [2]. Therapies used in the clinic for the successful treatment of RA target various aspects of these inflammatory pathways (e.g. corticosteroids, methotrexate, anti-TNF agents, interleukin [IL]-1β and IL-6 pathway blockade, B-cell depletion via targeting of CD20, and blockade of lymphocyte co-stimulation via cytotoxic T lymphocyte antigen 4 [1,3,4,5,6,7,8,9]). It is also noteworthy that bisphosphonates are also used to target bone destruction in RA resulting from inflammatory processes as well as from commonly used anti-inflammatory treatments, particularly glucocorticoids. These are pyrophosphate analogues that target osteoclasts, and are currently considered the standard of care for reducing bone loss in postmenopausal osteoporosis. Although anecdotal reports of bisphosphonate use in RA patients are widespread, there is a dearth of appropriately designed clinical trials that specifically investigate the effects of bisphosphonates in RA [10,11]. Therefore, while most of these therapies have been shown to slow progressive joint damage as determined by X-ray imaging [5,9,12,13], not all patients respond robustly to these therapies with respect to bone erosion. Hence, in addition to targeting synovitis, it is highly desirable to find mechanisms to stop and ultimately reverse bone erosion in RA.

The normal mechanism by which bones are formed and resorbed is mediated via the interaction between two cell lineages, bone-forming osteoblasts and bone-resorbing osteoclasts (Figure 1). Osteoblasts differentiate from the mesenchymal cell lineage under the control of key signals such as parathyroid hormone, the canonical Wnt–β catenin pathway and the bone morphogenetic protein (BMP) pathway [14,15]. These signaling pathways produce key bone matrix products that are subsequently mineralized. Osteoclasts differentiate from myeloid lineage precursors under the control of the key pathways involving colony stimulating factor 1 and the RANKL–RANK axis, which act at early and terminal differentiation stages respectively [2,16,17]. These cells degrade bone via expression of effector molecules such as cathepsins, matrix metalloproteinases, and local production of hydrogen ions. Thus, these two types of effector cells, derived from independent precursor lineages and with opposing functions, act in concert to maintain normal bone metabolism. Cross regulation of these cell types can occur; for example, the RANKL decoy receptor osteoprotegerin (OPG) is expressed by osteoblasts, induced by Wnt signaling [18], and acts to repress the osteoclast axis by regulating RANK signaling. Much effort has been dedicated to the study of these cell lineages given their important contribution to human diseases such as osteoporosis, osteoarthritis, ankylosing spondylitis and RA [2,19,20].

In the case of RA, the osteoblast–osteoclast axis is severely disrupted due to ongoing inflammatory processes resulting in enhanced osteoclast function [21,22]. Key macrophage-derived and T cell-derived proinflammatory cytokines such as TNF, IL-1β, IL-6 and IL-17 act in a pleiotropic network to induce expression of RANKL by synovial fibroblasts, osteoblasts and bone marrow stromal cells leading to enhanced osteoclast differentiation [23,24,25,26,27]. In addition, RANKL is expressed by activated $CD4^+$ T cells infiltrating the synovium, providing other cellular sources of this osteoclast-generating protein under inflammatory conditions [23,28,29]. TNF also acts directly on osteoclast formation via signaling through TNFRSF1A (tumor necrosis factor receptor superfamily, member 1A), and IL-1βcan upregulate the expression of RANK on osteoclast progenitors [30,31]. Increased signaling through RANK on differentiating osteoclasts results in recruitment of TNF receptor-associated factor 6 and subsequent activation of the transcription factor NFATc1 (nuclear factor of activated T-cells, cytoplasmic, calcineurin-dependent 1), which is a master regulator of osteoclastogenesis [32,33]. This inflammation-driven activation of the RANKL–RANK pathway increases osteoclast levels in the joints and thus drives bone destruction in RA.

Perturbations to the osteoblast axis have also been implicated in RA pathogenesis because of the lack of bone repair activities after formation of erosions in this disease. Indeed, a recent murine arthritis study showed that inflammation within arthritic bone resulted in a decrease in mature osteoblast lineage cells and concomitant bone formation [34]. The BMP and canonical Wnt–β catenin pathways have been implicated in osteoblast function and bone formation [35, 36,37]. BMPs signal through heteromeric BMP type I and type-II serine-threonine kinase receptor complexes, and activate downstream osteoblast transcriptional pathways via phosphorylation of receptor-regulated Smad complexes [15,37]. Wnt proteins bind to the membrane receptors low density lipoprotein receptor-related protein (LRP)5 and LRP6 together with the coreceptor frizzled (Figure 2). Formation of this transmembrane complex results in activation of an intracellular signaling pathway that releases β-catenin, via changes in its phosphorylation status, from a destruction complex comprising the proteins axin, glycogen synthase kinase 3 (GSK3) and adenomatosis polyposis coli (APC) via activation of disheveled (DVL). β-catenin accumulates in the cell and translocates to the nucleus, where it induces expression of target genes including alkaline phosphatase and CCN family genes via the β-catenin–T cell factor/lymphoid enhancer binding factor 1 complex and drives differentiation of mesenchymal cells into osteoblasts [18,38,39,40].

Importantly, there are several endogenous inhibitors of the canonical Wnt pathway that regulate osteoblast function. Dickkopf1 (DKK1), a member of a family of cysteine-rich proteins, functions as an inhibitor of Wnt signaling via competitive binding to the LRP5/LRP6 receptor complex, and by recruiting kringle containing transmembrane protein 1, a negative coreceptor [41,42]. Levels of DKK1 are increased in both experimental arthritis and in human RA synovial tissue and serum, and expression of DKK1 is induced by TNF signaling [43]. Another protein of interest, sclerostin, is a secreted glycoprotein of the evolutionary conserved differential screening-selected gene aberrant in neuroblastoma family of proteins containing a cysteine knot motif, and has been implicated as an inhibitor of both the Wnt and BMP pathways driving osteoblast formation [44,45]. Unlike DKK1 and other Wnt antagonists, sclerostin is unique in that it is produced by osteocytes and so is an endogenous mediator in the bone formation/ destruction axis [46]. Sclerostin binds to BMPs as well as to LRP5/LRP6, thus antagonizing BMP and Wnt signaling and hence osteoblast formation [47,48,49]. Inherited genetic mutation of the *SOST* gene (which encodes sclerostin in man) results in sclerosteosis, a progressive bone dysplasia disorder [50].

Given the important roles of the RANKL–RANK pathway in bone resorption, and the Wnt and BMP pathways in bone formation and repair, strategies targeting both RANK signaling as well as targeting antagonists of the Wnt and BMP pathways, such as DKK1 and sclerostin, might provide avenues both to inhibit bone destruction and potentially to repair erosions in RA.

Pre-clinical data

Inhibition of RANK signaling has been shown to be beneficial in several animal models of arthritis. In a TNF-induced model of arthritis, inflammation and bone erosion could be blocked by simultaneous treatment with anti-TNF and the RANKL decoy receptor OPG versus anti-TNF alone [51]; OPG treatment alone slowed joint destruction, but did not affect inflammation [52,53]. In a rat adjuvant-induced arthritis model, treatment with OPG at time of disease onset resulted in decreased bone erosion, but did not affect inflammatory progression [28]. Using the K/BxN mouse model of serum transferred arthritis, RANKL-deficient mice were protected against bone loss but not inflammation [54]. Similarly, in a rat adjuvant-induced arthritis model, treatment with OPG resulted in decreased loss of bone mineral density, but did not affect inflammation [55]. Blockade of intracellular signaling pathways downstream of RANK also prevented bone loss or destruction in inflammation-induced bone destruction and ovariectomy-

induced bone loss mouse models [56]. Therefore, inhibition of RANK signaling is beneficial for protection against bone damage in multiple animal arthritis models, but has little benefit in inhibiting the inflammatory axis of the disease.

Blockade of DKK1 using an antibody resulted in a decrease in bone erosion but did not affect inflammatory signs of disease in the TNF-induced mouse arthritis model and the collagen-induced mouse arthritis model [43]. Interestingly, blockade of DKK1 also resulted in new bone formation due to formation of osteophytes, indicating elevated differentiation of osteoblasts. In addition, blockade of DKK1 decreased osteoclast numbers in the joints; this finding was ascribed to upregulation of OPG levels via Wnt signaling. Further, targeted deletion of one allele of DKK1 in mice was shown to be sufficient to result in increased osteoblast number and bone formation [57]. Importantly, these data suggest that boosting osteoblast function through blockade of Wnt antagonists can overcome and repair ongoing bone destruction in arthritis independent of inflammation.

Targeted deletion of sclerostin in mice results in increased bone volume, bone mineral density and bone strength compared with wild type controls [58]. Sclerostin-deficient animals had increased osteoblast activity as well as increased serum levels of osteocalcin, an osteoblast marker, but the osteoclast axis was unaffected. A further study using the ovariectomized rat model of postmenopausal osteoporosis showed that administration of an antibody against sclerostin resulted in increased bone formation on multiple bone surfaces after 5 weeks of treatment [59]. Further, antibody-mediated blockade of sclerostin in a murine model of chronic colitis was recently shown to halt inflammation-induced bone loss [60]. These data support the notion that sclerostin, like DKK1, is an important antagonist of osteoblast formation and that inhibition of this pathway has potential benefit for boosting bone repair.

Clinical data

A monoclonal antibody against RANKL, denosumab, has been developed and tested in the clinic. The development of this antibody has focused on postmenopausal osteoporosis, and has reported positive outcome data [61]. A 12 month placebo-controlled phase 2 study of this antibody was also conducted in RA [62]. This study showed a dose responsive effect of denosumab on decrease of MRI-determined erosion scores compared with placebo as early as 6 months after treatment, and both tested doses of denosumab decreased X-ray determined Sharp erosion scores after 12 months of treatment. In addition, RANKL blockade resulted in rapid and sustained suppression of the systemic markers of bone turnover C-telopeptide of type I collagen (CTX-I) and N-propeptide of type I collagen (PINP), but only transiently decreased the cartilage marker C-telopeptide of type II collagen (CTX-II) after 3 months of treatment. Consistent with preclinical observations, denosumab treatment had no effect on measures of RA disease activity, such as components of the American College of Rheumatology (ACR) response, Health Assessment Questionnaire (HAQ) scores or the Disease Activity Score 28 (DAS28), as compared with placebo treated patients, suggesting that it does not substantially impact on the inflammatory component of RA.

Future clinical development

As discussed above, there is considerable evidence that inhibiting osteoclast function via blockade of RANKL, and enhancing osteoblast function via blockade of Wnt/BMP antagonists DKK1 and sclerostin, might have benefits for reducing ongoing bone erosion and repairing existing joint damage; however, blockade of these pathways is not likely to have an effect on inflammation-dependent signs and symptoms of RA such as joint swelling and tenderness. These mechanisms of action raise two implications for clinical development of these potential therapies: first, they will likely have to be administered in combination with well established anti-inflammatory therapies such as methotrexate or TNFα antagonists; second, appropriate

endpoints such as radiographic and MRI-defined erosion scores rather than signs and symptoms endpoints such as change in American College of Rheumatology and Disease Activity Score 28 scores must be utilized during clinical trials to determine the contribution of these therapies to meaningful clinical outcomes.

The patient population most likely to benefit from such a therapeutic intervention would also have to be carefully considered. For example, there have been reports of RA patients who are in considered to be in a state of disease remission, but yet have progressive radiographic joint destruction [63]. Such patients presumably have an inflammation-independent bone destructive disease manifestation, and might benefit more from alteration of the osteoclast–osteoblast balance rather than from other therapies targeting only the inflammatory cascade.

We need to optimize the therapeutic window for these therapies to avoid adverse effects such as pushing bone resorption or bone formation too far in the opposite direction, resulting in inappropriate bone formation characteristic of anabolic bone disorders (e.g. osteoarthritis and ankylosing spondylitis) and neoplastic transformation resulting from excessive activation of the Wnt or BMP pathways, or both [64,65]. The risk of development of oncogenesis caused by long term activation of the Wnt pathway through antagonism of DKK1 or sclerostin must also be considered [66].

Outlook

The progress made over the past few years in defining the molecular basis of control of osteoblast and osteoclast differentiation has presented new opportunities to target bone disorders in the clinic. Since bone destruction and concomitant lack of new bone formation is a major clinical manifestation of RA, therapeutic intervention to reverse these pathogenic processes should have considerable clinical benefit for patients with RA. Such therapies will likely need to be administered in combination with well established and cost effective anti-inflammatory therapies such as methotrexate, and might benefit patients who have clear evidence of bone destruction even though their inflammatory disease is well controlled. Since at present joint destruction in RA is irreversible and highly debilitating, this alternative therapeutic intervention, if successful, would be a major addition to existing anti-inflammatory treatment paradigms for this disease.

KEY POINTS

- A hallmark of RA pathogenesis is an imbalance of the osteoblast–osteoclast axis that is driven by inflammatory processes

- Current treatments for RA are targeted at synovitis but do not adequately target bone repair

- The RANKL pathway is a key driver of osteoclastogenesis, whilst the Wnt and BMP pathways drive osteoblastogenesis

- DKK1 and sclerostin have been shown to act as endogeneous inhibitors of the Wnt and BMP axes

- Targeting RANKL, DKK1 & scelerostin may have clinical benefit in reducing bone destruction and enhancing repair of erosions

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Biographies

Yongwon Choi received his Ph.D. from University of Illinois College of Medicine, Chicago, USA, and carried out postdoctoral research with John Kappler and Philippa Marrack (National

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Joseph Arron earned a PhD in immunology from The Rockefeller University in 2002 and an MD from Cornell University in 2003. After a postdoctoral fellowship at Stanford University, he started at Genentech in 2006. He directs a laboratory focusing on molecular and clinical heterogeneity and biomarker discovery in allergic and inflammatory diseases.

Michael Townsend gained a PhD in Immunology from the University of Cambridge in 2001 followed by a postdoctoral fellowship at Harvard University. He now directs a laboratory at Genentech that studies disease mechanisms and heterogeneity focused on discovery of biomarkers in autoimmune diseases.

Acknowledgments

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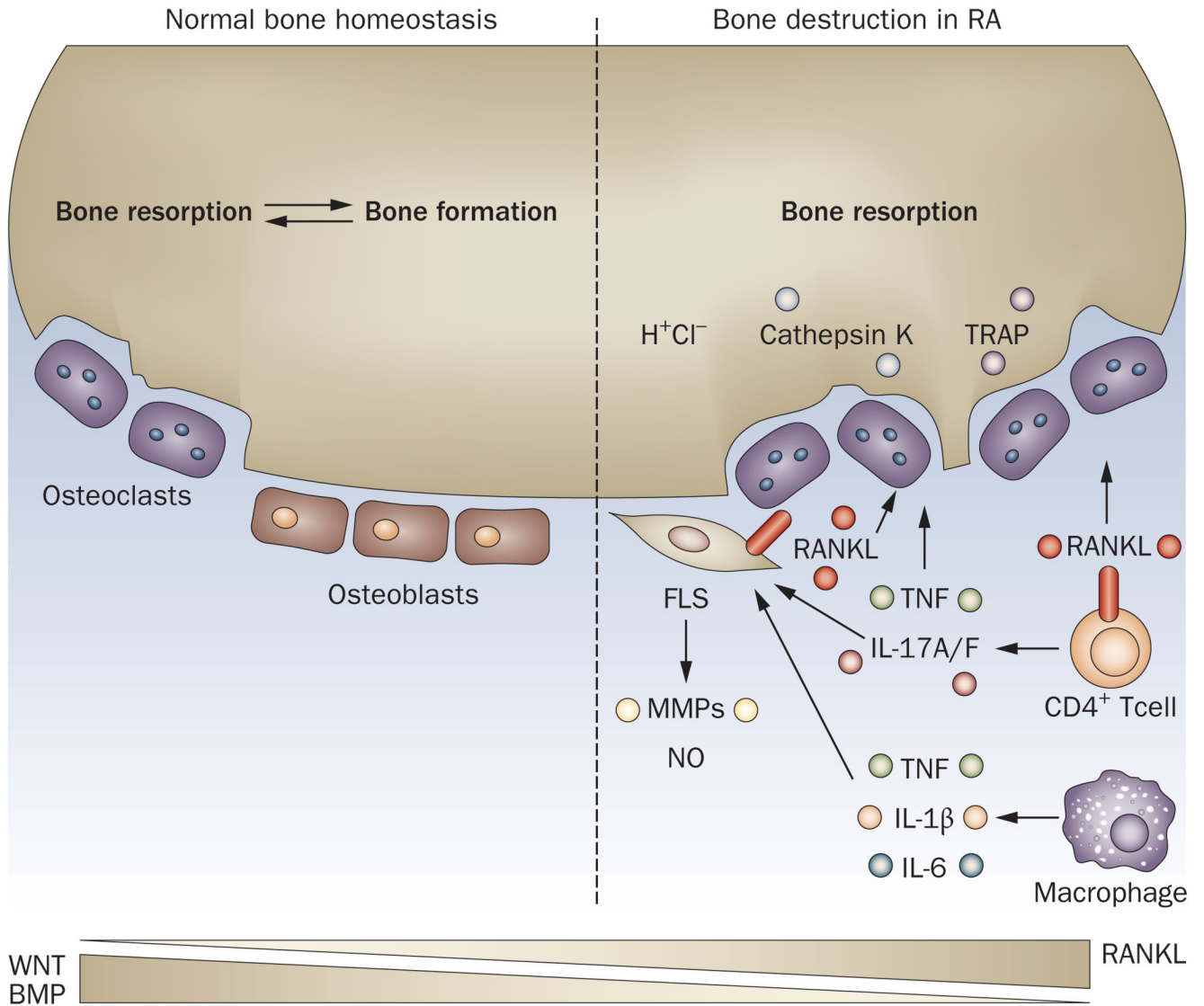
Figure 1. Bone homeostasis in healthy and RA joints
In normal joints, bone formation and bone resorption are maintained by the balanced function of osteoblasts and osteoclasts. The molecular basis of this homeostasis is controlled in part by the opposing actions of Wnt and BMP pathways on osteoblasts and the RANKL pathway on osteoclasts. Under the inflammatory conditions of RA, activity of infiltrating macrophages and CD4^{+} T cells results in expression of proinflammatory cytokines such as TNF that drive osteoclast formation via induction of RANKL in the synovium. In addition, RANKL is expressed on synovial fibroblasts and infiltrating T cells. The resulting osteoclasts, and associated local production of H^{+} ions and cathepsin K, result in increased bone resorption and joint destruction.
BMP, bone morphogenetic protein; FLS, fibroblast-like synoviocyte; IL, interleukin; MMPs, matrix metalloproteinases; NO, nitric oxide; RA, rheumatoid arthritis; RANKL, Receptor activator of NF-κB Ligand; TNF, tumor necrosis factor; TRAP, tartrate-resistant acid phosphastase
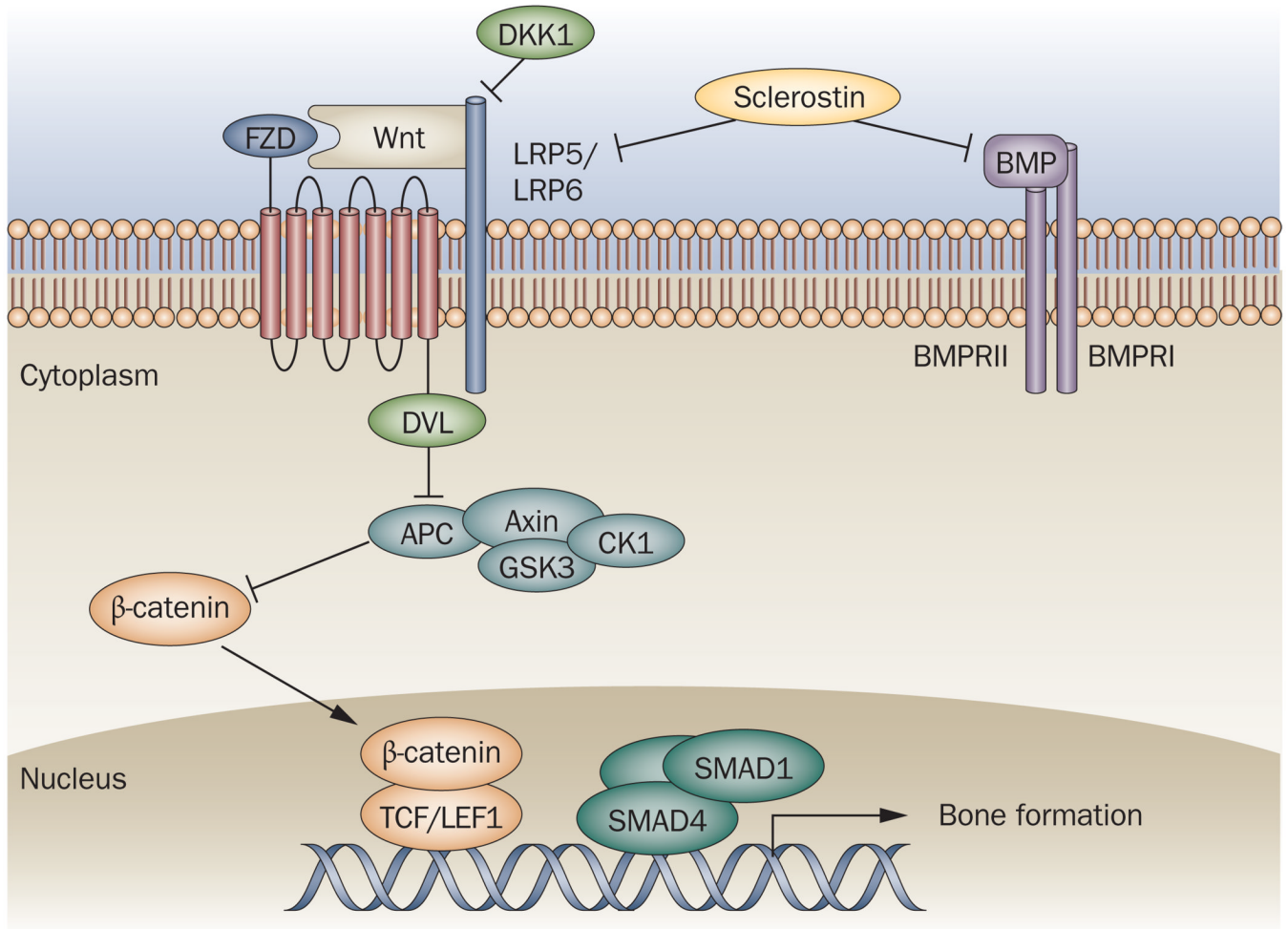
Figure 2. Osteoblast differentiation pathways
Osteoblast formation from mesenchymal progenitors is controlled by the canonical Wnt–β catenin and the BMP pathways. Engagement of plasma membrane Wnt receptors LRP5 and LRP6 and the coreceptor FZD by Wnt leads to activation of DVL, which blocks a protein complex comprising axin, APC and GSK3. This allows the translocation of β-catenin to the nucleus, where it complexes with TCF/LEF1 and binds DNA. In addition, binding of BMP to its membrane receptor complex activates a signaling cascade involving heteromeric SMAD protein complexes, which translocate to the nucleus and bind DNA. Both of these transcriptional complexes then drive osteoblastogenic gene expression programs. DKK1 and sclerostin are endogenous proteins that inhibit osteoblast differentiation by binding to the LRP receptors and blocking Wnt docking. In addition, sclerostin binds BMPs and so can potentially block this pathway too.
APC, adenomatosis polyposis coli; BMP, bone morphogenetic protein; BMPR, bone morphogenetic protein receptor; CK1, casein kinase 1; DKK1, Dickkopf1; DVL, human homolog of the Drosophila dishevelled gene; FZD, frizzled; GSK3, glycogen synthase kinase 3; LRP, LDL receptor-related protein; SMAD, TCF/LEF1, T cell factor/lymphoid enhancer binding factor 1.