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# Emerging role of lncRNA DANCR in progenitor cells: beyond cancer

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**Abstract. – Long noncoding RNAs (lncRNAs) are important participants in biological processes including cell proliferation, differentiation and death, as well as pathogenesis of various diseases. LncRNA differentiation antagonizing non-protein coding RNA (DANCR) is an emerging regulator in cell metabolism and many diseases besides cancers. DANCR is negative in epidermal, osteoblastic and endoderm differentiation, but positive in chondrogenic differentiation of progenitor cells. It is protective for calcification of the ligamentum flavum, stroke, acute myocardial infarction and arterial calcification, but a risk factor for bone loss, fracture healing and idiopathic pulmonary fibrosis. In addition, DANCR is a potential target for improving tissue regeneration. Mechanically, DANCR, a cytoplasmic lncRNA, sponges corresponding microRNAs or interacts with various proteins. This review aims to summarize the role of DANCR in progenitor cells and provide perspectives for further studies.**

*Key Words:*
LncRNA DANCR, Differentiation, Progenitor cells, Gene expression.

### Abbreviations

LncRNAs: Long noncoding RNAs; DANCR: Differentiation antagonizing non-protein coding RNA; ANCR:Anti-differentiation non-protein coding RNA; MAF: MAF bZIP transcription factor; MAFB: MAF bZIP transcription factor B; GRHL3: Grainyhead like transcription factor 3; ZNF750: Zinc finger protein 750; KLF4: Kruppel like factor 4; PRDM1: PR domain zinc finger protein 1; BMSC: Bone marrow mesenchymal stem cells; BMD: Bone mass density; EZH2: Enhancer of zeste homolog 2; FOXO1: Forkhead box protein O1; PRC2: Polycomb repressive complex 2; Runx2: Runt-related transcription factor 2; MAPK: Mitogen-activated protein kinase; SphK2: Sphingosine Kinase 2; IL-6: Interleukin 6/8; ceRNA: competitive endogenous RNA; JAK2: Janus kinase 2; STAT3: Signal Transducer And Activator Of Transcription 3; SMSCs: Synovium-derived mesenchymal stem cells; Sox4/9: SRY-related HMG box 4/9; Cdk2/4: Cyclin Dependent Kinase 2; AGC1: Aggrecan1; Col2: Collagen2; MMP13: Metalloproteinase13; PDL: periodontal ligament; DPSCs: dental pulp stem cells; PDLSC: periodontal ligament stem cells; OIIRR: Orthodontically induced inflammatory root resorption; AMSCs: Adipose-derived mesenchymal stem cells; PTBP1: polypyrimidine tract-binding protein 1; ID2: Inhibitor of DNA binding 2; IPF: Idiopathic pulmonary fibrosis; CLF: ligamentum flavum; BMECs: Brain microvascular endothelial cells; OGD: Oxygen-glucose deprivation; XBP1: X-box binding protein 1 spicing; PI3K: Phosphoinositide 3-kinase; AKT: Protein kinase B; mTOR: mammalian target of rapamycin; ERK: Extracellular-signal-regulated kinase; VSMCs: Vascular smooth muscle cells; BMP2: Bone morphogenetic protein-2.

## Introduction

Approximately 70-90% of the genome can be transcribed into RNA in a cell-specific manner, in which less than 2% is encoded into protein[1]. Vast majority of transcribed genome produces long noncoding RNAs (lncRNAs) comprised of transcripts over 200 nucleotides in length, lacking capacity of coding protein[2]. Numerous studies revealed that lncRNAs are important participants in biological processes including cell proliferation, differentiation, senescence, canceration and death by regulating gene expression at the epigenetic, transcriptional, and post-transcriptional levels[3].

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LncRNA differentiation antagonizing non-protein coding RNA (DANCR), a single 855-bp RNA transcript, has been demonstrated to play an important role in many diseases including tumorigenesis[4]. Jin et al[5] summarized the oncogenic functions of DANCR and concluded that DANCR is an emerging therapeutic target for various human cancers. Nevertheless, DANCR is more than an onco-lncRNA. It participates in several diseases of skeletal system, cardiovascular system, dental tissue and so on. In addition, DANCR is a potential target for improving tissue regeneration. Thus, this review aims to summarize the physiological and pathological roles of DANCR in progenitor cells based on present studies.

## *LncRNA DANCR in Epidermal Differentiation*

Epidermis is a physical barrier to external environment, which undergoes constant renewal[6]. Keratinocytes located in the basal layer migrate outward as they were withdrawn from cell cycle and engaged in epidermal differentiation, then forming new spinous, granular, and cornified layers[7] (Figure 1A). The healthy epidermis needs a balance between the self-renewal and terminal differentiation of progenitor cells.

Khavari et al[8] analyzed the lncRNA expression in epidermal progenitor populations *vs.* differentiating cells, finding that NR_024031, a single 855-bp RNA transcript, is significantly down-regulated upon the terminal differentiation of keratinocytes. Then they named this lncRNA as ANCR (anti-differentiation ncRNA), which was renamed as DANCR in subsequent studies[9]. The depletion of DANCR in epidermal progenitor cells could lead to rapid differentiation gene induction without extra stimuli[8]. Lopez-Pajares et al[10] further revealed that DANCR suppression enhances the transcription of MAF and MAFB, which initiate the expression of key transcription factors, including GRHL3, ZNF750, KLF4, and PRDM1, to engage terminal epidermal differentiation (Figure 1B).

In addition, Khavari et al[8] also reported that DANCR is widely expressed in a variety of human tissues and cell lines. And they observed that DANCR levels decreased significantly in the differentiation of osteoblasts and adipocytes[8]. Their innovative work stimulated subsequent investigations on DANCR's functions in multiple biological processes.

## *LncRNA DANCR in BSMCs and Osteogenesis*

Bone is an organ undergoing dynamic balance between bone formation by osteoblasts and bone resorption by osteoclasts[11]. Mature osteoblasts *in vivo* are not able to divide, only supplemented by the constant proliferation and osteogenic differentiation of bone marrow mesenchymal stem cells (BMSCs)[12] (Figure 2A). Thus, malfunctions in osteogenesis of BSMCs could result in the weakness in bone formation,

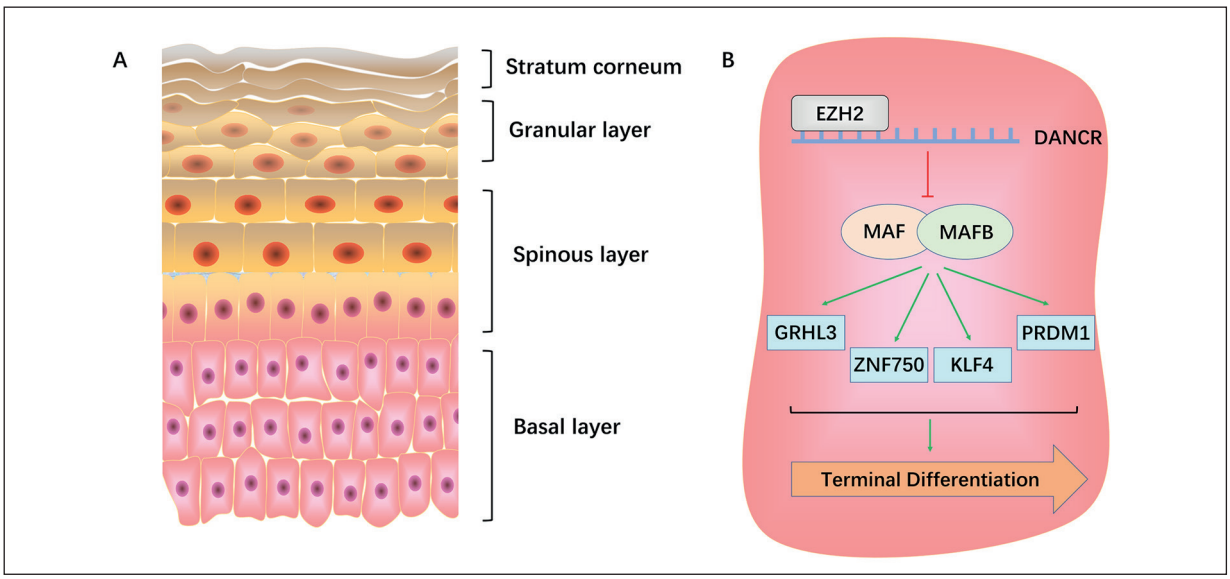


**Figure 1. A,** The histological structure of epidermis. **B,** The role of DANCR in the network of epidermal differentiation regulation.

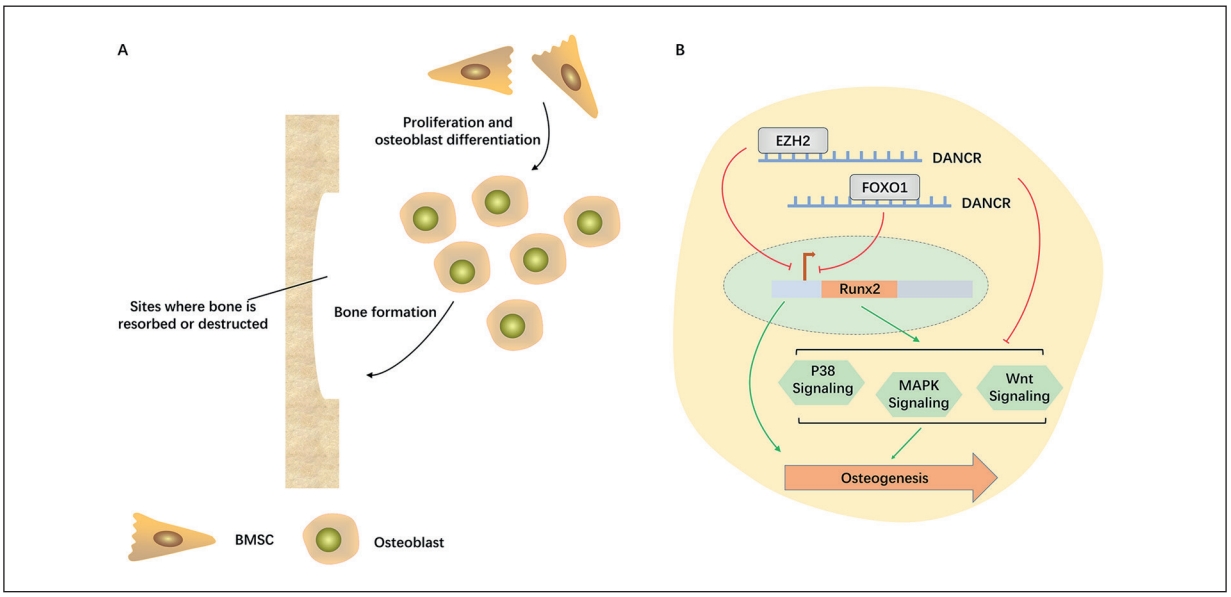


**Figure 2. A,** A diagrammatic sketch for the basic process of bone repair. When a site in bone were resorbed or destructed, BMSCs were activated and differentiated into osteoblast to form new bone tissue. **B,** DANCR in the regulation of BMSC osteogenesis.

leading to bone diseases, such as osteogenesis imperfecta, osteoporosis, and delayed healing in bone fractures[13].

The level of DANCR was proven to be associated with osteoporosis and fractures. In mice model of postmenopausal osteoporosis, DANCR is significantly enhanced in ovariotomized mice[14]. In addition, DANCR level in serum samples from fracture patients with relatively low bone mass density (BMD) is significantly higher than those from healthy controls with normal BMD[15,16]. Besides, some patients undergoing total hip replacement, the most common methods for the treatment of femoral neck fracture, may suffer from aseptic loosening of artificial hip joint resulting from osteolysis of tissue surrounding the prosthesis[17] and DANCR expression is upregulated in periprosthetic tissues compared with the surrounding normal tissues[18]. These data suggest DANCR is a risk factor for osteoporosis and delayed fracture healing.

In consistence, DANCR is significantly down-regulated in BMSCs during osteogenic differentiation[19]. And several studies demonstrated that DANCR knockdown significantly enhances the osteogenic differentiation of BMSCs, and DANCR overexpression has inhibitory effects. The known direct targets of DANCR are Enhancer of zeste homolog 2 (EZH2) and Fork box transcription factor 1 (FOXO1)[18,19]. (1) EZH2 is the catalytic subunit of PRC2, which catalyzes H3K27me3, leading to the repression of corresponding gene transcription[20]. DANCR is able to physically interact with EZH2 and recruit EZH2 to Runx2 gene promoters, resulting in the depression of Runx2 expression and subsequent osteoblast differentiation suppression[19]. (2) FOXO1 belongs to fork box protein family, involving in metabolism, cell proliferation, oxidative stress (OS) and cell death[21]. Previously, FOXO1 was reported to interact with the promoter of Runx2 to enhance osteogenic differentiation[22]. DANCR binds to FOXO1, which increases its ubiquitination and quickens its degradation, leading to Runx2 downregulation and osteoblast differentiation inhibition[18] (Figure 2B).

Proliferation of osteoblast progenitors is also promoted by DANCR knockout and suppressed by DANCR overexpression[15,23]. And studies demonstrated that DANCR downregulation activates p38, MAPK and Wnt/β-catenin pathways, contributing to the enhancement of both osteoblastic differentiation and proliferation of BMSCs[15,23] (Figure 2B).

Given all the above, DANCR seems to be a negative regulator in osteogenesis of BMSCs. However, there were still two major limitations in present studies. (1) The association of DANCR and BMSCs haven't been proven by *in vivo* studies, such as transgene mice model. (2) It was showed significant upregulation of DANCR in the blood mononuclear cells from low-BMD pa-

tients, suggesting DANCR might also participate in the differentiation of osteoclasts[24]. However, no study about the influence of DANCR on osteoclastogenesis were reported. Therefore, more high-quality studies are needed to reveal the effects of DANCR on bone metabolism.

## LncRNA DANCR in Chondrocytes and Chondrogenesis

### *DANCR Alterations in OA Chondrocytes*

Osteoarthritis (OA) is the most common chronic joint disease in the elderly population, characterized by progressive destruction of cartilage, subchondral sclerosis, and synovial inflammation[25] (Figure 3A). Though the pathogenesis of OA is complicated, chondrocyte as the only cell type in mature cartilage plays a main role in the degenerative process of cartilage in OA patients[26]. In the early stage of OA, chondrocytes near the superficial layer divide, form clusters, and exert enhanced anabolic effects in order to repair the tissue[27]. But as the disease progresses, the chondrocyte phenotype is lost and transformed towards matrix destruction and inflammatory cytokine production, resulting in eroded cartilage[27,28].

A higher level of DANCR was found in serum and cartilage specimen of OA patients[29-31]. Intriguingly, overexpression of DANCR promotes the proliferation and inhibited the apoptosis of OA chondrocytes *in vitro* through competitively binding to miR-577 to upregulate SphK2, while DANCR knockdown suppresses the viability of chondrocytes[29]. Nevertheless, DANCR also promotes the production of inflammatory cytokines including IL-6 and IL-8 by acting as a competitive endogenous RNA (ceRNA) for miR-216a-5p to activate JAK2/STAT3 signaling pathway[30] (Figure 3B). Considering that OA arises from an imbalance between the repair and destruction of joint tissues, DANCR may have bidirectional effects on the progression of cartilage deterioration. The increase of DANCR may activate the division of chondrocytes to repair the joint, which alleviates the development of cartilage erosion. On the other hand, DANCR could boost the production and release of inflammatory cytokines, contributing to synovial inflammation which induces chondrocyte senescence and phenotype loss. Further *in vivo* studies should be performed to confirm the functions of DANCR in the pathogenesis of OA.

### *DANCR Regulates the Chondrogenesis of Synovium-Derived Stem Cells (SMSCs)*

For now, existing clinical therapies for OA focus on relieving symptoms rather than reversing the pathological condition[32]. Developing alternative treatment strategy to restore damaged cartilage is quite necessary. Autologous chondrocyte implantation is promising for treating cartilage defects, but its clinical application was limited owing to the insufficient donor sources[33].

SMSCs, existing in joint synovium, are considered as a possible source for cartilage regeneration[34]. SMSCs have high chondrogenic potential and were successfully used for cartilage repair proved by improved MRI features and clinical

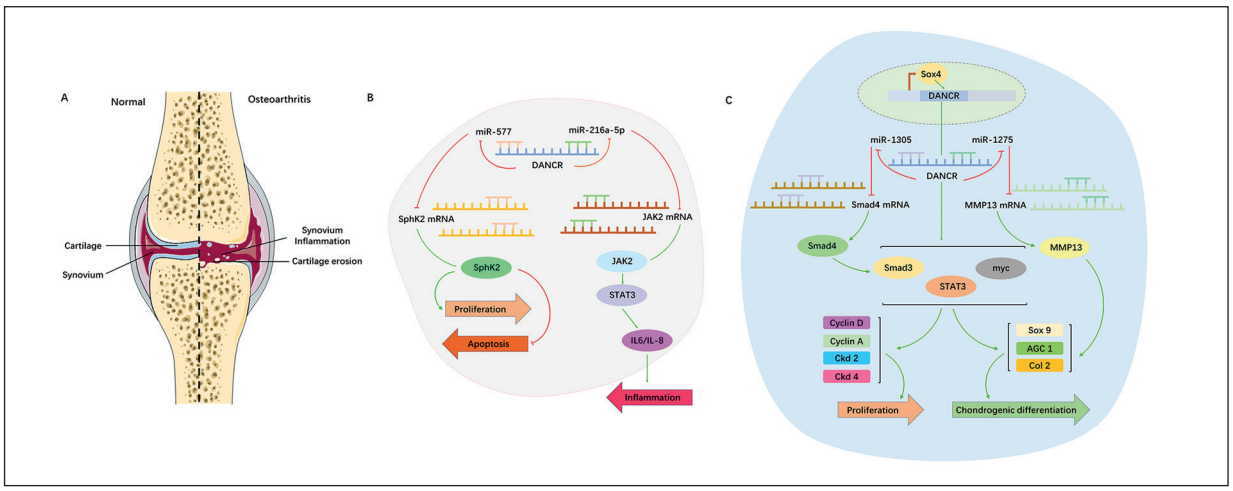


**Figure 3. A,** A diagrammatic sketch for osteoarthritis. Osteoarthritis is characterized by cartilage erosion and synovial inflammation. **B,** The functions of DANCR in OA chondrocytes. **C,** The role of DANCR in chondrogenesis of SMSCs.

outcomes in pigs[35]. And it would be helpful to promote the proliferation and chondrogenic differentiation capacity of SMSCs through molecular regulation for tissue regeneration.

Several studies reported that DANCR increases chondrogenesis in SMSCs[36-39]. Sox4, a member of SRY-related HMG-box (Sox) family, was revealed to enhance chondrogenic differentiation and proliferation of human SMSCs. DANCR level is upregulated after Sox4 directly binding to its promoter[39]. DANCR overexpression results in increased proliferation rate and heightened proliferation markers expression including Cyclin D, Cyclin A, Cdk2 and Cdk4[39]. Mass formation experiment showed that DANCR overexpressed SMSCs could form larger tumor compared to SMSCs with control vector when injected into the nude mice[39]. In addition, DANCR raises chondrogenic markers including Sox9, AGC1 and Col2 in SMSCs[38,39] (Figure 3C). Besides, DANCR knockdown suppressed cell proliferation and chondrogenic differentiation[37,39].

The following downstream mechanisms of DANCR are discovered in SMSCs: (1) DANCR interacts and stabilizes the mRNA transcripts of myc, STAT3, and Smad3, all of which are acknowledged positive regulators of chondrogenesis[37]. (2) DANCR can also directly sponge to miR-1305 to remove its inhibition on Smad4 expression. Then Smad4 is involved in TGF-β signaling via Smad2/3[38]. (3) DANCR can serve as ceRNA of miR-1275, which binds to and unstablize the mRNA of Metalloproteinase (MMP)13[36]. MMPs are key regulators in collagen degradation, and MMP13 was reported to regulate chondrogenesis positively (Figure 3C).

## LncRNA DANCR in Dental Regeneration

Teeth are organ containing two mineral layers, enamel and dentine, which form an integrated complex with the soft inner pulp tissue[40] (Figure 4A). Dental diseases, such as periodontitis, could cause massive tissue destruction including alveolar bone, periodontal ligament (PDL), and root cementum[41]. Adult stem cells derived from dental tissues, such as dental pulp stem cells (DPSCs) and periodontal ligament stem cells (PDLSCs), are thought to be good sources of dental regeneration for damaged tissue[42]. Because of the complexity of dental tissue structure, achieving directional differentiation is one major concern for stem-cell based therapy[43]. Moreover, the present studies revealed that DANCR is a regulator in differentiation of dental tissue derived stem cells, which makes it a target for improving tooth regeneration[44].

### DANCR in Odontoblast Differentiation DPSCs

The repair of severe dental damage, especially those penetrating dentine into pulp, need odontoblasts, which form new dentin-pulp complex to restore the lesion[45]. DPSCs are the progenitor of odontoblasts, of which high proliferation rate and strong differentiation ability make it a favorable source for tooth regeneration[46]. In addition, transplantation into nude mice revealed that DPSCs are able to generate functional dental tissue in the form of dentine/pulp-like complexes[47,48].

DANCR expression is significantly down-regulated in odontoblast differentiation of DPSCs

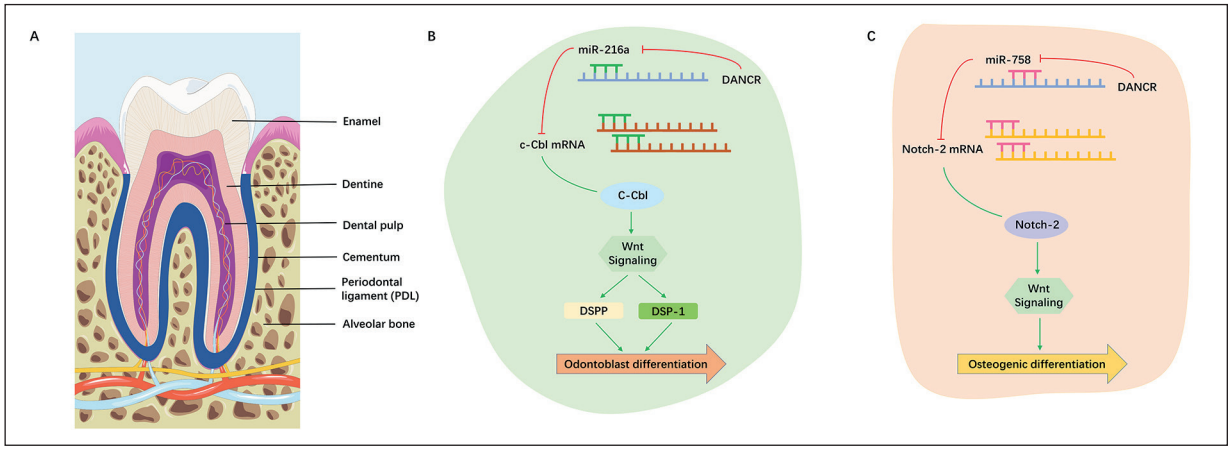


**Figure 4. A,** The histological structure of teeth. **B,** DANCR in the regulation of DPSCs odontoblast differentiation. **C,** DANCR in the regulation of PDLSCs osteogenic differentiation.

in a time-dependent manner[49]. DANCR knockdown promotes the ability of mineralized nodule formation and the expression of DSPP and DMP-1 in DPSCs, while DANCR overexpression blocks odontoblast differentiation of DPSCs[49]. It is showed that DANCR could sponge miR-216a, competitively relieving its binding to the 3'-UTR of c-Cbl[50]. Thus, DANCR downregulation leads to the decrease of c-Cbl, further stimulating the Wnt signaling, resulting in enhanced odontoblast differentiation[49,50] (Figure 4B).

### DANCR in Osteogenic Differentiation of PDLSCs

PDL is a connective tissue connecting the tooth *via* cementum and the periodontal ligament with alveolar bone[51]. PDLSCs within PDL express mesenchymal stem cell markers and are capable of differentiating along mesenchymal cell lineages to produce cementoblast-like cells, which makes it important for the homeostasis and regeneration for periodontium[52]. *In vivo* transplantation showed that PDLSCs could form adjacent bone tissue with cementum-periodontal ligament complex[53].

The osteogenic differentiation of PDLSCs is generally accepted to be similar with the differentiation of BMSCs into osteoblasts[54]. As expected, DANCR decreases notably in osteogenic differentiation and DANCR knockout enhances the proliferation and osteogenesis of PDLSCs[55]. DANCR serves as the sponge of miR-758 which binds to 3'-UTR of Notch-2[56]. DANCR/miR-758/Notch2 axis then regulates Wnt/β-catenin signaling that activates the osteogenic differentiation and regeneration of PDLSCs[56,57] (Figure 4C).

Based on the present studies, DANCR regulates the differentiation of stem cells derived from dental tissues, which may be a novel therapeutic target for tooth regeneration. Besides, DANCR also participates in the pathogenesis of certain dental diseases. For example, Orthodontically induced inflammatory root resorption (OIIRR) is an unavoidable pathologic consequence of orthodontic treatment, which causes destruction of the root apex structure[58]. An *in vivo* rat study reported that DANCR in PDL cells increases in respond to compression force, which results in enhanced Jagged production, then stimulating osteoclast formation and root resorption[59]. Thus, the role of DANCR in dental diseases and regeneration need further research.

## Others

### Endoderm Differentiation

The endoderm is one of the primary germ layers during embryogenesis and can give rise to organs including the thyroid, lungs, pancreas, liver, and intestines[60]. The irreversible damage to these tissues often need organ transplant, which is difficult, expensive and with complications[61]. Thus, the generation of endoderm-derived lineages could be beneficial for regenerative medicine, as well as drug testing and toxicological studies[62].

Adipose-derived mesenchymal stem cells (AMSCs) are reliable regenerative resources, which could be induced to endoderm lineages and organs. DANCR level dramatically declines during the differentiation of hAMSCs to endoderm. DANCR interacts with RNA-binding polypyrimidine tract-binding protein 1 (PTBP1) to facilitate its association with ID2 mRNA, leading to increased ID2 mRNA stability, then restricting the endoderm differentiation[63] (Figure 5A).

### DANCR in Idiopathic Pulmonary Fibrosis (IPF)

IPF is an aggressive type of lung disease resulting in scarring of lung tissues whose etiology remains largely unknown[64]. The scarring full of abnormally proliferated myofibroblasts/fibroblasts and excessive deposition of extracellular matrix progresses quickly, which could lead to respiratory failure[65].

DANCR is upregulated in the fibrogenesis of alveolar type II epithelial (RLE-6TN) cells. DANCR overexpression increases FOXO3 levels by binding to and guiding AUF1 to activate the translation of FOXO3 mRNA, leading to promotion of the proliferation and fibroblastic differentiation of RLE-6TN cells and the initiation of IPF66 (Figure 5B).

### DANCR in Calcification of the Ligamentum Flavum (CLF)

CLF is a spinal disease that mostly occurs in the aged Asian population[67]. CLF is characterized by the formation of ossific-calcific components in the ligamentum flavum, leading to posterior spinal cord compression, which may cause myelopathy and successive neurological deficits[68].

Decreased DANCR levels in ligamentum flavum from CLF patients are found compared with healthy control. The knockdown of DANCR enhances chondrogenic differentiation and calcification of ligamentum flavum-derived mesenchy-

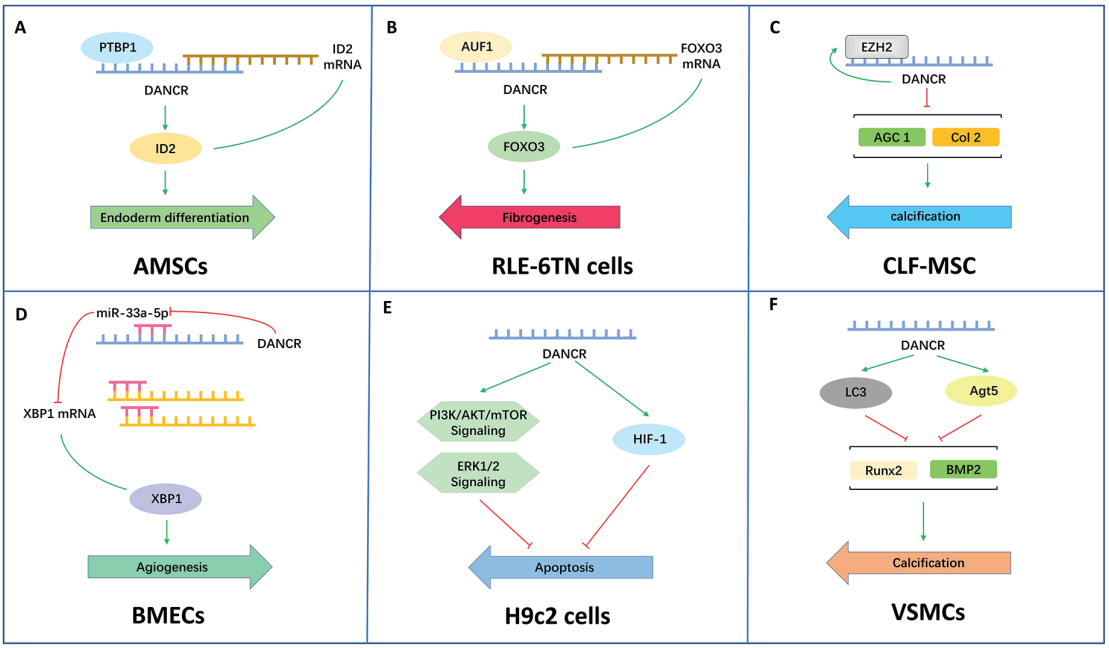


**Figure 5.** Functional properties and molecular mechanisms of DANCR in cell types and different diseases.

mal stem cells (CLF-MSCs), then contributing to the CLF pathogenesis. This study revealed that DANCR enhances the EZH2 level to form more DANCR/EZH2 complexes, which suppress calcification markers including AGC1 and Col2. However, the direct target of DANCR/EZH2 complex remains uncovered[69] (Figure 5C).

### *DANCR in Stroke [Brain Microvascular Endothelial Cells (BMECs)]*

Stroke occurs when the blood supply of the brain was suddenly interrupted or reduced, of which 85% are caused by ischemic stroke[70]. BMECs are critical components of brain microvasculature, which form blood-brain barrier and maintain brain integrity[71]. Ischemic stroke causes BMECs death, leading to brain edema and poor prognosis of patients[72].

Oxygen-glucose deprivation (OGD) treatment is an *in vitro* model of ischemic stroke. Increased DANCR expression is shown at 2, 4, 6, and 8 h after OGD-treated BMECs. DANCR sponges miR-33a-5p, inhibiting its binding to 3′-UTR of XBP1, leading to the increase of XBP1 which exerts protective effects on the survival and angiogenesis of BMECs[73] (Figure 5D).

### *DANCR in Acute Myocardial Infarction*

Acute myocardial infarction (AMI) is caused by sudden blockage of blood supply to heart muscle, resulting in oxygen deficiency in myocardial cells. Then hypoxia cause myocardial cell necrosis, heart failure and even death[74].

In hypoxia-induced heart muscle (H9c2) cells, which is an *in vitro* model for AMI, DANCR expression is negatively regulated compared to the control. Overexpression of DANCR could alleviated obvious cell activity inhibition and apoptosis in hypoxia-induced H9c2 cells, by activating PI3K/AKT/mTOR and ERK1/2 pathways and enhancing HIF-1α expression[75](Figure 5E).

### *DANCR in Arterial Calcification*

Aged people or patients with hypertension, chronic kidney disease and diabetes mellitus are susceptible to arterial calcification, a risk factor for cardiovascular diseases and mortality[76]. Vascular calcification is caused by osteoblastic differentiation of vascular smooth muscle cells (VSMCs), characterized by expression of key osteoblast transcription factors including Runx2 and BMP2[77].

DANCR overexpression could suppress β-glycerophosphate (β-GP)-induced VSMCs osteoblastic differentiation and mineralization by promoting LC3 and autophagy protein 5 (Agt5) levels, then down-regulating Runx2 and BMP2 expression. In addition, injection of DANCR overexpressing lentivirus attenuates high calcitriol-induced mice model of arterial calcification[78](-Figure 5F).

**Table I.** Real time PCR primers.

| Diseases type | DANCR expression | Protective or progressive | Functional properties (validated) |
|---|---|---|---|
| Osteoporosis | ↑ in BMSCs | Progressive | Inhibiting proliferation and osteogenic differentiation |
| Fracture | ↑ in BMSCs | Progressive | Promoting proliferation, |
| osteoarthritis | ↑ in chondrocytes | NA | inflammation and inhibiting apoptosis |
| OIIRR | ↑ in PDL cells | Progressive | Promoting inflammation and osteoclastogenesis |
| IPF | ↑ in lung cells | Protective | Inhibiting fribrogenesis |
| CLF | ↓ in CLF | Protective | Inhibiting chondrogenic differentiation and calcification |
| Stroke | ↑ in OGD-treated BMECs | Protective | Inproving survival and angiogenesis |
| AMI | ↓ in hypoxia-induced H9c2 cells | Protective | Inhibiting apoptosis |

## Conclusions and Future Perspectives

From this review, we could conclude that one major function of DANCR is regulating differentiation of progenitor cells. DANCR was named for its differentiation antagonizing effects in keratinocytes. However, many studies revealed that DANCR is not just a suppressor of differentiation, but more like a controller of differentiation orientation. For example, DANCR promotes chondrogenesis of SMSCs and inhibits osteoblastic differentiation of BMSCs. Another complexity is that DANCR is a protective factor in some diseases but a risk factor in others. Many investigations focused on the association of DANCR and osteoblastic/osteoblastic-like differentiation in MSCs and indicated that DANCR is a negative regulator for osteogenesis in various cell types. Thus, high DANCR expression could be a risk factor for osteoporosis and delayed fracture healing, but protective for diseases caused by heterotopic ossification such as CLF and arterial calcification (Table I). From all the above, it seems that DANCR is not a perfect therapeutic target for systematic treatment. But still the researchers on DANCR could reveal the mechanisms of multiple biological processes and diseases, which is very helpful for seeking new molecular therapeutic targets.

A promising clinical appliance of DANCR is in tissue regeneration. The transplant of progenitor cells into damaged tissue is a rising method for tissue repair. Adjustment of certain factors such as lncRNA DANCR *in vitro* to enhance the differentiation of progenitor cells is a relatively safe way to promote treatment efficiency. Present studies showed that DANCR overexpression activates chondrogenesis of synovium-derived stem cells and DANCR knockdown enhances osteoblastic-like differentiation of DPSCs and PDLSCs, making it possible to be applied into skeletal and dental regeneration. Because DANCR is widely expressed in numerous cell types, it may also be a target for modulating regeneration of other tissues. Therefore, more high-quality, especially *in vivo* studies are needed in this area.

Besides, DANCR also participants in the regulation of inflammation. And we noticed that DANCR is relatively highly expressed in lymphocytes, suggesting it may be important in lymphocyte differentiation and immune functions[8]. But there is no relative study so far. Many studies confirmed that DANCR is higher in tumors compared to paraneoplastic tissue[4]. It is possible that DANCR is not only important regulator inside cancer cells, but also an important factor in tumor micro-environment, which is an interesting direction for future studies.

### Conflict of Interest

The Authors declare that they have no conflict of interests.

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