

Molecular Mechanisms of Osteoporosis: A Road Map for Osteoporosis Therapeutics

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Abstract: Osteoporosis is widely spread throughout the world and becomes a serious public health problem. It is mainly caused by the imbalance of the bone remodeling process, that bone resorption (led by osteoclasts) overwhelms bone formation (led by osteoblasts). This review summarizes several important molecular mechanisms in osteoporosis and their corresponding treatment. Among them, drug therapy and cell therapy are two major therapies that are commonly used. Drug therapy is clinically mature, but there are some side effects that cannot be used for a long time. Cell therapy can cure osteoporosis, and it does little harm to the human body. However, cell therapy is clinically immature, and there may be ethical issues. More detailed molecular mechanisms require further investigation and could provide a promising direction for osteoporosis treatment and prevention.

1 INTRODUCTION

Osteoporosis has become a common public health concern in human society, especially in aged people. It is a chronic metabolic bone disease defined by low bone mineral density (BMD), which could further cause increased bone fragility and risk of bone fracture. More than 200 million people are affected by osteoporosis and the number would continuously ascend because of an aging population and prolonged life in human society (Macias 2020). Figure 1 shows the prevalence of osteoporosis in aged populations in several countries, indicating a huge number of distribution of the disorder. Currently, drug therapies are the most useful clinical intervention strategies for

osteoporosis patients. However, medicine would still have various adverse effects that may be harmful to people's health. More effective therapies are therefore needed to treat osteoporosis. Molecular mechanisms could specifically indicate the target for the cause of diseases. Numerous intervention strategies developed from molecular mechanisms have shown significant effect in clinic. Molecular factors play a key role in the study of osteoporosis, but few were clearly understood. Figuring out the mechanisms of osteoporosis development would provide promising therapeutic directions and molecular mechanisms exhibit their great significance. This review aims to overview several molecular mechanisms of osteoporosis, as well as discuss their current and further therapeutic approaches accordingly.

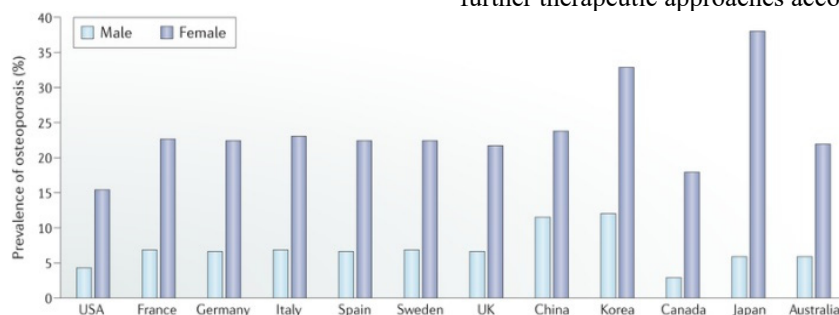


Figure 1: (derived from Yang, T.L., et al.) Prevalence of osteoporosis in populations of age 50 years and older in selected countries.

2 OVERVIEW

Osteoporosis occurs due to the imbalance of bone homeostasis. Osteoclasts clear away bone tissue (bone resorption) while osteoblasts form bone tissue (bone formation) to maintain the homeostasis process. Increased bone resorption or reduced bone formation would lead to osteoporosis (Yang, et al. 2020). A large number of molecules could take part in osteoporosis development and therefore, lead to various molecular mechanisms. Figure 2 shows a schematic diagram of

bone homeostasis and molecular components involved, including mesenchymal stem cell (MSC), parathyroid hormones (PTH), calcitonin and estrogen. Nevertheless, most molecular mechanisms focus on the bone homeostasis process, affecting bone resorption or bone formation to cause osteoporosis. For instance, MSC, PTH and estrogen have important roles in modulating osteoblasts, while calcitonin is revealed to have inhibitory function in osteoclasts. Consequently, here the author will detailly review molecular mechanisms that are related to the bone homeostasis.

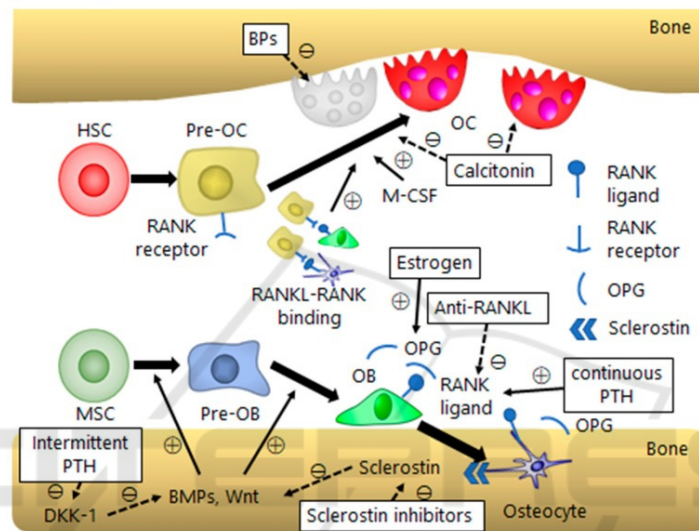


Figure 2: (derived from Ukon, Y., et al.) Schematic summary of bone homeostasis and related molecular mechanisms. BP, bisphosphonate; DKK, dickkopf; M-CSF, monocyte/macrophage colony-stimulating factor; HSC, hematopoietic stem cell; OB, osteoblast; OC, osteoclast.

2.1 Mesenchymal Stem Cells (MSCs) in Osteoporosis

2.1.1 Mechanism MSCs Are Progenitors of Adipocytes and Osteoblasts

They were primarily found in the bone marrow and therefore, considered to be involved in bone regeneration. Similar to bone homeostasis, the differentiation of MSCs to adipocytes and osteoblasts is also a balanced process. Ascending adipocytes differentiation could significantly suppress osteoblasts differentiation. Subsequently, fewer osteoblasts generation could induce osteoporosis development. Three main molecular mechanisms regulate the differentiation of MSCs: signaling pathways, microRNAs and transcription factors (Hu, et al. 2018). Two signaling pathways have been demonstrated and are well-understood in past decades. The bone morphogenic protein (BMP)

signaling pathway could induce specific complex formation with other molecules, translocating into the nucleus and promoting the osteogenic gene expression. A previous study also showed that a high concentration of BMP could cause osteoblasts differentiation while a low concentration of BMP could lead to adipocytes differentiation. Another important signaling pathway of MSCs differentiation is the Wnt signaling. Like BMP signaling, Wnt signaling also has positive effects on the osteogenic process. Wnt signaling could inhibit the phosphorylation of β -catenin. Unphosphorylated β -catenin in the cell nucleus could facilitate osteoblasts' differentiation to form bone tissue.

MicroRNAs (miRNAs) are short non-coding RNA sequences but are widely involved in molecular and cellular activities. In the MSC differentiation process, they are found to be anti-osteogenic. Some miRNAs could indirectly regulate transcription factors to control MSC differentiation while some

miRNAs could directly suppress osteogenic gene expression. Notably, in different types of cells miRNAs exhibited opposite regulatory effects on MSC differentiation, and most detailed mechanisms of miRNAs remain unexplored. Distinct from two former molecular mechanisms, transcription factors are revealed to directly modulate osteogenic or adipogenic gene expression with specified mechanisms. As their name suggests, transcription factors are series of molecular factors that regulate gene expression via regulating the transcription process. Runt-related transcription factor 2 (runx2) and osterix significantly participate in osteogenic

gene expression. In runx2-deficient cells and osterix-deficient mice, MSCs could not differentiate into osteoblasts and express adipogenic phenotypes. In contrast, peroxisome proliferation-activated receptor γ (PPAR γ) could facilitate adipogenic differentiation in MSCs, indicating that both positive and negative transcription modulators play key roles in MSC differentiation. Taken together, molecular mechanisms of MSCs are briefly summarized in figure 3. Both signaling pathways and microRNAs are upstream of transcription factors. Involved with other molecules, all three mechanisms could significantly regulate the differentiation of MSC.

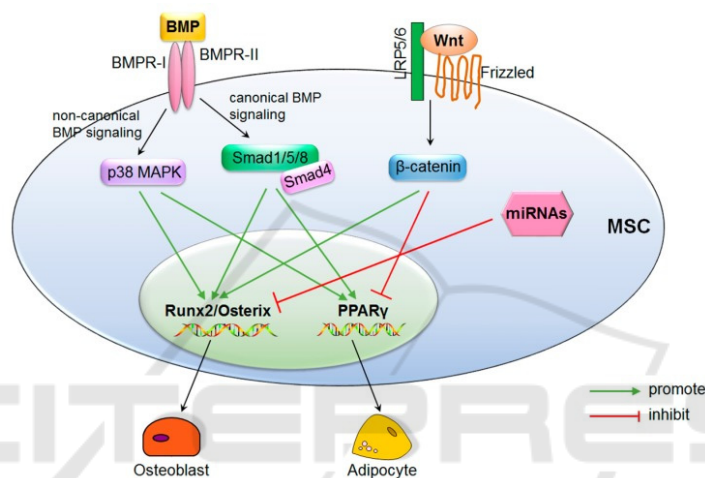


Figure 3: (derived from Hu, L., et al.) Schematic summary of molecular mechanisms in MSC differentiation.

2.1.2 Current and Future Therapeutic Applications

The differentiation of MSCs to adipocytes instead of osteoblast is important in osteoporosis development. Based on molecular mechanisms of MSC differentiation, current therapeutic strategies are mainly focusing on inducing the osteogenic differentiation process of MSCs.

Two types of MSCs are now conducted in clinical trials. They are derived from bone marrow tissue and adipose tissue, respectively. Both of them prefer to differentiating into osteoblasts, confirmed by transplantation experiments in previous animal model research (including mice and rabbits). Though these MSCs have promising therapeutic potential in vivo, their applications in clinical trials have not come up with positive results yet. This may be due to some ethical problems because some clinical trials ended up halfway without any reported results. Moreover, the differences (such as microenvironment and cellular interaction) between the human body and

animals may also hinder the normal function of transplanted MSCs.

For future therapies, according to the molecular mechanisms of the MSC differentiation process in osteoporosis, more available treatment could be performed even though there is no reliable experimental evidence yet. For instance, specific drugs could be developed to promote the level of transcription factors in the human body. Besides, by gene-editing methods, osteogenic-inducing miRNA sequences could be added to the MSC genome. Such engineered cells may supply the loss of osteoblasts in osteoporosis patients.

2.2 Calcium and Parathyroid Hormones (PTH) in Osteoporosis

2.2.1 Mechanism Calcium Absorption is Critical in Preventing Osteoporosis

A reduced level of calcium in serum could trigger an increased secretion level of parathyroid hormones

(PTH), which would induce bone resorption. In other words, if serum has less calcium than normal level, it will try to gather more calcium from bone tissue by PTH. Then increased bone resorption induces osteoclasts differentiation and suppresses osteoblasts differentiation (bone formation), leading to osteoporosis.

2.2.2 Current and Future Therapeutic Applications

Teriparatide, which consists of part of amino acids sequence of PTH, exhibited positive function in bone marrow density increase. It is an anti-osteoporosis drug. In clinical trials teriparatide significantly decreased the risk of bone fracture. However, its underlying mechanism which accounts for bone homeostasis remains unknown. Genetic studies via mouse model suggested that various molecules include Fos, Runx2, and insulin-like growth factor are important in bone formation and closely related to the action of PTH. Also, in PTH-induced bone regeneration, the SOST gene and its protein product sclerostin are revealed to mainly express in osteocytes. Additionally, another PTH-related peptide abaloparatide is used to treat osteoporosis in the US, with the function of increasing BMD and decreasing osteoporotic fractures (Tanaka 2019).

Similarly, the future direction of osteoporosis therapies could be based on exploring more PTH-related peptides isoforms, since several of them have been proved to be effective in treating osteoporosis. Other methods to increase the calcium absorption in serum could also help to decrease bone resorption and therefore, avoid osteoporosis.

2.3 Calcitonin in Osteoporosis

2.3.1 Mechanism Calcitonin

Mechanism Calcitonin is a type of hormone secreted by the thyroid gland. It could bind to the receptors on osteoclasts' membrane, inhibiting osteoclasts' capacity of bone resorption and their maturity. Thus, it maintains bone tissue to prevent or treat osteoporosis. Moreover, calcitonin was demonstrated to have capacity for pain relief, via modulating serotonergic systems, sodium channel and alleviation peripheral circulatory disturbance.

2.3.2 Current and Future Therapeutic Applications

The function of eel calcitonin was investigated in several clinical experiments. Besides increasing

BMD, inhibiting bone absorption and decreasing fractures, eel calcitonin was also demonstrated to significantly reduce osteoporosis patients' bone pain and improve their life quality. Calcitonin could be a preferred option of acute osteoporotic fractures (Ukon, et al. 2019).

Future therapeutic efforts could be performed to explore calcitonin from other animal sources. Eel calcitonin is effective but still has various adverse effects, especially it may have risk in oncogenesis (though experiments only showed weak correlation).

2.4 Estrogen in Osteoporosis

2.4.1 Mechanism Postmenopausal

women were originally reported to have a higher risk of osteoporosis, which may be due to their reduced level of estrogen. In vivo experiments suggested that after removing the estrogen receptors in osteoclasts of female mice, the phenotype of osteoporosis was observed. Also, estrogen was found to directly regulate the survival of mature osteoclasts. It suppresses osteoclastic bone resorption. Two cytokines named M-CSF and RANKL could activate osteoclasts. Estrogen could therefore inhibit osteoclasts via reducing the expression of those cytokines which produced in marrow cells and osteoblasts. Though its detailed molecular mechanisms are not well-understood, several signaling pathways (including Wnt signaling) are regulated or involved with estrogen. In general, estrogen might control BMD in a positive manner (Chen 2019).

2.4.2 Current and Future Therapeutic Applications

Estrogen could prevent or treat postmenopausal osteoporosis. However, long-term use of estrogen could induce serious negative effects, such as cancer and cardiovascular disease. Estrogen may also have potential impact on the endometrium for women with an intact uterus. Thus, estrogen is seldom directly used in the clinic and other estrogen-like hormones are used instead. These hormones show similar therapeutic effects as estrogen with mild adverse effects. Still, most of them could only be used for prevention or relief, rather than treatment.

Even estrogen-like hormones could have numerous side effects, new directions may target estrogen's mechanism in osteoporosis. The estrogen receptors in osteoclasts are taking a critical role in it. Specific drugs or molecules could be injected into bone tissue to increase the affinity (activity) level of

those receptors. Moreover, engineering osteoclasts to express more receptors on their membrane may also help to reduce bone resorption.

2.5 Bisphosphonates in Osteoporosis

2.5.1 Mechanism Bisphosphonates (BPs)

BPs are the most commonly used drugs nowadays. BPs exhibit a high binding affinity to bones (hydroxyapatite). The interaction between BPs and osteoclasts could suppress bone resorption. Initially, non-nitrogen-containing BPs were used to induce osteoclasts apoptosis, while later nitrogen-containing BPs with stronger anti-resorption ability replaced former BPs to inhibit the function of osteoclasts (Langdahl 2021).

2.5.2 Current and Future Therapeutic Applications

BP's family has various types. Different types may show different aspects of treating or preventing abilities. For example, zoledronate was characterized to prevent vertebral fractures while risedronate was characterized to prevent non-vertebral fractures. Alendronate is one of the most popular bisphosphonates for treating and preventing postmenopausal osteoporosis, approved by FDA. It shows a remarkable decrease of vertebral fracture in clinical patients. Furthermore, as long-term use of BP may cause adverse effects, a group of patients with low risk of bone structure were asked to stop using alendronate for five years. Meanwhile, their key bone parameters remain normal, indicating that stop alendronate therapy in a proper period would have

little effect on bones. Scientists also used nanoparticles to deliver BPs. Nanoparticles predominantly increased the targeting efficiency and did not induce an immune response. Nevertheless, from its investigation to application, there is still a long way to go.

Taking BP medicine via digestion system could have side effects on gastrointestinal diseases. Therefore, delivery methods could be improved. Except for nanoparticles, injection and inhalation may be effective, but the dose usage must be carefully conducted. In addition, BPs could be coated by some molecules that will not be digested in the gastrointestinal tract. Only in serum or cellular environment would those molecules be degraded and BPs could perform their function.

3 DISCUSSION

Nowadays, drug therapies are still mostly used in the clinic. Due to different molecular mechanisms of osteoporosis, drug therapy obtains various types and targets. During different stages of bone remodeling, drug therapies interact with numerous molecules (figure 4). For example, denosumab and some cytokines (RANK and RANKL) contribute to the activation of osteoclast precursor. Bisphosphonates could decrease bone resorption. In bone formation, calcium and phosphate are mainly involved. Moreover, with further investigation of other cell-related molecular mechanisms, cell therapy could also be a novel option for patients. These two major therapies (as two examples) both have advantages and limitations in distinct aspects, respectively.

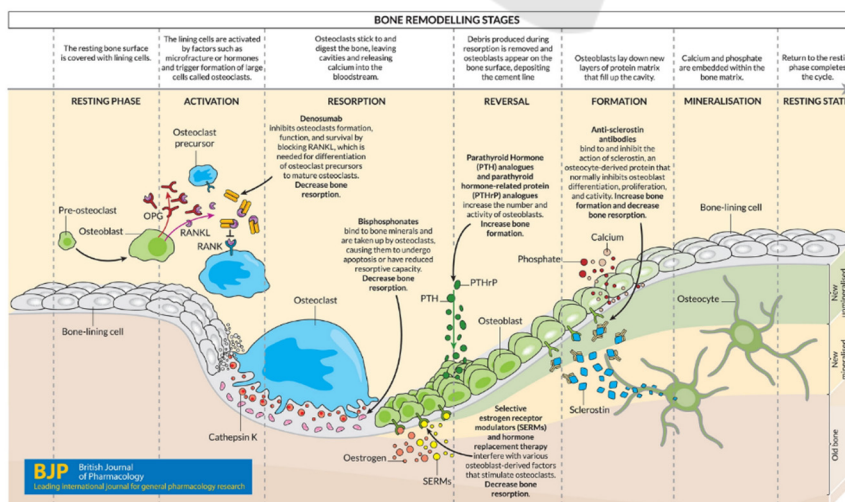


Figure 4: (derived from Langdahl, B.L.) Different molecular treatments in distinct stages of bone remodeling.

3.1 Drug Therapy

The advantages of drug therapy have been widely identified according to clinical trials in the past decades. Drug therapies are quite effective and have mature application guidance, including dose usage, delivery methods and applicable population. Different drugs or molecules could also be mixed to perform on the same patient. Clinical results suggested that combining different drugs could

significantly increase therapeutic effectiveness and decrease adverse effects. Up to now, several chemical anti-osteoporotic drugs have been produced to regulate bone metabolism (figure 5). Bisphosphonate, oestrogen and selective estrogen receptor modulator (SERM) could inhibit osteoclast development as well as the coupling factors. Besides, denosumab, teriparatide and romosozumab mainly suppress osteoblast development. Further investigation based on current drugs may provide novel therapeutic strategies.

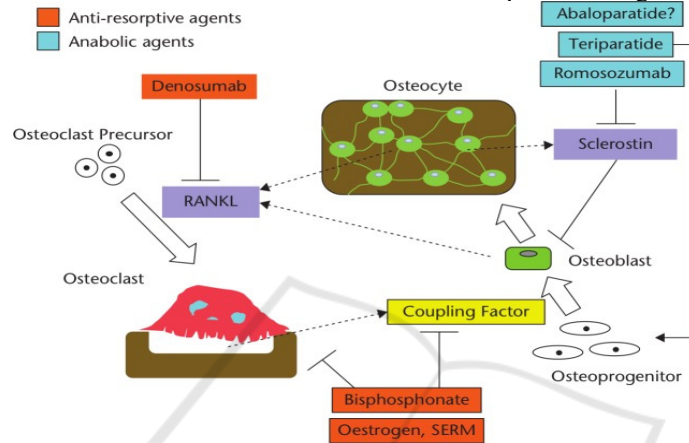


Figure 5: (derived from Tanaka, S.). Regulation of bone metabolism and mechanisms of anti-osteoporotic drugs.

However, adverse effects are still an unavoidable problem that is extensively existing in drug therapies (Vandenbroucke 2017). Most drugs would exhibit more than one adverse event (AE), and those AEs have various types, distributing in different drugs. Even worse, drugs have risk in causing withdrawals and death. (Table 1) Some drugs could only be used for short-term or even prevention rather than treatment, just to reduce the harmful effects that medicine brings to the human body. Some drugs are gender-limited such as estrogen, which is not suitable to apply to males. Besides, the delivery efficiency of

medicine requires to be improved. Since most drugs have to go through the digestion system, serum, cellular environment and finally to specific tissues, a high dose of drugs would damage other parts of the human body while a low dose would have unsatisfying efficacy. Besides, the molecular mechanisms of some drug therapies have not been clearly understood yet. These drug therapies are only known to be effective in clinic and may interact with some molecules. Identifying the mechanisms would contribute to find out more novel therapeutic strategies.

Table 1: (derived from Vandenbroucke, A., et al.) Summary of several most relevant adverse events from the currently available osteoporosis treatments (risedronate, zoledronic acid and teriparatide) in very elderly women.

AE	Risedronate			Zoledronic acid			Teriparatide		
	Placebo	Risedronate	P-value	Placebo	Risedronate	P-value	Placebo	Risedronate	P-value
≥1 adverse event	89.7%	90.9%		91.8%	92.6%	0.34	91%	83%	
Nausea	8.3%	9.4%		5.9%	7.5%	0.05	9%	8%	
Dyspepsia	6.8%	6.8%					5%	4%	
Abdominal pain	7.7%	8.2%					13%	6%	
Diarrhea				5.6%	6.8%	0.11	3%	10%	
Death	7.1%	5.7%	0.276	7.5%	7.0%	0.58			
Withdrawals due to AEs	20.3%	20.6%	0.947						

3.2 Cell Therapy

Cell therapy could prevent most of the disadvantages of drugs. Mature cell therapies exhibit a relatively low level of side effects in the clinic. Specific stem cells could be engineered to suit every single patient. As cells are generally transplanted or injected into the human body, the delivery process is significantly reduced when molecules try to reach a specific target. Besides, as mentioned above, MSC could be a

potential target for cell therapies due to its important mechanisms in osteoporosis. By manipulating its paracrine secretion of molecules (mainly composed of several growth factors), both osteoblasts and osteoclasts could be specifically regulated or generated (figure 6). More importantly, instead of taking drugs for a long term, cell therapy could be performed only once to cure patients because it exactly targets on defective cells and fix or replace them to function normally (Arjmand 2020).

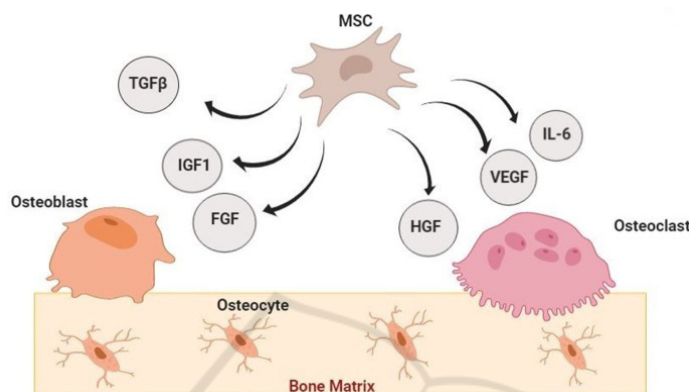


Figure 6: (derived from Arjmand, B., et al.) Paracrine effects of MSCs in bone regeneration. IGF-1: insulin-like growth factor; TGF-β: transforming growth factor β; VEGF: vascular endothelial growth factor; HGF: hepatocyte growth factor; IL-6: interleukin-6; FGF: fibroblast growth factor.

Despite those attractive advantages in cell therapy, limitations are also disturbing scientists. Ethical problems are often mentioned when applying stem cells from different donors. Cells from other donors could trigger an immune response in patients as well, causing worse situations. Further, most cell therapies are not mature enough to treat patients. Some therapeutic strategies have not been accepted to be used in the clinic yet. Overall, drug therapy and cell therapy are promising treatments, and they should be continuously developed to deal with osteoporosis.

detailed mechanisms (such as calcitonin) of the interaction between molecules and cells are still unknown. All these require in-depth exploration by researchers.

4 CONCLUSIONS

In conclusion, this review summarizes several molecular mechanisms that are significantly involved in osteoporosis, as well as discusses the current and future therapies. Drug therapy and cell therapy are taken as two major examples to compare their advantages and limitations.

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Based on molecular mechanisms, therapies have an effective function in the clinic but still require improvement. Researchers can further study more new treatment methods. It is worth noting that some

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