COMMENT
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Epigenetic modification of the Wnt signaling pathway: a potential target for intestinal aging

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Intestinal aging is emerging as a major public health problem

The world has entered an aging society, and it is expected that the global population aged 60 years or older will reach 1.5 billion by 2035 [1]. Aging is gradually becoming a major public health issue, posing a significant challenge to healthcare systems [2]. It is a complex natural physiological process involving the irreversible degeneration of body cells, tissues, and organs, and it is a major risk factor for many chronic diseases.

The gut performs crucial functions such as digestion, nutrient absorption, microbial mechanical barrier maintenance, and mucosal immunity, playing a vital role in normal life activities. Intestinal aging (IA) affects digestion and nutrient absorption and is a risk factor for conditions such as constipation, diarrhea, irritable bowel syndrome, and cancer [3]. Currently, commonly used clinical drugs to delay aging include rapamycin, β-nicotinamide mononucleotide, and metformin. However, the long-term use of these drugs poses risks such as immunosuppression, hyperlipidemia, and diabetes, limiting their clinical application [4].

The number and pluripotency of intestinal stem cells (ISCs) are closely related to IA

Maintaining a normal intestinal epithelial tissue structure is crucial for intestinal homeostasis [5]. The intestinal epithelium has a rapid cell renewal capacity, approximately every 3–5 days. The generation of new cells is achieved through the continuous proliferation and differentiation of ISCs [6]. ISCs are the primary source of various types of intestinal cells, providing the necessary renewal to keep the intestine fresh and vital.

In the intestine, there are several invaginations between the protruding intestinal villi called crypts. ISCs are located at the base of these crypts and are interspersed with Paneth cells. ISCs usually express the Leucine-rich repeat-containing G-protein coupled receptor 5 (Lgr5), making Lgr5 a critical marker of these cells [7]. ISCs can self-renew through characteristic asymmetric divisions and differentiate into various cell types, such as absorptive cells (intestinal epithelial cells and M-cells) and secretory cells (Paneth cells, goblet cells, tuft cells, and enteroendocrine cells) (Figure 1). These differentiated epithelial cells form an ordered intestinal structure to maintain intestinal homeostasis and essential functions such as absorption, secretion, barrier formation, and antimicrobial properties [8].

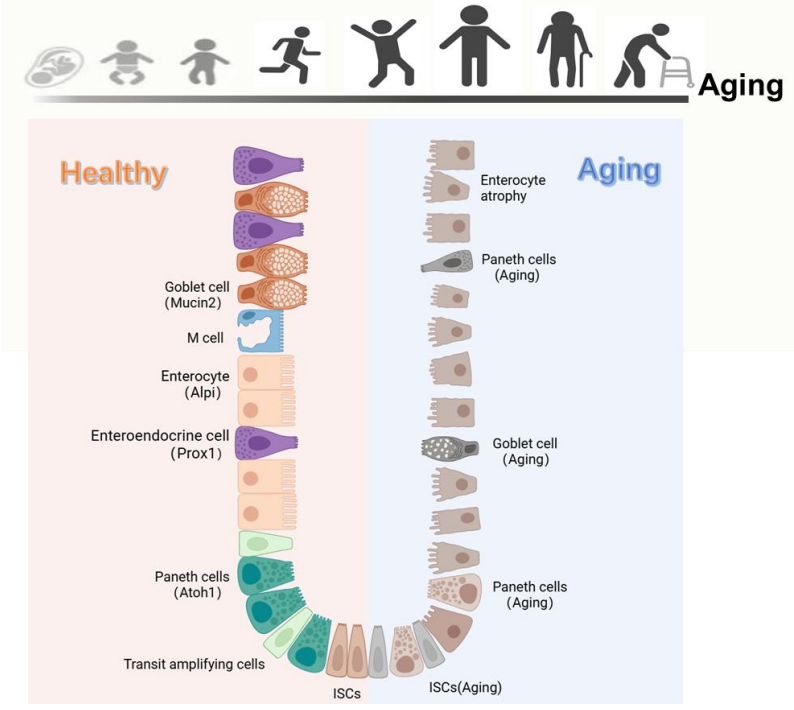

Figure 1 Gradual decrease in the number and function of ISCs, absorptive and secretory cells in intestinal tissue during aging. ISCs, intestinal stem cells.

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A recent study published in Nature found that compared to young mice, aged mice exhibited a disorganized intestinal structure, reduced nutrient absorption and barrier immunity, a decreased number of ISCs, and a significant reduction in stem cell pluripotency in the basal part of the intestinal crypts [9]. Another study found that after knocking out ISCs at the base of the intestinal crypts in young mice, the small intestinal villi became loosely structured and atrophied, the crypts became shallower, the number of crypts was reduced, and the degree of IA in young mice was comparable to that in old mice [10]. These studies indicate that the number and pluripotency of ISCs are closely related to IA, and maintaining these aspects of ISCs can help delay IA.

Pluripotency of ISCs is regulated by the Wnt/β-catenin signaling pathway

The Wnt signaling pathway is a classical and highly conserved pathway that controls the differentiation and development of organisms. Wingless/Integrated (Wnt) proteins are secreted proteins that induce the activation of intracellular signaling cascades, including both classical (Wnt/β-catenin pathway) and non-classical (β-catenin-independent pathway) pathways [11]. The Wnt/β-catenin pathway comprises Wnt ligands, Frizzled class receptors (FZD), co-receptors, the β-catenin degradation complex, β-catenin/transcriptional co-chaperones, and other regulatory components.

The Wnt/β-catenin signaling pathway plays a crucial role in regulating the proliferation and pluripotency of ISCs. Studies have shown that when the Wnt/β-catenin signaling pathway is in the "ON" state, it enhances the proliferation and pluripotency of ISCs by regulating transcriptional coactivators that mediate the transcriptional activation of genes such as *Wnt*, *Lgr5*, *Moloneymurine leukemia virus integrationsite 1 fi (Bmi1)*, and *recombinant olfactomedin 4 (Olfm4)*. Conversely, the "OFF" state of the Wnt/β-catenin signaling pathway is also important in regulating ISC proliferation and pluripotency [12]. When the pathway is "OFF", there is a decrease in the number and pluripotency of ISCs, which induces intestinal senescence [13]. The specific processes of "Wnt ON" and "Wnt OFF" are as follows.

"Wnt ON" state

In the presence of the Wnt ligand, the Wnt protein binds to the heterodimeric receptor complex FZD-LRP5/6, formed by the FZD receptor and LRP5/6. This promotes the aggregation of disheveled protein (DSH) to the tail of the FZD protein, inhibiting glycogen synthase kinase 3β (GSK3β). GSK3β phosphorylates β-catenin, reducing β-catenin degradation. The accumulated β-catenin in the cytoplasm subsequently translocates into the nucleus, where it interacts with T cell factor/lymphoid enhancer factor (TCF/LEF) family members, activating the transcription of downstream target gene core-binding factors and initiating the expression of target genes such as *Wnt* (Figure 2).

"Wnt OFF" state

In the absence of Wnt ligands, a complex consisting of GSK3β, casein kinase I (CKI), recombinant axis inhibition protein (Axin), and adenomatous polyposis coli (APC) promotes cytoplasmic β-catenin phosphorylation. APC mediates the binding of phosphorylated β-catenin to the ubiquitin-mediated protein hydrolysis pathway in the cytoplasm (Figure 2), thereby reducing the expression of *Wnt* target genes. Impairment of the Wnt/β-catenin signaling pathway has been found to reduce the number and pluripotency of ISCs, exacerbating intestinal senescence.

The "FZD8-Wnt" complex is a central switch that mediates the "Wnt ON" state of the Wnt/β-catenin signaling pathway

As one of the key molecules in this pathway, the FZD is a type of transmembrane G-protein-coupled receptor with a 7-transmembrane structural domain (7TM) and an extracellular N-terminal cysteine-rich domain (CRD). FZD8 is a member of the FZD family, which regulates various biological functions. When Wnt proteins are present outside the cell, they bind to the CRD of the FZD8 receptor to form the "FZD8-Wnt" complex [14]. This allows LDL receptor related protein (LRP) to be phosphorylated by GSK3β and other kinases, causing Axin to bind to LRP. This binding leads to the dissociation of the cytoplasmic complex mediated by the scaffolding protein Axin, thereby preventing β-catenin phosphorylation and degradation [15]. The free β-catenin accumulates in the cytoplasm and then translocates to the nucleus, where it binds to corresponding transcription factors. This binding activates the transcription of genes such as *Wnt*, *Lgr5*, and *Bmi1*, enhancing the proliferation and pluripotency of stem cells.

YTHDF1 regulates FZD8 expression in an m6A-dependent manner

m6A is one of the most common forms of RNA methylation modification, which regulates the eukaryotic transcriptome by affecting mRNA splicing, export, localization, translation, and stability [16]. The level of m6A modification is dynamically and reversibly regulated by methylating enzymes ("writers") and demethylating enzymes ("erasers"). m6A methylation

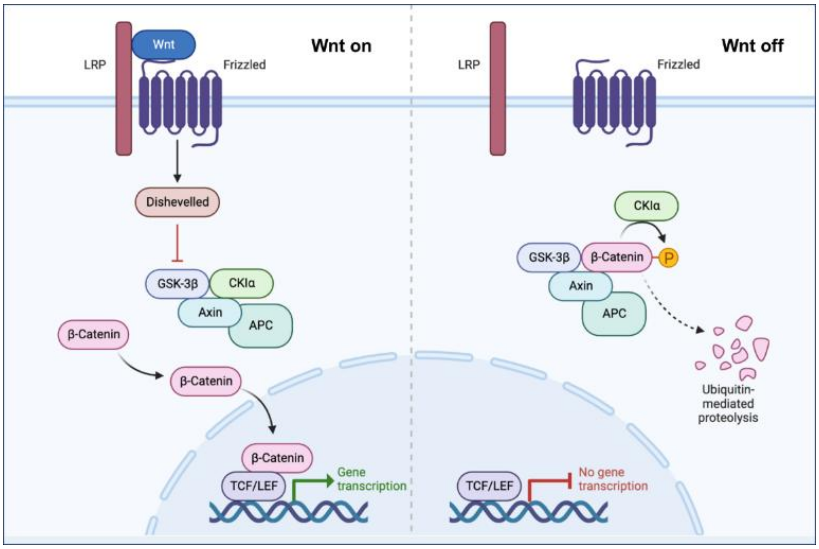

Figure 2 Signaling process of Wnt/β-catenin in "ON" and "OFF" states. GSK3β, glycogen synthase kinase 3β; TCF/LEF, T cell factor/lymphoid enhancer factor; APC, adenomatous polyposis coli; Axin, recombinant axis inhibition protein; Wnt, Wingless/Integrated; LRP, LDL receptor related protein.

is recognized by m6A reader proteins, such as the YTH domain family. proteins (YTHDF1, 2, 3), which influence mRNA degradation and translation by recognizing m6A [17].

YTH domain family proteins are the most common "readers" of m6A and act as m6A-binding proteins. m6A modification plays a significant role in the maintenance, proliferation, and differentiation of ISCs. YTHDF1, in particular, promotes the translation of FZD8, a key molecule in the Wnt/β-catenin signaling pathway. Intracytoplasmic FZD8 then migrates to the cell membrane to form a CRD domain, which recruits Wnt to form the "FZD8-Wnt" complex, promoting Wnt/β-catenin activation and enhancing the pluripotency of ISCs [18].

Researchers found that YTHDF1 protein was highly expressed in ISCs, and conditional knockdown of YTHDF1 in mouse small intestinal epithelium led to intestinal senescence. Inhibition of YTHDF1 in ISCs in vitro suppressed the activity of the Wnt/β-catenin signaling pathway, significantly reducing the pluripotency of ISCs. This suggests that YTHDF1 plays an important role in maintaining Wnt/β-catenin activation.

Future prospects

Traditional Chinese medicine and its active components have demonstrated broad therapeutic potential in the treatment of IA. For example, *Eucommia ulmoides* Oliv., Huankuile suspension and Gamisoyo-san can improve IA by inhibiting inflammatory response, regulating intestinal flora and Wnt signaling pathway [19–21]. The discovery of the dynamic changes of Wnt signaling pathway in IA can help to elucidate the mechanism of action of traditional Chinese medicine in treating IA.

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Author contributions

Jida Wang: Writing – original draft, Investigation. Yuxuan Shi: Validation, Investigation, Data curation. Ogbe Susan Enechojo: Investigation, Writing – original draft. Ying Wang: Visualization, Validation, Investigation. Abankwah Joseph Kofi: Validation, Investigation, Formal analysis. Huantian Cui: Investigation. Xiangling Wang: Visualization. Yuhong Bian: Supervision, Conceptualization. Xiaoqian Chu, Yuhong Bian: Supervision, Conceptualization.

Competing interests

The authors declare no conflicts of interest.

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Abbreviations

IA, intestinal aging; ISCs, intestinal stem cells; Lgr5, Leucine-rich repeat-containing G-protein coupled receptor 5; Wnt, Wingless/Integrated; FZD, Frizzled class receptor; GSK3β, glycogen synthase kinase 3β; Axin,

recombinant axis inhibition protein; APC, adenomatous polyposis coli; LRP, LDL receptor related protein; CRD, cysteine-rich domain.

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