The potential role of mechanically sensitive ion channels in the physiology, injury, and repair of articular cartilage

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Abstract
Biomechanical factors play an extremely important role in regulating the function of articular chondrocytes. Understanding the mechanical factors that drive chondrocyte biological responses is at the heart of our interpretation of cascade events leading to changes in articular cartilage osteoarthritis. The mechanism by which mechanical load is transduced into intracellular signals that can regulate chondrocyte gene expression remains largely unknown. The mechanically sensitive ion channel (MSC) may be one of its specific mechanisms. This review focuses on four ion channels involved in the mechanotransduction of chondrocytes, exploring their properties and the main factors that activate the associated pathways. The upstream and downstream potential relationships between the protein pathways were also explored. The specific biophysical mechanism of the chondrocyte mechanical microenvironment is becoming the focus of research. Elucidating the mechanotransduction mechanism of MSC is essential for the research of biophysical pathogenesis and targeted drugs in cartilage injury-related diseases.

Keywords
chondrocytes, ion channel, mechanotransduction, membrane protein, Piezo1, Piezo2, TRPV4

Introduction
The weight-bearing joints (knee joint, hip joint, and the temporomandibular joint) and the spinal vertebral cones are always subject to cyclic mechanical loads. As a result of being in this special mechanical environment for a long time, the cartilage tissue between the two bones gradually formed basic characteristics such as high sensitivity to mechanical signals and adaptive response to stimuli. In addition, there is no blood supply to cartilage tissue, so the mechanotransduction process of cartilage tissue is essential to maintain the morphology of cartilage and the homeostasis of the intra-articular environment. Chondrocytes are extremely sensitive to mechanical loads such as dynamic compression, fluid shear, tissue shear, and hydrostatic pressure. Through the response to mechanical signals, chondrocytes cooperate with environmental factors and genetic factors to regulate cell metabolism. Moderate mechanical...
load is a necessary factor to maintain the homeostasis of normal chondrocytes. These mechanical factors can stimulate chondrocytes to synthesize aggrecan and collagen, thereby converting mechanical stimulation into the biochemical signal for output distributing the stress. It can regulate the development, integrity, and long-term maintenance of chondrocytes.

Different degrees of damage occur when cartilage tissue is subjected to mechanical overload. From a relatively narrow point of view, cartilage injuries consist of two main types. One is an acute injury caused by violent impact load on cartilage tissue caused by trauma or improper high-intensity exercises, such as osteochondral fracture, meniscus tear, and exfoliative osteochondritis; the other is chronic degenerative wear and tear of cartilage tissue due to long-term braking or long-term abnormal static load accumulation (obesity), such as osteoarthritis (OA). The latter is mainly caused by an imbalance between degradation (catabolism) and promotion (anabolism), which is characterized by overexpression of matrix metalloproteinases (MMPs). Moderate exercise can not only produce benefits such as alleviating chronic pain and enhancing body balance function but also inhibit the expression of MMPs to reduce joint injury. Thus, the essence of cartilage injury is more likely to be the final coupling of biochemical and biomechanical effects. Besides, the occurrence and development of osteosarcoma and chondrosarcoma are also closely related to the abnormal transmission of mechanical signals. Mechanical stress induces cartilage formation and promotes differentiation and maturation during fetal development. The lack of certain mechanical stimulation may lead to osteochondral dysplasia.

However, the exact role of chondrocytes in mechanotransduction is unclear, and it still needs to be explored. On the one hand, chondrocytes may respond to biochemical signals produced by mechanical stimulation. On the other hand, it has a direct and rapid response to mechanical signals. The mechanically sensitive ion channel (MSC) protein plays an important role in the mechanotransduction of the latter. However, there is no close relationship between the various channels, for each channel is independent. Whether there is a dependent progressive relationship between them or other cell signal transduction pathways and whether each channel antagonizes or promotes each other are still unknown. In this article, we will explore the effects of mechanical loading on articular cartilage and the mechanism of mechanical stress transduction under normal physiological and pathological conditions, with emphasis on the synergistic effects and cascade reactions of various MSCs in this process.

**Biological structure and mechanical microenvironment in articular cartilage**

Chondrocytes are the only cell type in mature cartilage. Its density in cartilage tissue is very low, and its proliferation and metabolism are very slow. And chondrocytes must survive under mechanical loading. Taking the knee joint as an example, the biomechanical model of articular cartilage can be simplified to a smooth sphere placed on a smooth plane. It is subjected to a variety of dynamic mechanical stimuli, including tensile, compression, shear, and torsional loads. Compressive load is the main source of stress. Therefore, the structure of cartilage tissue is always adapted to the mechanical environment. In the process of chondrocytes subjected to periodic stress loads, the shape of chondrocytes gradually becomes larger and round. Its adaptive behavior can be used as a mechanical trigger for biological signal transduction. This mainly depends on the interaction between the cytoskeleton and the extracellular matrix (ECM).

Cartilage matrix accounts for more than 90% of adult cartilage tissue. The elastic modulus increased linearly from pericellular matrix (PCM) to territorial matrix and then to ECM. ECM plays a leading role in the long-term mechanical conduction response of chondrocytes. The mechanical stimulation of ECM participates in the mechanical signal transduction of cartilage units by regulating PCM and PC. Collagen with a special triple helix structure is the main component of ECM. The stiffness of ECM is one of the most important sources of environmental signals to stimulate cells. The intertwined collagen fibers are closely bound to the proteoglycan polymer attached in the antiparallel direction and maintain the stiffness of the ECM by intercepting solute and water molecules. At the same time, due to the existence of the fixed charge density of proteoglycans, the negatively charged groups have a strong attraction to cations, resulting in a large number of positive charges in the intercellular solution. In addition, the ion concentration in cartilage tissue is higher than that in the synovial fluid around the joints, resulting in pressure difference, leading to tissue expansion, leading to changes in cartilage tissue water content and extracellular osmotic pressure. This results in chondrocytes living in a special ion and osmotic microenvironment. Therefore, ECM as a special mechanical signal sensor, its unique biomechanical properties make cartilage maintain good viscoelasticity and hydrostatic pressure (HP).

Mechanotransduction is a dynamic process that allows chondrocytes to quantitatively regulate the rate of matrix synthesis and degradation and change the composition of the ECM. The process itself is mainly mediated by transmembrane integrin molecules and mechanical sensitive proteins such as MSC, which bind to the ECM on the outside of the cell, while actin binds to the cytoskeleton on the inside of the cell through binding proteins. The mechanical connection needs to be exerted by the contraction of actomyosin adhered to the newly attached matrix. With the applied force and the resistance of the matrix, the adhesion changes its size, strength, and mechanics. This results in greater adhesion and more mechanical signals.
on the rigid matrix. The formation of strong adhesion will cause actin flow to act on ankle protein in an adhesion-sliding way, resulting in a stretch–relaxation cycle. At the same time, additional plate pseudopodia expansion and contraction as well as the next round of mechanotransduction of matrix stiffness are activated. There is a direct correlation between the stimulation of HP on cells and the behavior of cell membrane channels. HP enhances Ca\(^{2+}\) and Na\(^+\) channels and Na\(^+-K^+\) pumps that interact with protein kinase A. Besides, HP increases the activity of Na\(^+-H^+\) pump. These two phenomena eventually increase the concentration of intracellular Ca\(^{2+}\). The change of Ca\(^{2+}\) signal transduction is closely related to the decomposition and synthesis balance of ECM. Excessive or inappropriate mechanical load, inflammatory microenvironment, or the accumulation of alkaline calcium phosphate crystals may lead to changes in membrane potential and electrical conductance of chondrocytes, and then affect the changes of MSC channel group of chondrocytes regulated by Ca\(^{2+}\).

**Mechanically sensitive ion channels**

MSCs are the membrane protein structural fundament for chondrocytes to perceive and react to mechanical factor stimuli. The mechanical force conduction process it mediates is the fastest conduction system in organisms known so far. In the process of mechanotransduction, the metal ions in the intercellular fluid play an important role as a mediator along with the change of cell osmotic pressure. Depending on the type of metal ion selected for penetration in the channel, the MSC on the surface of chondrocytes mainly includes the following four different types: Piezo channel, transient receptor potential (TRP) channel, degenerative protein (DEG)/epithelial sodium channel (ENaC), and double-pore potassium (K2P) channel.

**Take the second messenger-calcium ion as the leading role to play the role of mechanotransduction**

**TRP ion channel vanillin receptor subfamily.** TRP is a non-selective cation channel, most of which have good selective permeability for calcium ions.\(^{24}\) According to the sequence similarity of TRP protein, it can be divided into seven subclasses: transient receptor potential channel, transient receptor potential vanilloid (TRPV), transient receptor potential melastatin, transient receptor potential A (TRPA), transient receptor potential polycystin no mechanoreceptor potential C, transient receptor potential polycystin, and the mucolipin subfamily of transient receptor potential channels.\(^{22}\) Although the yeast vacuolar transient receptor potential channel in *Saccharomyces cerevisiae* has the most distant genetic relationship compared with other TRP channels, it is still classified as the eighth subclass. Most of them can be activated by local mechanical pressure, such as TRPV1,\(^{26}\) TRPV2,\(^{27}\) TRPV4,\(^{28}\) and TRPA1.\(^{29}\) At present, the studies focus on TRPV1 and TRPV4, which are widely expressed in cartilage, bone, synovium, and other musculoskeletal tissues.\(^{30,31}\) They also can be activated by osmotic pressure, mechanical stimulation, phorbol ester, and other factors. In recent years, it has been found that TRPV4 can mediate the metabolic process of chondrocytes and participate in the occurrence of OA.\(^{32}\) TRPV4-mediated Ca\(^{2+}\) influx activates the intracellular signal pathway, which is very important for maintaining bone homeostasis. Although TRPV4 is mediated by the second messenger, the other channels are directly gated. TRPV4 is unable to sense the mechanical force of membrane conduction and may be functionally coupled with other force-conducting factors.\(^{33,34}\) What is significantly reduced is the Ca\(^{2+}\) response of chondrocytes caused by external mechanical effects and the catabolism of the matrix using TRPV4 inhibitors.\(^{35}\) It is worth noting that TRPV4 is expressed on the whole membrane structure of chondrocytes (including PC). PC is the most important mechanoreceptor organelles of chondrocytes.\(^{36}\) The integrity of the structure and function of the PC is the physiological basis for mediating calcium response.\(^{37}\) TRPV4 was activated by a mechanical signal, and the Ca\(^{2+}\) microdomain was formed. At the same time, many Ca\(^{2+}\) were induced to flow into the PC, which caused the downstream cascade reaction and affected the function of chondrocytes. Normally, TRPV4 also has a certain feedback regulation mechanism. When the channel was opened and calcium ion flowed in, the concentration of intracellular calcium ion increased, and then the speed and amplitude of activation of the channel by different factors could be significantly increased. After a while, the intracellular calcium ion increased to saturation, and the channel could be blocked by the negative feedback mechanism of Ca\(^{2+}\) dependence, which reflected the phenomenon of calcium overload and protected the cell itself from abnormal because of excessive voltage difference.\(^{38}\) Therefore, the signals sensed by chondrocytes can in turn regulate the metabolism of chondrocytes, the dynamic balance of the cartilage matrix, and the health of the whole joint through MSC.\(^{39}\)

The mechanism by which TRPV4 regulates chondrocyte apoptosis through mechanotransduction may be as follows. On the one hand, the matrix stiffness changes under abnormal mechanical load, and mechanical signals are transmitted to the chondrocytes to activate TRPV4, causing a large amount of calcium influx. It induces intracellular signal transduction, which triggers caspase-3/6/7/8 expression or upregulates the death domain of Fas-associated proteins. This will eventually lead to chondrocyte apoptosis or inhibition of proliferation.\(^{40}\) On the other hand, TRPV4 itself can affect the condition of the cartilage matrix to regulate indirectly. It also promotes the expression of A disintegrin and metalloprotease 10 to make CD44 cleavage, resulting in abnormal hyaluronic acid metabolism and matrix stiffness changes.\(^{41}\) Hence, it can regulate the changes of matrix stiffness and shape of chondrocytes as well as the concentration of calcium in chondrocytes, so it
can form a vicious circle to aggravate chondrocyte apoptosis in an abnormal state. Studies have found that TRPV4 gene mutations can cause joint functional disorders in people. The knockout of the TRPV4 gene in mice significantly increased the risk of OA in mice due to obesity and aging.  

At the same time, some studies have suggested that knockout of TRPV4 has a certain effect on age-related and post-traumatic OA, but it cannot prevent the progression of OA after medial meniscus instability.  

To conclude, TRPV4 plays an important role in the mechanical stimulation of chondrocytes and the synthesis and degradation of the cartilage matrix. Therefore, a further investigation into the role of TRPV4 channels in the process of chondrocytes receptive to mechanical microenvironment can help understand the molecular mechanism of the interaction between cells and the mechanical microenvironment.  

**Piezo MSCs.** Piezo is a novel MSC discovered from the mouse Neuro2A cell line by RNA interference technique. The two proteins with similar structures and genes, Piezo1 and Piezo2, were found by the siRNA gene knockout technique. It is composed of about 2500 and 2800 amino acids. It has the most transmembrane regions, including 24–36 transmembrane regions. It has no homology with other known MSC or voltage-activated calcium channels. It can nonselectively pass through divalent ions Ca$^{2+}$, Mg$^{2+}$, Mn$^{2+}$, Ba$^{2+}$ and monovalent basic ions K$^{+}$, Na$^{+}$. But it has an obvious preference for Ca$^{2+}$. The three-dimensional structure of Piezo ion channel proteins was successfully analyzed from recent research, which further revealed the mechanism of mechano-induced ion channels. Several studies have shown that cells can sense external environmental stimuli and regulate cation influx caused by mechanical stimulation with the help of Piezo MSCs. Furthermore, it is also actively involved in the regulation of various functional metabolic processes of cells, such as differentiation and cell migration. Piezo is mainly expressed in tissues that are more mechanically stimulated, such as the lung, colon, bladder, kidney, blood vessels, and ganglia. Piezo1 ion channel can feel mechanical stimulation, and excessive activation can promote the process of apoptosis and cause mechanical injury. Piezo2 ion channel can reduce the physiological response caused by harmful stimulation, increase nerve sensitivity, and decrease pain threshold. It can also participate in the migration of immune cytokines and regulate the dynamic balance of nerve end plate current mediated by mechanical stimulation. At present, it is found that Piezo is sensitive to mechanical loads such as compression load, tensile load, and ultrasonic stimulation, but there may be a certain preference. Lee et al. reported that the precise application of compressive stress on chondrocytes by atomic force microscope cantilever can activate Piezo1/2, to induce Ca$^{2+}$ influx. At the same time, it has been found that ECM makes Piezo1 protein more sensitive to mechanical force. Wrapping of the ECM protein beads at the cantilever end can promote its interaction with the cells. This interaction will form a network of mechanical connections between the cells so that the force pulling the cell membrane can activate the Piezo1 channel protein more efficiently. After experiencing tensile stress, chondrocytes first activate Piezo1 and then result in Ca$^{2+}$ influx. Pan et al. activated the Piezo1, on the surface of T cells to induce downstream gene transcription for chimeric antigen receptor T cell immunotherapy.  

Piezo1 and Piezo2 were significantly expressed in mice’s articular cartilage. At the same time, Piezo channel inhibitor GsMTx4 (tarantula extract) could reduce the apoptosis rate of chondrocytes. Rocio Servin-Vences et al. found that Piezo1 can mediate the intracellular current induced by tension by high-speed pressure clamp, which further confirmed the role of Piezo1 in the mechanical stress signal transduction of chondrocytes. However, the role of Piezo2 in the mechanism of mechanical signal transduction of chondrocytes and its relationship with cartilage-related diseases is not clear. We speculate that Piezo1 and Piezo2 have a synergistic effect in the transmission of mechanical signals, and these channels may be involved in the transmission of harmful mechanical stimuli. At present, it has been found that Piezo channel protein will be activated when the high-strain mechanical load is transferred to chondrocytes. A large amount of calcium influx is caused by the assistance of type L voltage-gated calcium channels. This phenomenon will break the balance of endoplasmic reticulum calcium concentration in chondrocytes and lead to endoplasmic reticulum stress. Furthermore, it activates caspase-12 protein and promotes the synthesis of Bcl-2-related X protein and Bcl-2-related proapoptotic protein. The decrease of antiapoptotic factor Bcl-2 ultimately leads to chondrocyte apoptosis. Other studies suggest that MAPK/ERK5 and MAPK/ERK1/2 are downstream signal molecules mediated by Piezo1 protein ion channels to mediate late excessive apoptosis of chondrocytes under high-strain stimulation. From the current research, there may be many pathways in the excessive apoptosis of articular cartilage in patients with OA. Whether the MSC Piezo1 protein is intrinsically related to other pathways needs further analysis. In our previous studies, we also found that GsMTx4 did not completely block the Piezo1 protein. It is necessary to further explore the efficient blockers of Piezo1.

**Synergistic cascade relationship between Piezo and TRPV4.** In chondrocytes, both Piezo and TRPV4 can be activated by mechanical stimulation. There is a certain relationship between the two channels. The MSC on the surface of the chondrocyte membrane is sensitive to different amplitude periodic tensile strain stimuli in the mechanical microenvironment. The specific manifestations were as follows: TRPV4 $\rightarrow$ Piezo1/2 $\rightarrow$ Piezo2. They were involved in the force transduction process of chondrocytes from low strain to high strain. TRPV4 $\rightarrow$ Piezo1/2 was involved in the...
sensory transduction of chondrocytes from high matrix stiffness (approximately 197 kPa) to low matrix stiffness (approximately 2 kPa). Considering the regulation of cyclical tensile strain (CTS) and matrix stiffness on cartilage matrix synthesis and degradation, TRPV4 mediates the calcium concussion of chondrocytes stimulated by medium- and low-amplitude CTS. Piezo1 and Piezo2 synergistically mediate the calcium concussion of chondrocytes stimulated by high-amplitude CTS. Furthermore, it participates in the synthesis of cartilage matrix, which is beneficial to the health of cartilage. Piezo2 may mediate the calcium concussion of chondrocytes stimulated by high-amplitude CTS. Moreover, it participates in the degradation of the cartilage matrix, causes damage to cartilage, and induces joint disease. At the same time, these two channels mediate the separation but overlap of electromechanotransduction pathways. Piezo1 responds to stretching and substrate deflection, while TRPV4 only responds to substrate deflection. Both TRPV4 and Piezo1 play a role in the mechanism of ECM-induced currents, but only Piezo1 plays a role in mechanically induced currents.

Mechanical load perception by affecting the internal and external balance of sodium and potassium ionic membranes

K2P channel. Potassium channel is the ion channel of intracellular potassium ion outflow, which can cause an extroverted or inward current. It is the most abundant subtype and the most complex ion channel found at present. Among them, K2P channel is an important type of cell potassium channel protein family. K2P channel protein is composed of homologous or heterodimeric subunits, and each subunit contains four transmembrane fragments, two-pore domains, and intracellular N-terminal and C-terminal. The sequence homology in the K2P channel family is very low, and only the pore domain has the highest degree of conservation. Several subfamilies of K2P channels can be roughly divided into four categories, namely weak inward rectifiers K channel (TWIK), tandem pore domain acid-sensitive K channel (TASK), TWIK-related K channels (TREK), and TWIK-related arachidonic acid-stimulated K channels (TRAALK). Among them, it has been proved that TREK-1, TREK-2, and TRAAC have mechanical gating characteristics. The heterologous TREK-1 can respond to the membrane tension of intact cells and plaques in vitro, which proves that the mechanical force can control the channel. Similar mechanical stimulation can also activate the heterologous expression of TREK-2 and TRAAC channels. Their range of induction to mechanical force is very wide, and the response threshold is very low (0.5–12 MN/m). The stronger the mechanical force is, the higher the possibility of opening the channel is. However, there is no detailed study on the expression of related channel proteins in chondrocytes. However, potassium channels are often considered to be an important part of the function of chondrocytes, and they can involve in the regulation of membrane potential level, metabolic activity, and the concentration of calcium ion in chondrocytes. TASK-2 belongs to the TALK subgroup of the K2P protein family. It is expressed in human articular chondrocytes, and its expression decreases significantly with the development of OA. It has been found that it stabilizes the membrane potential of chondrocytes, but the mechanotransduction process of this channel protein needs to be further explored, which may play an important role in regulating the activity of chondrocytes and the value of PH inside and outside the membrane. At the same time, it may also be used as one of the pathological markers of OA.

Degenerin/epithelial sodium channels. ENaC is a voltage-independent ion channel protein discovered in 1994. Its expression is not limited to epithelial cells but can exist on the membrane of non-epithelial cells, which is responsible for regulating the transport of Na⁺ inside and outside the cells, to maintain the balance of Na⁺ in vivo. It can respond to a variety of stimuli, including mechanical forces. The DEG/ENaC channel protein family has the same structure, including two transmembrane domains, an extracellular ring rich in cysteine, and the N-terminal and C-terminal in the cell. ENaC has three subunits: α, β, and γ, while α2 β γ is a widely distributed composition of ENaC. Among the three homologous subunits, α subunit is the main functional unit, while β and γ subunits are accessory subunits. ENaC is also distributed in osteoblasts and chondrocytes. α and β subunits were expressed in chondrocytes of normal cartilage tissues, but the expression of ENaC was low or not in the process of OA. ENaC may be involved in the mechanotransduction process by sensing the mechanical signals transmitted by the cytoskeleton. Many studies have reported that cytoskeleton-related proteins have been linked to the regulation of ENaC activity. MARCKS can act as a linker between ENaC and cytoskeleton. Besides, co-localization of β1 integrin with ENaC and voltage-activated calcium channels in chondrocytes. Therefore, we hold a positive view that ENaC may be either directly involved in the osteoblast response to stress stimulation through the signal transduction pathway to regulate bone growth or itself functions as a signal transduction factor of bone physiological response.

Conclusions

As we learn about the complexities of the mechanosensory mechanisms that eventuate in chondrocytes, we cannot be surprised by the thorny nature of cartilage tissue engineering and surgical treatment in cartilage damage. Mechanotransduction is one of the important cornerstones for maintaining the health and normal tissue remodeling of articular cartilage. The study on the mechanism of MSC in chondrocytes is highly clinically relevant. Cartilage
injury is not caused solely by the change of the channel itself, but by the change of channel activation. Under physiological conditions, joint loading can be used to counteract the inflammatory pathway and restore anabolic activity by inhibiting the expression of MSC. Cartilage tissue also has a certain mechanical feedback regulation mechanism. MSC can protect cartilage by adjusting osmotic pressure and matrix stiffness to prevent the deformation caused by the excessive overflow of cartilage matrix. The high-intensity load is a high-risk factor for cartilage injury. Trauma or joint overload will lead to chondrocyte MSC dysfunction causing cartilage degeneration and OA. We need to further investigate the molecular pathways that support bone remodeling and mechanotransduction of chondrocytes in health and disease. The information obtained will not only elucidate the molecular mechanism related to cartilage injury but also provide clinical surgeons with new treatment strategies to eliminate adverse side effects. MSCs located on the surface of the chondrocyte membrane form a network of mechanical force-sensing pathways centered on Piezo and mediate clinical characterizations such as inflammation and local pain. Therefore, it is necessary to investigate all the potential therapeutic procedures that can lead to MSC-specific desensitization. Inhibition of Piezo1/2, TRPV4, and other downstream cytokines and translational factors can reverse joint inflammation or pain. Careful clinical studies are required to differentiate if primary non-responders to either Piezo1/2 or TRPV4 inhibition will respond more effectively to other classes of therapy. After all, it has been found that among these four kinds of MSC, one relatively single-channel protein inactivation, other ion channels may have a certain compensatory phenomenon. Based on the current clinical application, some calcium antagonists may have beneficial clinical side effects in patients with OA. However, the long-term effects of calcium antagonists on chondrocytes and cartilage are not clear. Besides, in the field of cartilage tissue regeneration medicine, mechanotransduction is also important for the transcriptional mechanism of stem cells and matrix scaffold-induced transplantation. This is an area where there is still a lot of information to be mined. Clarifying molecular signal events related to these pathological conditions will contribute to the development of reliable prognostic/diagnostic tools and treatment strategies for a variety of bone diseases.

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Author contributions

XBY and JY contributed the central idea, designed, and wrote the initial draft of the article. The remaining authors contributed to refining the ideas, carrying out additional analyses, and finalizing this article. GY and ZD reviewed and edited the manuscript. All authors read and approved the manuscript.

Availability of data and materials

Data sharing does not apply to this article as no data sets were generated or analyzed during the current study.

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