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EDITORIAL

A crucial physiological role of Piezo1 channel in differentiation rather than proliferation during myogenesis

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Mechanical signals are critical for the growth, maintenance and repair of skeletal muscle. However, it remained elusive whether the mechanically activated ion channels, notably the novel Piezo1 channel, mediate these functions in the muscle. Although an earlier report[1] suggested that Piezo1 channel could be important for myoblast cell fusion and myotube elongation in the C2C12 cell line, the current study by Bosutti et al[2] made significant progress in determining the physiological roles of Piezo1 channel in myogenesis and adult myofibre activity. The novelty presented by this paper exists in three aspects. Firstly, the functional roles of Piezo1 channel were thoroughly investigated in primary cells and skeletal muscle under physiological condition for the first time. Not only the differentiation, but also the proliferation and contraction were analysed against Piezo1 channel in this paper. Secondly, a precise

time-window for intervening in the myogenic differentiation was identified, generating new understanding of how temporal modulation of Piezo1 would promote myogenesis. Thirdly, the chemical activator of Piezo1 channel, Yoda1, was proved to be effective in inducing differentiation without causing any obvious side effect after the long-term treatment, which provides the direct evidence that a novel pharmacological approach to skeletal muscle regeneration is achievable. These findings raise important questions in terms of functional significance of Piezo1 channel and its translational potential.

Mechanical signals generated from cellular environment initiate and promote myogenesis during which satellite cells (SC cells), the muscle precursor cells, are directed to proliferate, differentiate and migrate with temporal and spatial patterns. The scale and length of these mechanical signals can change dynamically, depending on the reciprocal interaction between the cells and their niche[3]. For this reason, the mechanosensors which translate the mechanical signals into biological signals are indispensable for the determination of the cell fate at any given time. As a relatively newly discovered mechanosensor, Piezo1 channel is a bona fide sensor of mechanical force in mammals[4], which has been unanimously agreed by independent groups across the world. Named after the Greek word for pressure, Piezo1 assembles as a trimer to form Ca^{2+}- and Na^{+}-permeable ion channels with a typical unitary conductance of ~25 pS[4]. Mouse Piezo1 consists of 2547 amino acids, while human Piezo1 is composed of 2521 amino acids. Physiological studies have previously revealed that Piezo1 channel contributes to the blood pressure regulation, exercise performance facilitation, vascular development, red blood cell volume sensation, baroreception, cartilage stress sensation, bone growth and osteogenesis[4]. But it still remains underexplored whether and how Piezo1 channel determined myogenesis and myofibre activities at physiological levels. The research on Piezo1 functional roles in these tissue or cells was also complicated by the co-expression of other mechanosensitive ion channels such as TRP channels, as all these channels could be simultaneously activated by a mechanical stimulus. Thus identification of a chemical agonist specific for Piezo1 channel would make the distinguishment possible. Screened from ~3.25 million compounds with an unbiased cell-based fluorescence assay, Yoda1 with its specificity against Piezo1 has been widely tested[4]. Many studies have convincingly demonstrated that Yoda1 is highly

selective for Piezo1 channel. Even Piezo2 channel, the only paralog of Piezo1, is completely insensitive to Yoda1. The research on the vasculature confirmed that Yoda1 could even mimic the effects of laminar shear stress (mechanical force) on both the physiological and pathophysiological functions of endothelial cells[4,5]. Therefore Yoda1 turns out to be a powerful tool for the functional investigation into Piezo1 channel.

In the current study, the SC cells characterised with Pax-7, an exclusively expressed transcription factor in these cells, were chosen for the dissection of Piezo1 physiological functions. Neither the total cell number nor the percentage of Pax-7-positive cells was changed by the treatment of Yoda 1 at a concentration which would activate most Piezo1 channels. In contrast, the SC cell differentiation was triggered by the same concentrations of Yoda1 as hallmarked by the morphology of cell elongation and visualization of myogenin (MyoG), the muscle-specific transcription factor. It is hence concluded that Piezo1 channel is only crucial for SC cell differentiation without playing any noticeable role in proliferation and apoptosis.

The further analysis of “time window” showed that the differentiation only occurred after the cells were treated with Yoda1 for 72 hours, indicating that sophisticated downstream events took place following the activation of Piezo1 channels during this process. Interestingly, the differentiation was accompanied with a highly-regulated alignment of SC cells as most cells were oriented towards the same direction. The alignment was also clearly observed in endothelial cells following the activation of Piezo1 channels by mechanical force[5], which implies that a general mechanism may underlie the Piezo1-guided alignment in all cell types. Future in-depth mechanistic research will determine how such spatial and temporal control is achieved in Piezo1-mediated differentiation of muscle precursor cells.

After the differentiation of muscle precursor cells into myofibres, it is also completely unknown whether Piezo1 channels regulate the myofibre physiology. In this paper, the authors measured the electrical activity and contractility before and after the activation of Piezo1 by Yoda1 in the adult muscle fibres. For the electrical activity, neither the amplitude of the miniature endplate current (MEPCs) nor the frequency of endplate current (fMEPCs) was altered by Yoda1 after the stimulation of the neuromuscular junction (NMJ) synapse. For the contractility, the muscle contraction following an electronical stimulation to the motor nerve was also not changed by

Yoda1. Further Ca^{2+} imaging experiments showed that Piezo1-mediated Ca^{2+} entry was nearly lost in adult skeletal muscle fibres. Only some sporadic Ca^{2+} events from Piezo1 channels could still be observed when a higher concentration of Yoda 1 was used. As positive control, other Ca^{2+} signals were normal and expected. So these novel results rule out a physiological role of Piezo1 in these investigated functions of adult myofibres. However, these findings should also be interpreted with caution as it is still possible for Piezo1 to play a role in other functions of myofibres such as chronic adaptation to exercise.

As complementary approaches, the methods of biochemical immunostaining and single-cell sequencing were adopted to validate the functional results of Piezo1 channels in myogenesis and myofibre physiology. To reduce any potential concern about the Piezo1 antibody specificity, the authors performed the antigen retrieval control experiments with blocking peptide which appear to be convincing. It was then found that Piezo1 expression level was gradually decreased when the differentiation progressed. When the differentiation of muscle precursor cells was completed and mature myofibres were formed, Piezo1 expression seemed to be the lowest. In mature myofibres, the Piezo1 channels were only located to a small endplate region where the channels also had the least Ca^{2+} permeability, which is consistent with the findings that the channels are not required for the investigated physiological functions of myofibres. The interesting expression pattern of Piezo1 during myogenesis reported in this paper makes one wonder how Piezo1 channels are expressed in myofibre dedifferentiation and what their functional roles are in that scenario. Follow-up studies will certainly be needed to answer these intriguing questions.

The current study reveals for the first time that the novel mechanically gated ion channel, Piezo1, determines the differentiation during myogenesis in a temporal manner. On the contrary, the channel doesn't play any physiological role in other functions such as proliferation during myogenesis (as shown in Figure 1). As Piezo1 activation can initiate the differentiation in muscle precursor cells, decoding the downstream cellular mechanism will potentially generate even deeper insight into the understanding of mechanical force in muscle physiology[6].

As mouse Piezo1 and human Piezo1 share high similarity at both protein structure and function, the knowledge arising from this study can be readily extended to any

future research which investigates Piezo1 channelopthies in human myogenesis abnormalities. For such investigation, sample genetic analysis from the patients suffering from these disorders will likely generate some important clinical information. In addition, the current study may open a new avenue for the treatment of skeletal muscle injury or progressive myopathies. Because the differentiation initiated by Piezo1 channels could be critical to muscle regeneration, fine-tuning of Piezo1 channel activity with pharmacological approaches will probably bring therapeutic benefits for the recovery.

Therefore, pharmacological modulation of Piezo1 channel activity could represent a novel strategy to regulate physiological or pathophysiological functions. The modulators of Piezo1 channel discovered so far include the agonist Yoda1 (its derivatives as well) and some lipid molecules[1, 7]. They make it highly likely to modulate the channel's activation (opening) or inactivation (closure) at a designated time. From this perspective, these modulators or their derivatives could have tremendous potential to be developed into therapeutic drugs in future for the treatment of some myogenic diseases or other Piezo1 channelopthies.

In conclusion, the important findings from this study highlight a novel physiological role of Piezo1 channel in skeletal myogenesis. The new information about the specific time window also sheds light on a potential therapeutic strategy to regulate skeletal muscle physiology and pathophysiology.

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CONFLICT OF INTEREST

The authors declare no conflict of interest.

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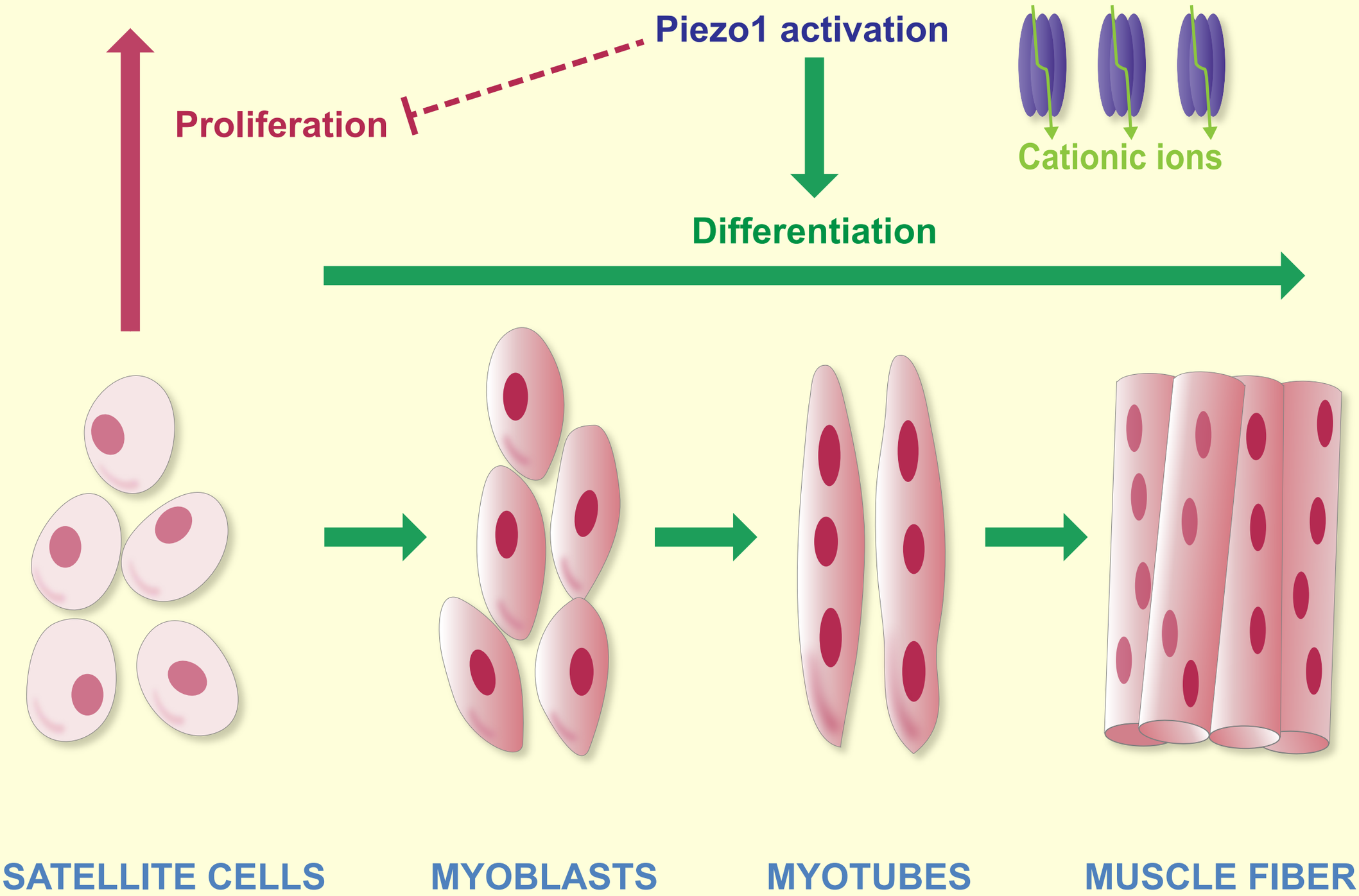
Piezo1 activation
Proliferation
Cationic ions
Differentiation
SATELLITE CELLS
MYOBLASTS
MYOTUBES
MUSCLE FIBER