

Synaptotagmin-4 promotes dendrite extension and melanogenesis in alpaca melanocytes by regulating Ca^{2+} influx via TRPM1 channels

Qiong Jia  | Shixiong Hu | Dingxing Jiao | Xiuqing Li | Shuhui Qi | Ruiwen Fan

College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, China

Correspondence

Ruiwen Fan, College of Animal Science and Veterinary Medicine, Shanxi Agricultural University, Taigu, 030801 Shanxi, China.
Email: ruiwenfan@163.com

Funding information

Shanxi Scholarship Council of China, Grant/Award Number: 2017-072; Aid Program for the Innovation Team in Shanxi Agricultural University, Grant/Award Number: CXTD201201

Synaptotagmin-4 (SYT4) is a membrane protein that regulates membrane traffic in neurons in a calcium-dependent or calcium-independent manner. In melanocytes, the intracellular free calcium ion (Ca^{2+}) may be important for dendrite growth and melanogenesis. Mammalian melanocytes originating from neural crest cells produce melanosomes. Therefore, we predicted that SYT4 might play a role in melanogenesis and the dendrite morphology of melanocytes. To investigate whether SYT4 is involved in melanocyte physiology, SYT4 was overexpressed in alpaca melanocytes and B16-F10 cells. The results showed that SYT4 overexpression resulted in a phenotype consistent with melanogenesis and dendrite extension. At the molecular level, SYT4 interacted with extracellular regulated MAP kinase (ERK) to decrease p-ERK activity, which negatively regulated CREB expression. Furthermore, cyclic AMP-responsive element-binding protein (CREB) was upregulated and caused the downregulation of the expression of melanogenic regulatory proteins, including microphthalmia-associated transcription factor (MITF), tyrosinase (TYR), tyrosinase-related protein-1 (TYRP1), dopachrome tautomerase (DCT), and transient receptor potential melastatin 1 (TRPM1). Intracellular free Ca^{2+} promoted the upregulation of calcium/calmodulin dependent protein kinase IV (CAMK4) expression, which phosphorylated CREB (p-CREB). In turn, p-CREB participated in the transcription of MITF. These results demonstrated that SYT4 promoted melanogenesis through dendrite extension and tyrosinase activity, during which the regulation of Ca^{2+} influx via the TRPM1 channel was a key factor.

Significance of the study: Intracellular Ca^{2+} is important for the function and survival of melanocytes and melanoma cells. SYT4 stimulated melanogenesis through calcium. These results provide evidence that SYT4 regulates Ca^{2+} influx through TRPM1 to cause melanogenesis and axonal elongation in alpaca melanocytes and further suggesting that the growth and metastasis of melanoma is controlled by the inhibited expression of SYT4 in melanoma cells.

KEYWORDS

Ca^{2+} , melanocyte, melanogenesis, SYT4, TRPM1

1 | INTRODUCTION

Synaptotagmins (SYTs) constitute a large family of membrane proteins that regulate membrane traffic in neurons and other types of cells¹, which are characterized by their distinct distributions patterns and different biochemical features.² The members of this protein family are likely to function as calcium sensors in calcium-regulated events in neurons.² One isoform, SYT4, serves as a postsynaptic Ca²⁺ sensor that releases retrograde signals to stimulate enhanced presynaptic function through the activation of the cyclic adenosine monophosphate (cAMP)-dependent protein kinase pathway.³ SYT4 inhibited SNAP [soluble N-ethylmaleimide-sensitive factor attachment protein] receptor (SNARE)-catalysed membrane fusion in both the absence and presence of Ca²⁺. Rat SYT4 could stimulate fusion in response to Ca²⁺ when the conserved Asp-to-Ser Ca²⁺ ligand substitution to the C2A domain was changed.⁴ SYT4 contains two internal repeats that have homology with the C2 domain of protein kinase C (PKC).⁵ This domain is known to regulate the calcium-dependent translocation of PKC to membranes and is present in many proteins with different functions.^{5,6} In SYTs, the two C2 domains are responsible for different calcium-dependent and calcium-independent reactions. SYT4 was shown to be expressed in muscle cells at the *Drosophila* neuromuscular junction.³ SYT4 is localized on insulin granules and transcriptional regulation of SYT4 affects insulin secretion.⁷ SYT4 is an integral vesicle protein present on brain-derived neurotrophic factor (BDNF)-harbouring dense core vesicles (DCVs) in neurons.⁸ SYT4 regulates the capture and spatial distribution the DCVs in hippocampal neurons.⁹ In a previous study, we found that SYT4 is expressed in mouse melanocytes and B16 melanoma cells.¹⁰

In the electron transfer system, calcium is important for both melanocyte function and survival.¹¹ An increase in intracellular free Ca²⁺ may be important in stimulating melanogenesis.¹² Transient receptor potential melastatin (TRPM) is a subfamily of ion channels that was originally identified as melanoma metastasis suppressor based on its expression in normal pigment cells in the skin and eye.¹³ TRPM1 (also known as melastatin) expression and intracellular Ca²⁺ levels are significantly lower in rapidly proliferating melanocytes compared with their levels in slow growing, differentiated melanocytes.¹⁴ Knocking down TRPM1 resulted in decreases in tyrosinase activity and intracellular melanin pigment levels.¹⁵ Calcium- and CAMK4, a cytoplasm and nucleus protein, is activated by Ca²⁺/calmodulin and is a multifunctional serine/threonine protein kinase,¹⁴ and nuclear effector of the Ca²⁺/calmodulin kinase (CAMK) pathway, where it coordinates transcriptional responses.¹⁵ CAMK4 phosphorylates CREB by acting upstream of CREB activation in β cells¹⁶ and in neurons.¹⁷ CREB activates transcription of MITF, which consequently triggers the transcription of downstream melanogenic genes such as TYR, TYRP1 and DCT.¹⁸ Mammalian melanocytes originate from neural crest cells.¹⁹ Therefore, we predicted that SYT4 leads to pigmentation in a process that is not completely understood.

2 | MATERIALS AND METHODS

2.1 | Construction of DNA recombination

The complete coding sequences (CDSs) of wild-type alpaca and mouse SYT4 were amplified by reverse transcription polymerase chain reaction (PCR) using a kit and gene-specific primers (Table 1) and subcloned into a pMD-19T vector to generate pMD-19T-SYT4 plasmids. The SYT4 inserts were liberated from pMD-19T-SYT4 using endonucleases NotI and XhoI and ligated into a pVAX1 expression vector to construct pVAX1-SYT4. The sequence of the construction was verified by restriction digestion and DNA sequencing. In addition, based on the mouse SYT4 CDS sequence, three SYT4 short hairpin RNAs (shRNAs) and negative controls (NCs) designed to target the mouse SYT4 mRNA were obtained from Sangon Biotech (Shanghai, China).

2.2 | Cell culture and transfection

Melanocytes used in this study were established by our lab and were maintained as previously described. B16-F10 cells are commercially got from Cell Research (Shanghai, China). Melanocytes and B16-F10 cells were transfected with pVAX1-SYT4, Sh-SYT4 and the negative control plasmid using Lipofectamine 2000 (Invitrogen) according to the manufacturer's instructions. Three days after transfection, the cells were collected. Cell lysates and total RNA were prepared and subjected to Western blot and real-time quantitative PCR analyses, respectively.

2.3 | Total RNA isolation and quantitative real-time PCR analysis

Total RNA from transfected cells was extracted with TRIzol reagent (Invitrogen) according to the manufacturer's instructions and treated with DNase I (MilliporeSigma, Burlington, MA, USA). For mRNA quantification, 1 μ g of total RNA was converted by a cDNA synthesis kit (TaKaRa, Dalian, China) according to the manufacturer's instructions, and qPCR was performed using SYBR Green PCR master mix (TaKaRa). For analyses, qPCR was performed with SYBR Green PCR Master Mix (TaKaRa) on a 7500 Fast Real-Time PCR system (Thermo Fisher Scientific). The primer sequences are listed in Table 1. Melting curves for each sample were analysed to validate the amplification specificity. All mRNAs were normalized to the amount of 18S mRNA. Quantification of mRNA transcript abundance was performed by using the comparative threshold cycle method. The significance the differences in the levels of mRNA was determined by ANOVA (SPSS 11.5 Software; IBM, Armonk, NY, USA).

2.4 | Western blotting

Protein samples from transfected cells were got by and were separated on 10% or 8% SDS-PAGE gels and transferred to nitrocellulose membranes. The membranes were blocked with 5% skim milk

TABLE 1 Primers used in this study

Primer Name	Primer Sequence 5'-3'	Annealing Temperature, °C
SYT4-F	ATAGCGGCCGCATGGCTCCTAT CACCACCAGCCGC	64
SYT4-R	ATCCTCGAGTATGGCTCCTAT CACCACCAG	
SYT4-F-RT	CCTCGTCTTCACTGTCTCTCTTT	58
SYT4-R-RT	TTCACCTCACTTTGTTCATCTCCTC	
MITF-F (alpaca)	TCCCAAGTCAAATGATCCAG	52
MITF-R (alpaca)	GAGCCTGCATTTCAAGTTCC	
DCT-F (alpaca)	CCAGGGCGCCCATACAAGGC	60
DCT-R (alpaca)	GCTGGTCTGTGCACACN(1/2G,1/2A)TCA	
18S-F (alpaca)	GAAGGGCACCACCAGGAGT	50-64
18S-R (alpaca)	CAGACAAATCACTCCACCAA	
MITF-F (mus)	GAGCAGAGCAGGGCAGAGAGTGAGT	60
MITF-R (mus)	TGGGAAGGTTGGCTGGACAGGAGTT	
TYR-F (mus)	TGAAAATCCTAACTTACTCAGCCCA	60
TYR-R (mus)	TCAAACCTCAGACAAAATCCACATC	
TYRP1-F (mus)	CCATTGCTGTAGTGCTGCGTTGTT	61
TYRP1-R (mus)	GGAGAGGCTGGTTGGCTTCATTCTT	
DCT-F (mus)	TTGCTCTGGGGTGTCTGGCTTTTC	61
DCT-R (mus)	TCCTCCGTGTATCTCTTGTCTGCTGA	
β -actin (mus)	TTGCTGACAGGATGCAGAAG	50-64
β -actin (mus)	ACATCTGCTGGAAGGTGGAC	

for 1 hour and incubated overnight at 4°C with the following diluted primary antibodies against SYT4 (1:1000, Abcam, Cambridge, UK), CAMK4 (1:1000, OmnimAbs), TRM1 (1:1000, Abcam, Cambridge, UK), ERK (1:1000, Cell Signaling Technology, Beverly, MA), MITF (1:1000, Abcam, Cambridge, UK), TYR (1:1000, Abcam, Cambridge, UK), TYRP1 (1:1000, Abcam, Cambridge, UK), DCT (1:1000, Abcam, Cambridge, UK), and β -actin (1:1000, CWBIO, Beijing, China). After the membranes were washed three times with TBST for 10 minutes and washed, they were incubated at 37°C for 1 hour with horseradish peroxidase-conjugated secondary antibodies against rabbit or mouse IgG (1:10 000, Invitrogen). After the membranes were washed again with TBST six times for 5 minutes per wash, the bound antibodies were visualized using enhanced chemiluminescence. Immunoblots were scanned on a ChemiDoc XRS+ Imager (Bio-Rad, Hercules, CA, USA), and protein levels were quantified with Image-Pro Plus Software (Olympus, Tokyo, Japan).

2.5 | Ca²⁺ imaging

Ca²⁺ imaging was performed according to the manufacturer's instructions. Melanocytes were harvested and rinsed with phosphate-buffered saline (PBS). Then, the melanocytes were labelled with Rhod-3 (Invitrogen) for 1 hour at room temperature, washed with PBS three times for 5 minutes per wash, and incubated for 1 hour to de-

esterify the dye.²⁰ Labelled cells are subjected to live cell imaging with laser scanning confocal microscope (FV1000, Olympus, Japan).

2.6 | Immunocytochemistry

Melanocytes were washed three times for 3 minutes per wash with PBS, fixed in 4% paraformaldehyde for 20 minutes, and incubated at room temperature in 3% hydrogen peroxide for 15 minutes to block the action of the endogenous peroxidases. After three 5-minute washes with PBS, the cells were immersed in bovine serum albumin (BSA) at 37°C for 25 minutes and incubated at 4°C overnight in rabbit anti-SYT4 antibody solution. After three 5-minute washes with PBS, the cells were incubated with horseradish peroxidase-conjugated anti-rabbit IgG for 30 minutes at 37°C and observed under a microscope (Leica Microsystems, Buffalo Grove, IL, USA). For the negative control (NC), IgG was substituted for the primary antibody.

2.7 | Tyrosinase activity measurement

Melanocytes were washed three times with PBS for 3 minutes per wash. Then, the cells were seeded into microtiter plates containing 100 μ L of 1% Triton X-100 in each well, and the plate was vortexed for 5 minutes. After a 1-hour incubation at room temperature, 50 μ L of 1% Levodopa was added (LJ350101; Cool Chemistry) and the plate was further incubated at 37°C for 2 hours. Tyrosinase activity was

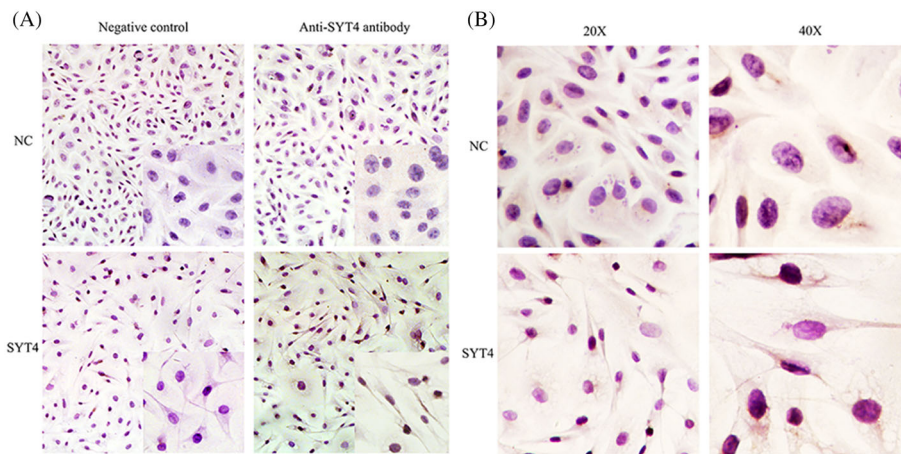


FIGURE 1 The effect of SYT4 on cell morphology. A, Analysis of SYT4 protein expression in melanocytes transfected with SYT4 overexpressing plasmids using immunocytochemistry. B, Overexpression of SYT4 in alpaca melanocytes makes cell synapse slender

determined spectrophotometrically by measuring the absorbance at 460 nm.

2.8 | Melanin measurement

Melanocytes transfected with SYT4-CDS and Sh-SYT4 were harvested and rinsed with PBS. For the assay of the total alkali-soluble melanin (ASM), 1 mL of 0.2M NaOH was added, and the absorbance of the solution was measured spectrophotometrically at 475 nm. For the assay of eumelanin (EM), the cells were hydrolysed in hot 30% hypophosphoric acid and hydriodic acid. After cooling, 50% ethanol was added, and the cell samples were centrifuged at 2, 234 g for 10 minutes. Insoluble eumelanin pigments were selectively solubilized in hot sodium hydroxide and hydrogen peroxide, and the solution was

cleared by centrifuging it at 10 700 g for 1 minute. The absorbance of the supernatant was measured at 350 nm. For pheomelanin (PM) assay, the cells were solubilized in phosphate buffer (pH 10.5) and cleared by centrifugation at 10, 700 g for 10 minutes before the determination of the absorbance at 400 nm. The melanin levels were normalized to the total number of cells. All experiments were performed in triplicate.

2.9 | Statistical analysis

Data related to the levels of mRNA, protein, tyrosinase activity, and melanin levels in the control and experimental groups were analysed using the Statistical Analysis System (GraphPad Prism 5). All

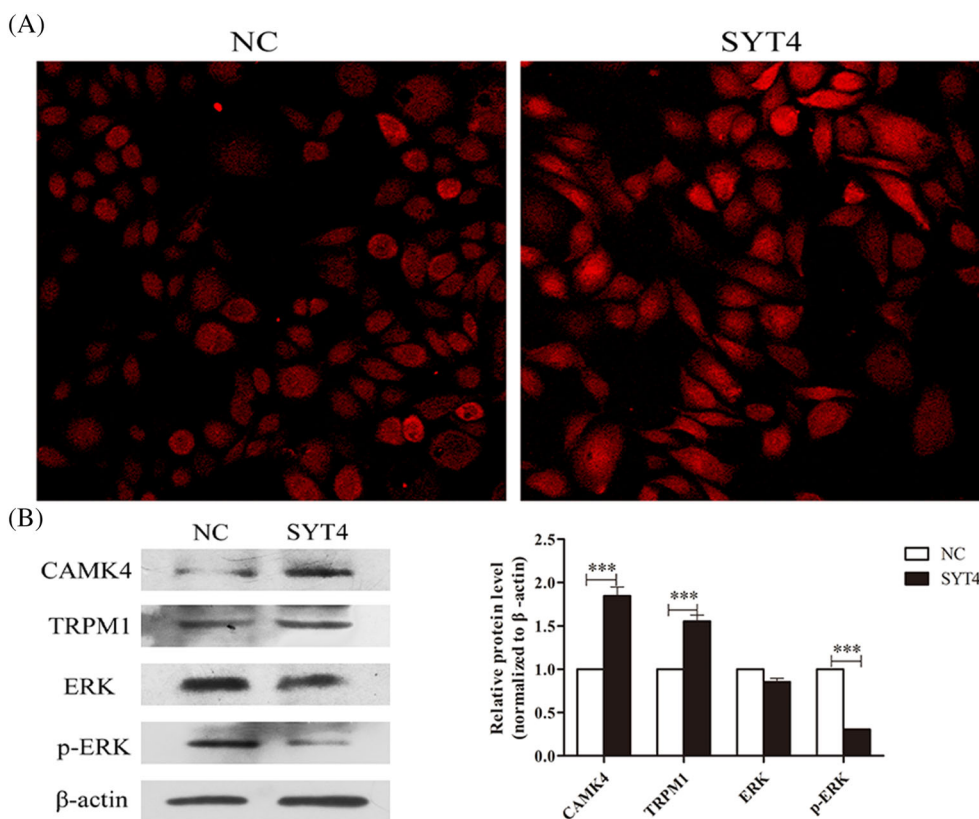


FIGURE 2 Effect of the overexpression of SYT4 on calcium influx and Ca^{2+} -related protein expression levels in alpaca melanocytes. A, Overexpression of SYT4 in alpaca melanocytes promotes calcium influx. B, The results from the Western blot and abundance analyses of the protein expression of CAMK4, TRPM1, ERK in SYT4-overexpressing melanocytes compared with that in the NC group. Data represent the mean \pm standard error (n = 3). ** $P < .05$. *** $P < .001$

experiments were repeated three times, and the data are expressed as the mean \pm standard error (SE). Statistical comparisons between different treatments were performed using one-way analysis of variance followed by a T test.

3 | RESULTS

3.1 | The effect of SYT4 on the phenotypes of the melanocytes with the overexpression of SYT4

To investigate the effect of SYT4 on cell morphology, an immunocytochemistry method was used. The results showed that SYT4 immunoreactivity was observed in the cytoplasm of melanocytes, and it was stronger in melanocytes overexpressing SYT4 than in NC cells (Figure 1A). At the same time, the melanocytes transfected with the NC plasmid showed a normal fusiform or polygonal shape, while the dendrites of melanocytes overexpressing SYT4 were obviously slender (Figure 1B).

3.2 | The effect of SYT4 on free intracellular Ca^{2+} and the protein expression of the related genes

By calcium imaging, the fluorescence signal of Ca^{2+} in melanocytes overexpressing SYT4 was significantly stronger than that in the

negative control (Figure 2A), which indicated that SYT4 overexpression could upregulate free intracellular Ca^{2+} . Furthermore, the protein expression of the genes related to Ca^{2+} , CAMK4, and TRPM1 was unregulated, while that of p-ERK was downregulated in the melanocytes overexpressing SYT4, compared with the expression of these proteins in the NC (Figure 2B).

3.3 | The effect of SYT4 on the tyrosinase activity and melanins production

To determine whether SYT4 affected tyrosinase activity, tyrosinase activity was measured after SYT4 was overexpressed or knocked down in alpaca melanocytes. The results showed that the tyrosinase activity was increased by >100% in alpaca melanocytes overexpressing SYT4 (Figure 3A). To determine whether SYT4 affected melanins production, the production of ASM, EM, and PM was measured in alpaca melanocytes after SYT4 was overexpressed or knocked down. The results showed that the production of ASM, EM, and PM was significantly higher in melanocytes overexpressing SYT4 than that in NC cells overexpressing SYT4 (Figure 3B). In contrast, the tyrosinase activity and the production of ASM, EM, and PM was significantly lower in melanocytes with SYT4 knocked down than that in the NC cells (Figure 3C,D).

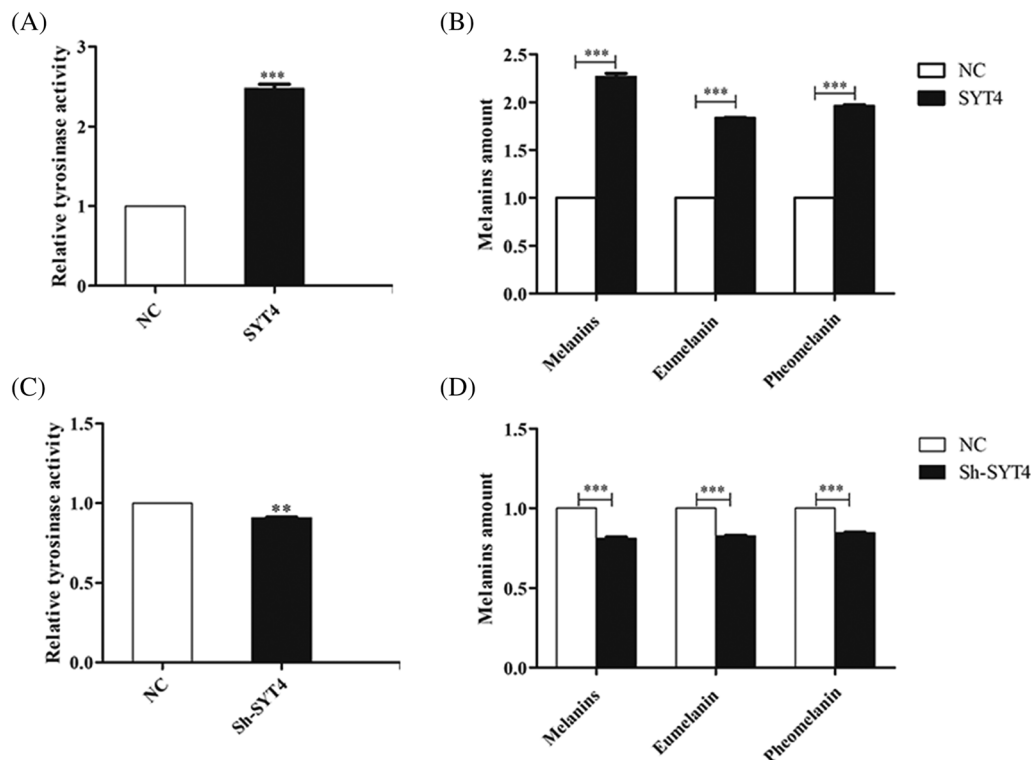


FIGURE 3 Effects of SYT4 on tyrosinase activity and melanins production. A, Effect of SYT4 overexpression on tyrosinase activity in alpaca melanocytes. B, Effect of SYT4 overexpression on the production of ASM, EM, and PM in alpaca melanocytes. C, Effect of SYT4 knock down on tyrosinase activity in alpaca melanocytes. D, Effect of SYT4 knock down on the production of ASM, EM, and PM in alpaca melanocytes. Data represent the mean \pm standard error ($n = 3$). ** $P < .05$. *** $P < .001$

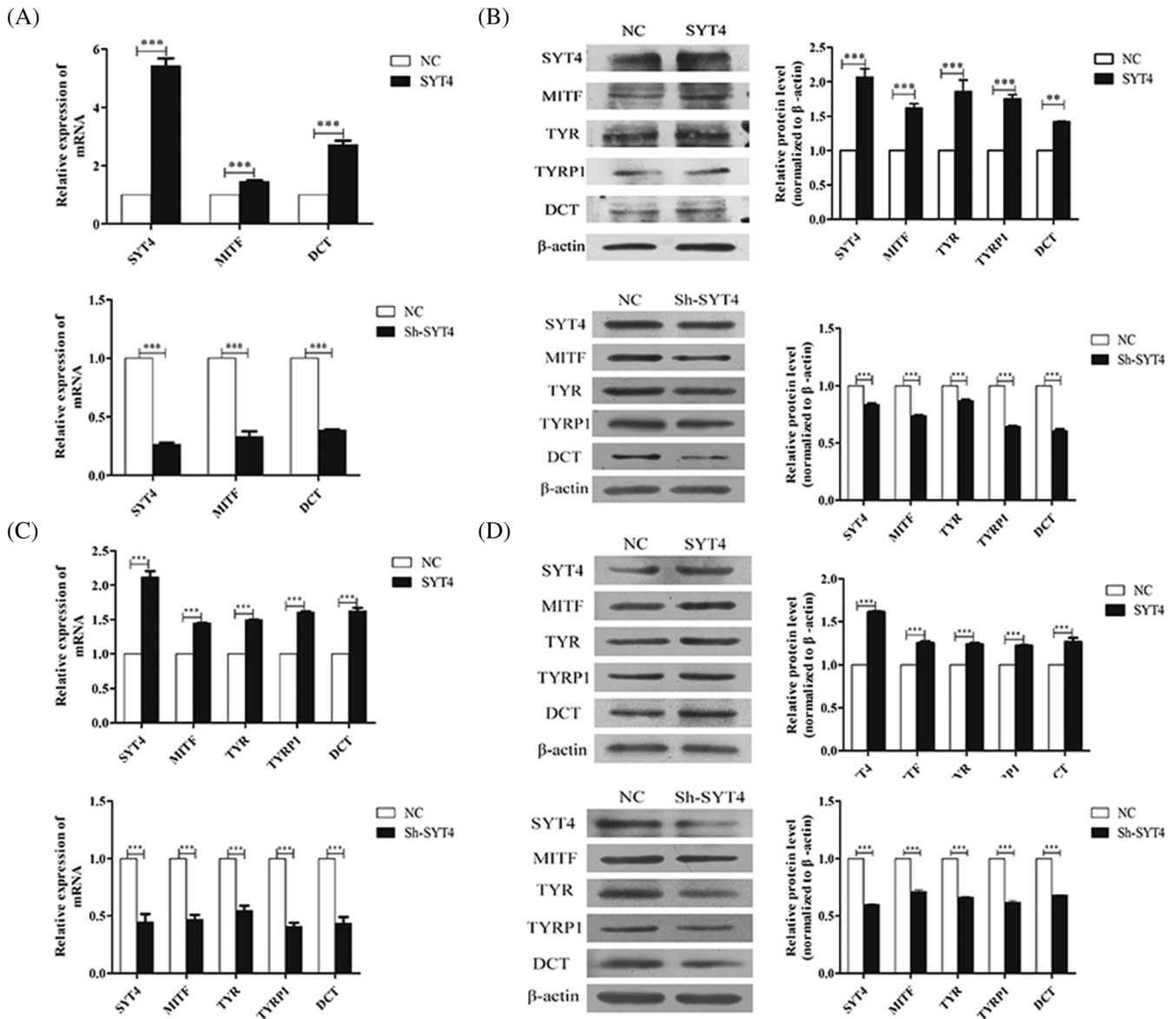


FIGURE 4 Effects of SYT4 overexpression on the protein and mRNA expression levels of melanogenesis-related genes in alpaca melanocytes. A, Results from the qPCR analyses of SYT4, MITF, and DCT mRNA expression in melanocytes with SYT4 overexpressed or knocked down compared with the expression in the cells of the NC groups. B, Results from the Western blot and abundance analyses of SYT4, MITF, TYR, TYRP1, and DCT protein expression in melanocytes with SYT4 overexpressed or knocked down compared with the expression in the cells of the NC group. C, Results from the qPCR analyses of SYT4, MITF, TYR, TYRP1 and DCT mRNA expression in melanoma B16-F10 cells with SYT4 overexpressed and knocked down compared with the expression in the cells of the NC groups. D, Western blot and abundance analyses of SYT4, MITF, TYR, TYRP1 and DCT protein expression in B16-F10 melanoma cells with SYT4 overexpressed or knocked down compared with expression in the cells of the NC groups. Data represent mean \pm standard error ($n = 3$). ** $P < .05$. *** $P < .001$

3.4 | SYT4 regulated the expression of melanogenesis related genes in alpaca melanocytes

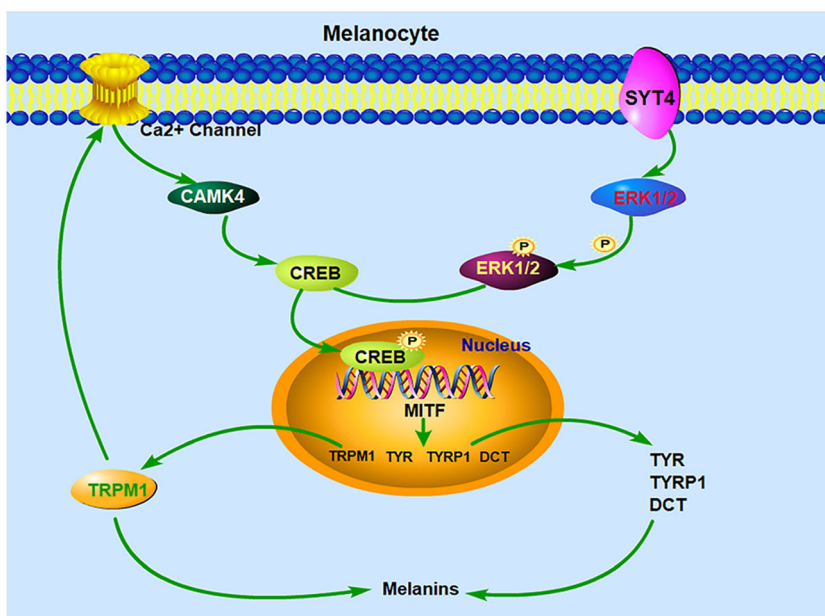
To explore the effects of SYT4 on melanogenesis in alpaca melanocytes, we investigated the expression levels of related genes. Overexpressed and knocked down SYT4 in melanocytes resulted in the increase and decrease of quantitative levels of mRNAs encoding MITF, and DCT, respectively (Figure 4A). The results of the Western blot analysis showed that the expression of the MITF, TYR, TYRP1, and DCT proteins was significantly increased and decreased in

melanocytes with SYT4 overexpressed or knocked down compared with that of the NC cell groups, respectively (Figure 4B).

3.5 | Verification of SYT4 in another melanoma cell line: B16-F10

To verify the effects of SYT4 on melanogenesis, SYT4 was overexpressed or knocked down in B16-F10 cells. The expression of mRNAs of MITF, TYR, TYRP1, and DCT was detected. The results showed that the expression levels of these genes were significantly

FIGURE 5 Graphic showing the signalling pathway by which SYT4 regulates melanogenesis via Ca^{2+} influx



increased and decreased in B16-F10 cells with overexpressed and knocked down SYT4 compared with that in the NC groups, respectively (Figure 4C). Similarly, results from the Western blot analysis showed that the expression of MITF, TYR, TYRP1, and DCT proteins was also significantly increased and decreased in B16-F10 cells with overexpressed and knocked down SYT4, respectively (Figure 4D).

4 | DISCUSSION

SYT family members with C2 domains were found to be related to dendrite lengthening.²¹ It has been reported that SYT14L containing the C2 domain induces significant elongation in dendrite length that was accompanied by the induction of melanocyte differentiation-related markers, including those for melanin synthesis, tyrosinase catalytic activity, and the expression of TYR, TYRP1, and DCT.²¹ Here, we investigated whether SYT4 overexpression induced the morphological phenotype of dendritic extension and the action of the melanocytes.

In mammalian skin, melanins are transferred within melanosomes through melanocytic dendrites to epidermal keratinocytes, which gives rise to the observed pigmentation, and it has been reported that dendrite formation is promoted by increases in intracellular Ca^{2+} in human melanocytes.²² Pigmentation can be regulated by Ca^{2+} influx via a transient receptor potential cation channel: the TRPM1 channel,²³ the downregulation of which has been correlated with a higher metastatic risk of skin melanoma.²⁴ Based on the results of the Rhod-3 calcium imaging kit, the Ca^{2+} influx was observed to be greater in melanocytes overexpressing SYT4. The results suggested that the opening of TRPM1 was promoted by SYT4 to increase free intracellular Ca^{2+} . Therefore, SYT4 induced Ca^{2+} to play important roles in the extended dendrites of melanocytes, which are responsible

for melanogenesis. In turn, we found that an increase in the amount of Ca^{2+} in the melanocytes, as induced by SYT4, led to upregulated CAMK4. The CAMK4/CREB cascade forms a signalling pathway in some cells, such as β cells¹⁶ and neurons,¹⁷ according to the extent by which CAMK4 phosphorylates CREB. In an interesting finding, SYT4 interacted with ERK and positively regulated p-ERK expression. The ERK signalling pathway may negatively affect the melanogenesis^{23,25} induced by the p53-POMC-MC1R signalling cascade to enhance the phosphorylation levels of ERK1/2 and CREB.²⁶ CREB activates the transcription of MITF, which consequently triggers the transcription of the downstream melanogenic genes TYR, TYRP1, and DCT,¹⁸ causing increases in the melanin content. However, TRPM1 expression was regulated by MITF in melanocytes.²⁷ Thus, SYT4 regulated melanogenesis by a crosstalk.

5 | CONCLUSION

Taken together, the findings indicate that SYT4 overexpression causes a positive loop depended on Ca^{2+} influx, a key factor that is regulated by TRPM1. Such functional morphological changes in the melanocytes of skin melanoma and the colour formation in hair and skin share the similar molecular mechanisms (Figure 5).

DATA AVAILABILITY STATEMENT

The data generated in this study are original and available, have not been published before, and are not currently under review elsewhere.

ACKNOWLEDGEMENTS

The authors thank their colleagues in the laboratory for their help with this study. This work was from a research project supported by the

Shanxi Scholarship Council of China (2017-072), a Special Fund for the Young Sanjin Scholars Distinguished Professor (to Ruiwen Fan) and the Aid Program for the Innovation Team in Shanxi Agricultural University (grant number CXTD201201).

CONFLICT OF INTEREST

The authors have no conflicts of interest to declare

ORCID

Qiong Jia  <https://orcid.org/0000-0003-2236-9995>

REFERENCES

- Hilbush BS, Morgan JI. A third synaptotagmin gene, Syt3, in the mouse. *P Natl Acad Sci*. 1994;91(17):8195-8199.
- Osborne S, Herreros J, Bastiaens P, Schiavo G. Calcium-dependent oligomerization of synaptotagmins I and II—synaptotagmins I and II are localized on the same synaptic vesicle and heterodimerize in the presence of calcium. *J Biol Chem*. 1999;274(1):59.
- Yoshihara M, Adolfsen B, Galle KT, Littleton JT. Retrograde signaling by Syt 4 induces presynaptic release and synapse-specific growth. *Science*. 2005;310(5749):858-863.
- Wang Z, Wang Z, Chapman ER. Rat and drosophila synaptotagmin 4 have opposite effects during SNARE-catalyzed membrane fusion. *J Biol Chem*. 2010;285(40):30759-30766.
- Pointing CP, Parker PJ. Extending the C2 domain family: C2s in PKCs δ , ϵ , η , θ , phospholipases, GAPS, and perforin. *Protein Sci*. 2010;5(1):162-166.
- Schiavo G, Osborne SL, Sgouros JG. Synaptotagmins: more isoforms than functions? *Biochem Biophys Res Commun*. 1998;248(1):0-8.
- Huang C, Walker EM, Dadi PK, et al. Synaptotagmin 4 regulates pancreatic β cell maturation by modulating the Ca^{2+} sensitivity of insulin secretion vesicles. *Dev Cell*. 2018;45:S1534580718302338.
- Dean C, Liu H, Mark Dunning F, et al. Synaptotagmin-IV modulates synaptic function and long-term potentiation by regulating BDNF release. *Nat Neurosci*. 2009;12(6):767-776.
- Bharat V, Siebrecht M, Burk K, et al. Capture of dense core vesicles at synapses by JNK-dependent phosphorylation of synaptotagmin-4. *Cell Rep*. 2017;21(8):2118-2133.
- Ji K, Fan R, Zhang J, et al. Differential expression of mRNA between murine melanocytes and B16 melanoma cells (in Chinese). *Chinese J Biochem Molec Biol*. 2019;35(1).
- Schallreuter-Wood KU, Pittelkow MR, Swanson NN. Defective calcium transport in vitiliginous melanocytes. *Arch Dermatol Res*. 1996;288(1):11.
- Carsberg CJ, Jones KT, Sharpe GR, et al. Intracellular calcium modulates the responses of human melanocytes to melanogenic stimuli. *J Dermatol Sci*. 1995;9(3):157-164.
- Devi S, Kedlaya R, Maddodi N, et al. Calcium homeostasis in human melanocytes: role of transient receptor potential melastatin 1 (TRPM1) and its regulation by ultraviolet light. *Am J Physiol-Cell Physiol*. 2009;297(3):C679-C687.
- Khattri A, Reddy VP, Pandey RK, et al. Novel mutations in calcium/calmodulin-dependent protein kinase IV (CAMK4) gene in infertile men. *Int J Androl*. 2012;35(6):810-818.
- Editor PE. IB4-binding sensory neurons in the adult rat express a novel 3' UTR-extended isoform of CAMK4 that is associated with its localization to axons. *J Comp Neurol*. 2013;522(2):308-336.
- Bo L, Helena BS, Jones PM, et al. The CAMK4/CREB/IRS-2 cascade stimulates proliferation and inhibits apoptosis of β -cells. *PLoS ONE*. 2012;7(9):e45711.
- Bilbao A, Parkitna JR, Engblom D, et al. Loss of the Ca^{2+} /calmodulin-dependent protein kinase type IV in dopaminergic neurons enhances behavioral effects of cocaine. *P Natl Acad Sci*. 2008;105(45):17549-17554. <https://doi.org/10.1073/pnas.0803959105>
- Vachtenheim J, Borovansky J. "Transcription physiology" of pigment formation in melanocytes: central role of MITF. *Exp Dermatol*. 2010;19(7):617-627.
- Kos L, Aronson A, Takayama H, et al. Hepatocyte growth factor/scatter factor-MET signaling in neural crest-derived melanocyte development. *Pigment Cell Res*. 1999;12(1):13-21.
- Ieda M, Fu JD, Delgado-Olguin P, et al. Direct reprogramming of fibroblasts into functional cardiomyocytes by defined factors. *Nihon Rinsho Japanese J Clin Med*. 2011;142(3):375-386.
- Yoo JC, Lim TY, Park JS, et al. SYT14L, especially its C2 domain, is involved in regulating melanocyte differentiation. *J Dermatol Sci*. 2013;72(3):246-251.
- Abdel-Naser MB, Seltmann H, Zouboulis CC. SZ95 sebocytes induce epidermal melanocyte dendricity and proliferation in vitro. *Exp Dermatol*. 2012;21(5):393-395.
- Hwang E, Lee TH, Lee WJ, et al. A novel synthetic Piper amide derivative NED-180 inhibits hyperpigmentation by activating the PI3K and ERK pathways and by regulating Ca^{2+} influx via TRPM1 channels. *Pigm Cell Melanoma R*. 2016;29(1):81-91.
- Moulin AP, Zografos L. TRPM1 and MITF expression in conjunctival melanocytic proliferations. *Acta Ophthalmol*. 2011;89(s246):0-0.
- Kim DS, Kim SY, Chung JH, Kim KH, Eun HC, Park KC. Delayed ERK activation by ceramide reduces melanin synthesis in human melanocytes. *Cell Signal*. 2002;14(9):779-785.
- Zhou D, Kuang Z, Zeng X, et al. p53 regulates ERK1/2/CREB cascade via a novel SASH1/MAP 2K2 crosstalk to induce hyperpigmentation. *J Cell Mol Med*. 2017;21(10):2465-2480.
- Miller AJ. Transcriptional regulation of the melanoma prognostic marker melastatin (TRPM1) by MITF in melanocytes and melanoma. *Cancer Res*. 2004;64(2):509-516.

How to cite this article: Jia Q, Hu S, Jiao D, Li X, Qi S, Fan R. Synaptotagmin-4 promotes dendrite extension and melanogenesis in alpaca melanocytes by regulating Ca^{2+} influx via TRPM1 channels. *Cell Biochem Funct*. 2020;38:275-282. <https://doi.org/10.1002/cbf.3465>