PIBB 生物化学与生物物理进展
Progress in Biochemistry and Biophysics
2017, 44(11): 1037~1040
www.pibb.ac.cn

The Effects and Mechanisms of Syntaxin-1 on The Differentiation of Murine Neuroblastoma

Dear Editor,

Synaptic vesicle fusion is mediated by the SNARE (for soluble N-ethylmaleimide-sensitive factor attachment receptor) proteins and SM (for "Sec1-Munc18 like") protein in neurons [1-2]. Syntaxin-1, SNAP25 and synaptobrevin-2 form a four-helical bundle to force presynaptic membrane fusion [3]. Among synaptic SNARE proteins, syntaxin-1 contains an N-terminal sequence (N-peptide), a three-helix bundle (H_{abc} domain), a linker region, a SNARE motif (H_3 domain), and a transmembrane region [4]. Besides the SNARE motif that can form SNARE complex, the H_{abc} domain has been revealed to fold back onto the SNARE motif of syntaxin-1 to maintain the "closed" conformation[5]. The transmembrane domain (TMR) of syntaxin-1 has suggested a role in fusion pore opening[6]. However, the essential role of syntaxin-1 in neurotransmitter release has been well demonstrated, whether it is involved in nerve cell differentiation process still remain unclear. The aim of this study was to determine the effects of syntaxin-1 in neuronal differentiation in the mouse neuroblastoma cells (N2a).

In order to investigate whether syntaxin-1 play a role in neuronal differentiation, N2a cells were transfected with GFP co-expressed empty vector or wild type syntaxin-1 ($Syntaxin^{WT}$), respectively. The cells were imaged 24 h after transfection using confocal microscopy. Among the transfected cells, those the total length of neurite is longer than 2 folds of the diameter of the cell were counted as differentiated N2a cells [7]. For each batch of cells, differentiation rate of the cell, number of branches and total length of the neurite were analyzed to determine the effects of syntaxin-1 in neuronal differentiation. As shown in Figure 1, the differentiation ratio of N2a cells was unchanged with or without syntaxin-1 overexpression. On the other hand, syntaxin-1 overexpression dramatically increased the branch numbers and total length of neurite. Since the differentiation ratio reflects the number of differentiated N2a cells, whereas the branch numbers and length of neurite reflect status of differentiated N2a cells, the results suggested that syntaxin-1 could facilitate rather than initiate N2a cell differentiation.

To further understand whether there is structure/function dependence of syntaxin-1 in neuronal differentiation, different truncated or inserted mutations of syntaxin-1 were expressed into N2a cells. To explore the role of H_{abc} domain of syntaxin-1 in N2a cell differentiation，as shown in Figure 2a and 2b, the H_{abc} domain truncated syntaxin-1 mutant ($Syntaxin^{\Delta Habc}$) was transfected into N2a cells. The results revealed that the mutant lacking H_{abc} domain of syntaxin-1 not only leave the differentiation ratio of N2a cells intact, but also lose the activity to increase the branch numbers and length of neurite as $Syntaxin^{WT}$, thus indicating the facilitation role of syntaxin-1 in N2a cell differentiation is mainly dependent on its N-terminal H_{abc} domain.

After investigating the function of N-terminal region of syntaxin-1 in N2a cell differentiation, the C-terminal domain of syntaxin-1 was analyzed by transfecting the TMR of syntaxin-1 truncated mutant into N2a cells. Interestingly, the TMR truncated but lipid-anchored of syntaxin-1 mutant ($Syntaxin^{\Delta TMR}$) [8] restored the increase in the branch numbers and length of neurite induced by $Syntaxin^{WT}$ overexpression

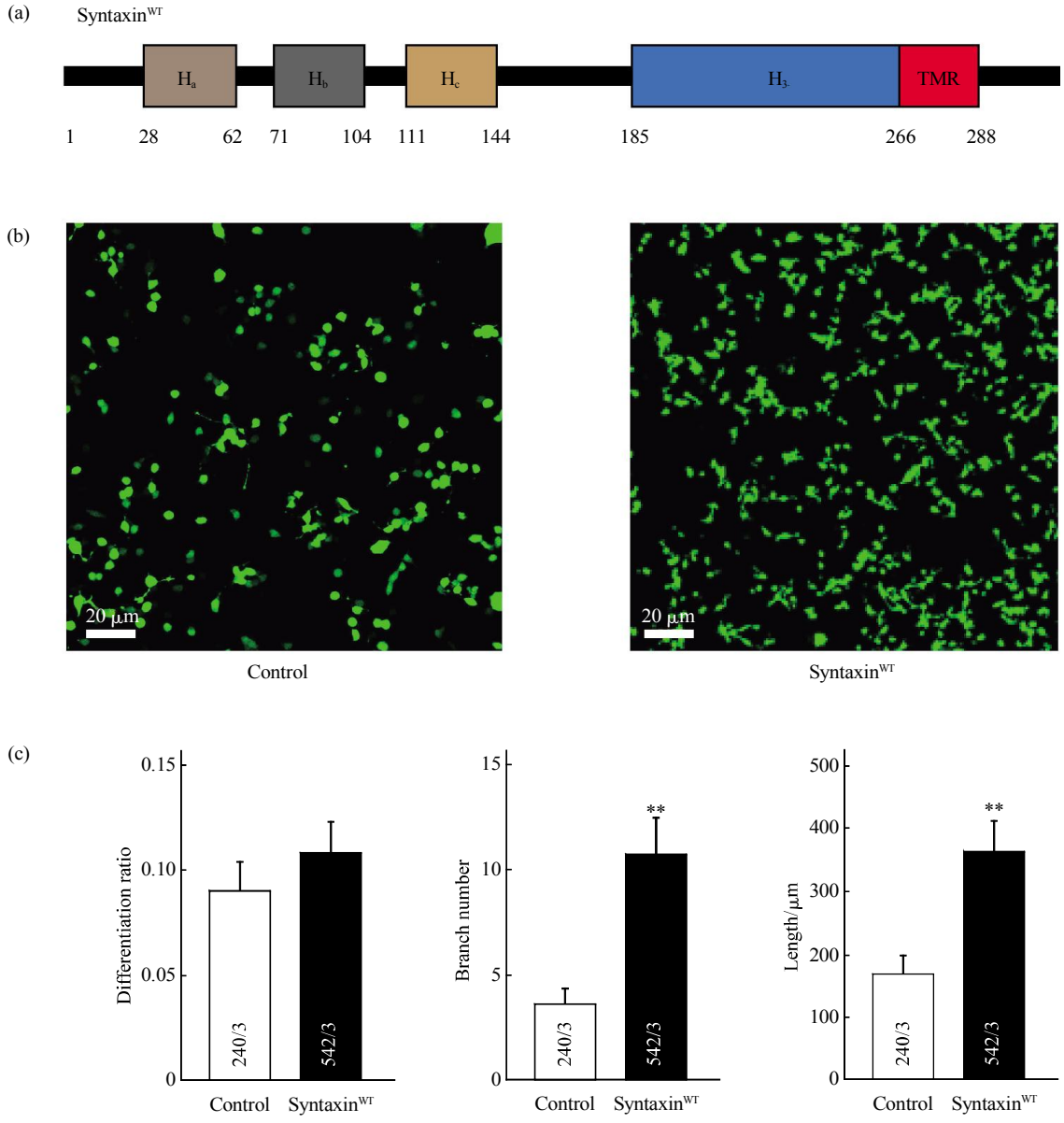

Fig. 1　The effect of syntaxin^{WT} overexpression on the differentiation of N2a cell

(a) Schematic structures of wild type syntaxin-1 (Syntaxin^{WT}). (b) Representative images of N2a cells transfected with GFP (Control) or Syntaxin^{WT}. (c) Summary graphs of differentiation ratio (left), branch numbers (middle) and length of neurite (right) analyzed from N2a cells as described for (b). Data are means±SEM; numbers of cells/independent experiments analyzed are listed in the bars. Statistical assessments were performed by Student's *t*-test comparing each condition to control (**$P < 0.01$).

(Figure 2c), suggesting the C-terminal region of syntaxin-1 is not required for promoting N2a cell differentiation. Previous studies have demonstrated the distance of the SNARE complex from the TMR in syntaxin-1 is crucial for its function in mediating membrane fusion [9]. In order to explore whether the distance of the SNARE complex from the TMR in syntaxin-1 is also important in N2a cell differentiation, a mutant contained seven residues inserted into syntaxin-1 at a position N-terminal to the TMR (Syntaxin^{7i}) [8] was introduced into N2a cells. As Figure 2d showed, Syntaxin^{7i} partially rescue the increase in the branch numbers but not in length of neurite comparing with Syntaxin^{WT}, indicating the

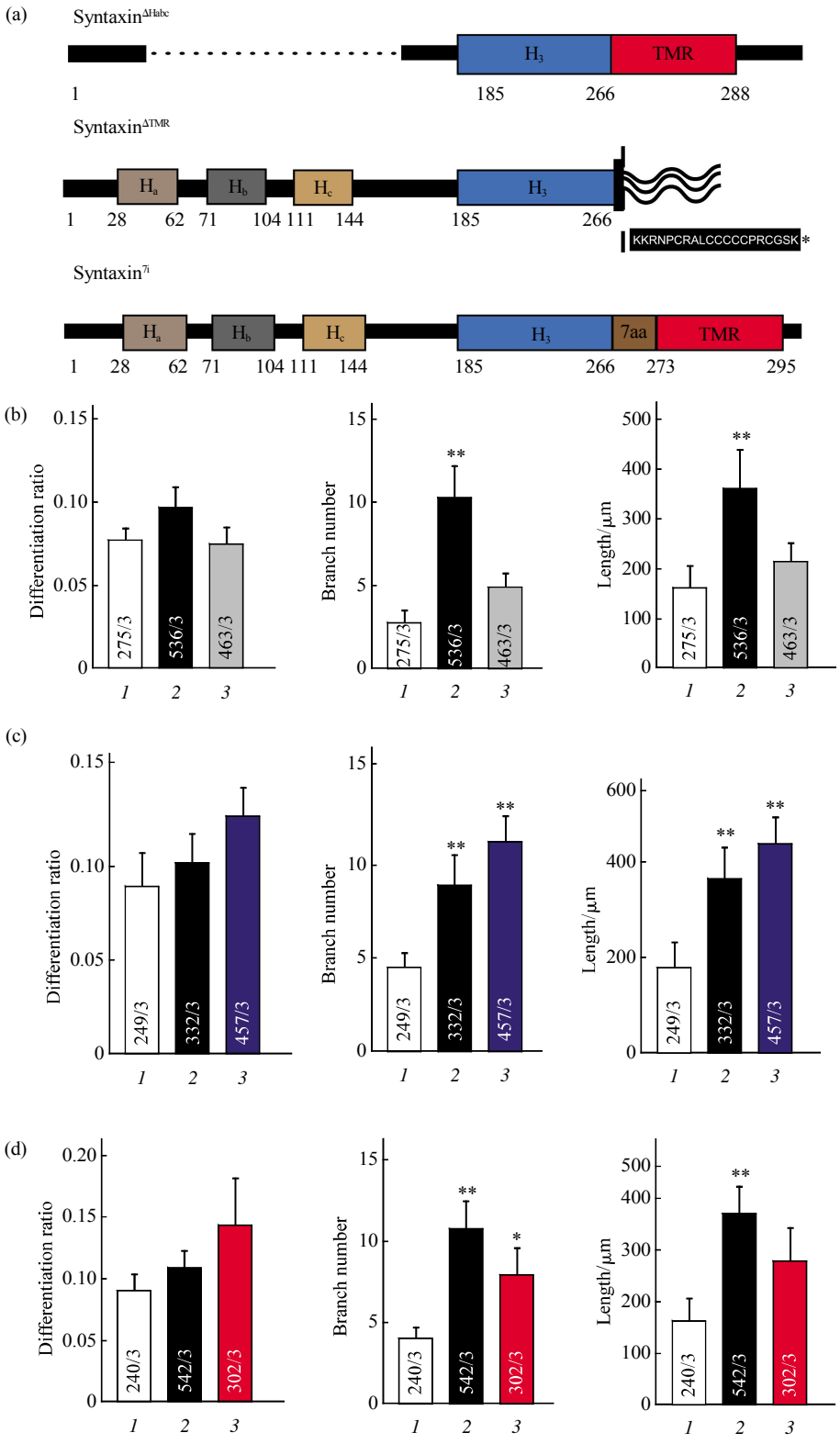

Fig. 2 The effects of mutants of syntaxin-1 on the differentiation of N2a cell

(a) Schematic structures of H_{abc} domain truncated syntaxin-1 (Syntaxin$^{\Delta Habc}$), transmembrane domain truncated but lipid-anchored syntaxin-1 (Syntaxin$^{\Delta TMR}$) and 7 amino acid inserted syntaxin-1 (Syntaxin^{7i}), respectively. (b-d) Summary graphs of differentiation ratio (left), branch numbers (middle) and length of neurite (right) analyzed from N2a cells for Syntaxin$^{\Delta Habc}$ (b), Syntaxin$^{\Delta TMR}$ (c), Syntaxin^{7i} (d), respectively. Data are means±SEM; numbers of cells/independent experiments analyzed are listed in the bars. Statistical assessments were performed by Student's *t*-test comparing each condition to control (*$P < 0.05$; **$P < 0.01$). (b) *1*: Control; *2*: Syntaxin^{WT}; *3*: Syntaxin$^{\Delta Habc}$. (c) *1*: Control; *2*: Syntaxin^{WT}; *3*: Syntaxin$^{\Delta TMR}$. (d) *1*: Control; *2*: Syntaxin^{WT}; *3*: Syntaxin^{7i}.

distance of the SNARE complex from the TMR in syntaxin-1 is involved in N2a cell differentiation process as well. In closing, the observations suggested that syntaxin-1 facilitated N2a cell differentiation. This facilitation is largely dependent on its N-terminal H_{abc} domain and the distance of the SNARE complex from the TMR in syntaxin-1.

Acknowledgements This work was supported by grants from The National Natural Science Foundation of China (31670850) and the Basic Research Project of SCUN (ZZ13002).

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DOI: 10.16476/j.pibb.2017.0331
Received: September 29, 2017 Accepted: October 23, 2017

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