primary fibroblasts was passage limited. Therefore, to establish a cell model will be convenient for some of the preliminary data collection. SMA is characterized by reduced expression of SMN proteins. Gene knockdown is a powerful technique to study the role of SMN in SMA. However, most of the articles published to study SMA with RNA interference used transient expression systems, and it is difficult to perform long-term studies with these transient expression systems. The stable knockdown cells can be used for long-term studies. In this article, we successfully established a stably inducible SMN knockdown cell model system. Different SMN protein levels were induced by 0.5 ug/mL and 1 ug/mL doxycycline. This indicates that, with this cell model, the SMN protein level can be knocked down to certain ranges by different concentrations of doxycycline. The cells with different levels of SMN proteins will be useful for SMN functional studies.

The data in Fig. 2 showed that the proliferative rate of cells with reduced SMN proteins only slightly decreased in HeLa cells. However, Trüzsch et al. [12] showed that when SMN gene expression was knocked down in the differentiated P19 cells, it showed a dramatic increase in the percentage of apoptotic cells but not in the undifferentiated cells. It is, therefore, likely that SMN knockdown in different cell types results in different effects. HeLa cells may represent some non-motor neurons cell types. It is possible that the non-motor neurons cells of SMA patients are weaker than those of normal people especially under certain conditions. Our data in Figs. 2 and 3 show that reduced SMN protein level only slightly decreased the proliferative rate in HeLa cells. However, H₂O₂ dramatically increased the cell death of SMN knockdown cells. The cell viability of HeLa treated with H₂O₂ in our experiment is also similar to previous results with or without doxycycline [21–23]. These results demonstrated that some of the non-motor neurons cells in SMA patients may be normal in phenotype, but they are vulnerable under stresses, such as oxidative stress. Oxidative stress has been reported to be one of the predisposing factors in the pathogenesis of motor neuron diseases, including amyotrophic lateral sclerosis and Parkinson’s disease [24–26]. Our results show that SMN plays an important role in non-neuronal cells under stressed conditions. It is still possible that motor neurons are also very sensitive under stresses, and the sensitivity to stresses of motor neurons is one of the major causes of the dysfunction in SMA patients. However, our results may be helpful to prevent some detrimental effects in SMA patients caused by defects in non-motor neurons. For example, it may be wise to advise SMA patients to avoid stresses, such as oxidative stress, in their daily health care.

Reactive oxygen species—generating agents, such as H₂O₂, menadione, and beta-lapachone, induce oxidative stress that inactivates the activity of SMN complex [27]. In addition, overexpressed SMN proteins protect cells against mutant superoxide dismutase 1 toxicity [28]. Superoxide dismutase 1 is an enzyme that is essential for scavenging of superoxide radicals. Thus, SMN may play a role in oxidative stress pathophysiology. Our data in Fig. 3 demonstrate that SMN participates in the cell death under H₂O₂ in vivo.

Overall, we successfully generated a cell model system with inducible SMN knockdown. With this cell model, we demonstrate that SMN participates in the cell death under oxidative stress. These findings may be helpful to prevent SMA patients from some detrimental effects. In addition to mechanistic studies, this cell model may be used for drug-screening assay to select the drugs that can improve the function of the cells with low levels of SMN protein. Our cell model may be a promising resource for studies on the mechanisms and clinical applications of SMA.

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