

# The $\alpha$ II spectrin is essential for nuclear envelope integrity

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## Introduction

The nuclear envelope consists of two membranes (INM and ONM), nuclear pore complexes, and an underlying protein structural support, the nuclear lamina network. In mammalian cells, the lamina is composed of A type lamin proteins (Lamin A and C) and B type lamin proteins which recruit and anchor most INM proteins, including emerin, LBR, nesprin-1  $\alpha$ , et al. At least twelve disease syndromes have been linked to mutations in lamin A and lamin-binding proteins. These diseases are termed as laminopathies including Emery-Dreifuss Muscular Dystrophy, Dilated Cardiomyopathy with Conduction System Disease, and Hutchinson-Gilford Progeria Syndrome, etc. The mechanisms of laminopathies have not been determined, but current evidence is consistent with defects in tissue-specific gene expression coupled to mechanical weakness of the nuclear envelope. The nuclear lamins are stiff proteins which make up a majority of the nuclear lamina. Our previous work showed the nuclear lamina is not a stiff network but has 'spring-like' memory, which can swell under osmotic pressure and shrink back to its original size and shape. Spectrins are a family of cytoskeleton proteins characterized with spring-like elastic structure. We hypothesize the spectrin repeat proteins play important roles in mechanical properties of nuclear envelope, especially for the nuclear lamina network.

In humans, spectrin repeat proteins  $\alpha$ II spectrin, nesprin-1 $\alpha$ , nesprin-2 $\beta$  and  $\beta$ SpIV $\Sigma$ 5 have been identified inside nucleus. Specifically, nuclear  $\alpha$ II spectrin is

under the study focus due to its nuclear interactions with the proteins associated with Fanconi anemia and its function in recruiting DNA interstrand crosslinks for DNA repair. Recently, the  $\alpha$ II spectrin was also revealed to interact with five groups of nuclear proteins *in vitro*, including structural proteins locate at nuclear envelope, DNA repair proteins, chromatin remodeling proteins, Fanconi anemia proteins, transcription and RNA processing proteins. All these suggest that  $\alpha$ IIS may be an important nuclear protein involve in both nuclear envelope architecture and nuclear function. However, little is known about the  $\alpha$ II spectrin roles in the nuclear envelope and its influence in nuclear lamina and laminopathies. Our present work will focus on the study of nuclear  $\alpha$ -II spectrin and our results suggest it is an essential structural component in nuclear envelope. Either overexpression or down-regulation of  $\alpha$ -II spectrin will influence the nuclear envelope integrity as well as the cell mitosis.

## **Results and discusstion**

### ***$\alpha$ II spectrin localized in nucleus***

In humans,  $\alpha$ II spectrin ( $\alpha$ IIS) is highly expressed in vertebrate nucleated cells especially brain, muscle and epithelial cells. It is mainly studied as a cytoskeletal protein that lines under the plasma membrane of many cell types, playing an important role in maintenance of plasma membrane integrity and cytoskeletal structure. In most cell types, the nuclear localized  $\alpha$ II spectrin is a small portion compared to the cytoplasmic localized  $\alpha$ II spectrin. In present work, western blot result reveals that  $\alpha$ II spectrin isoforms localized in the nucleus of Hela cell, but little in cytoplasm of Hela (figure to be shown in poster). It suggests that Hela cell is an

ideal cell line to analyze the  $\alpha$ IIS functions in nucleus, without been largely affected by cytoplasmic  $\alpha$ IIS. We also constructed the GFP-tagged cDNA of  $\alpha$ II spectrin and transfected into HeLa cell. The overexpressed  $\alpha$ II spectrin finally localized in nuclear envelope (NE) supported by both time-depend statistical analysis of green signal localization (figure to be shown in poster) and the GFP  $\alpha$ II spectrin dynamics tracked by live imaging (Fig. 1).

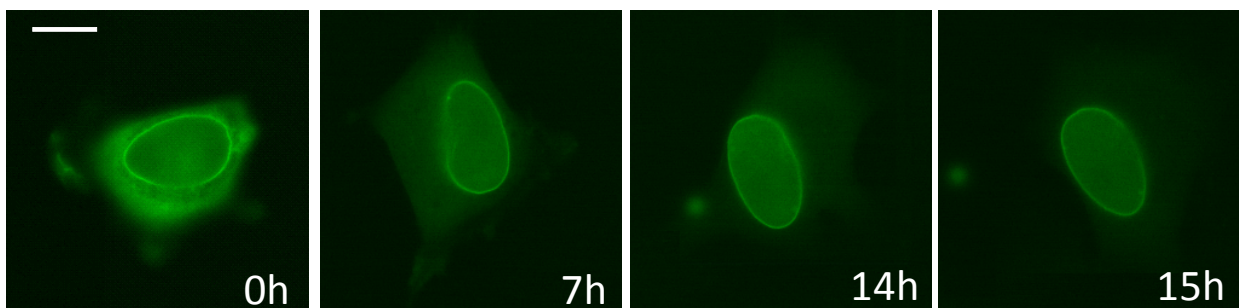


Fig.1 The time-lapse sequence of GFP-  $\alpha$ IIS localization tracking. The cytoplasmic GFP-  $\alpha$ IIS is transported into NE with time increase. Imaging taking started at three days after transfection labeled as 0h. Bar, 10  $\mu$  m.

### ***$\alpha$ II spectrin influence nuclear envelope integrity and lamina network***

Nuclear lamina is the major mechanical support of the cell nucleus. We examined the effect of  $\alpha$ II spectrin knockdown on lamina network. We labeled lamin A/C and lamin B in  $\alpha$ IIS knockdown cells. Most cells had lamina weakening area of the NE, present as both nuclear herniation or even leakage and reduced fluorescence (Figure 2 A, B). This reduced fluorescence signal was true for both lamin A/C and lamin B. The percentage of nuclear herniation and DNA leaking cells as well as the severity increased with the knockdown time increase. Such DNA-leaking cells are rarely found, even in the parallel control cells with lamin A/C knockdown (figure to be shown in poster). Moreover, statistical analysis ( $n > 200$ ) showed that the average

size of  $\alpha$ II spectrin knockdown nuclei is about 40% larger than normal cells and lamin A/C knockdown cells (figure to be shown in poster). And DNA signal of the large nuclei in  $\alpha$ II spectrin knockdown cell reduced comparing to normal size nuclei, suggesting the size increase of  $\alpha$ II spectrin knockdown nucleus may be due to nuclear expansion rather than proliferation of chromatin. Supporting this idea, the reduced fluorescence signal of lamin B in nucleus is correlated with the reduced fluorescent

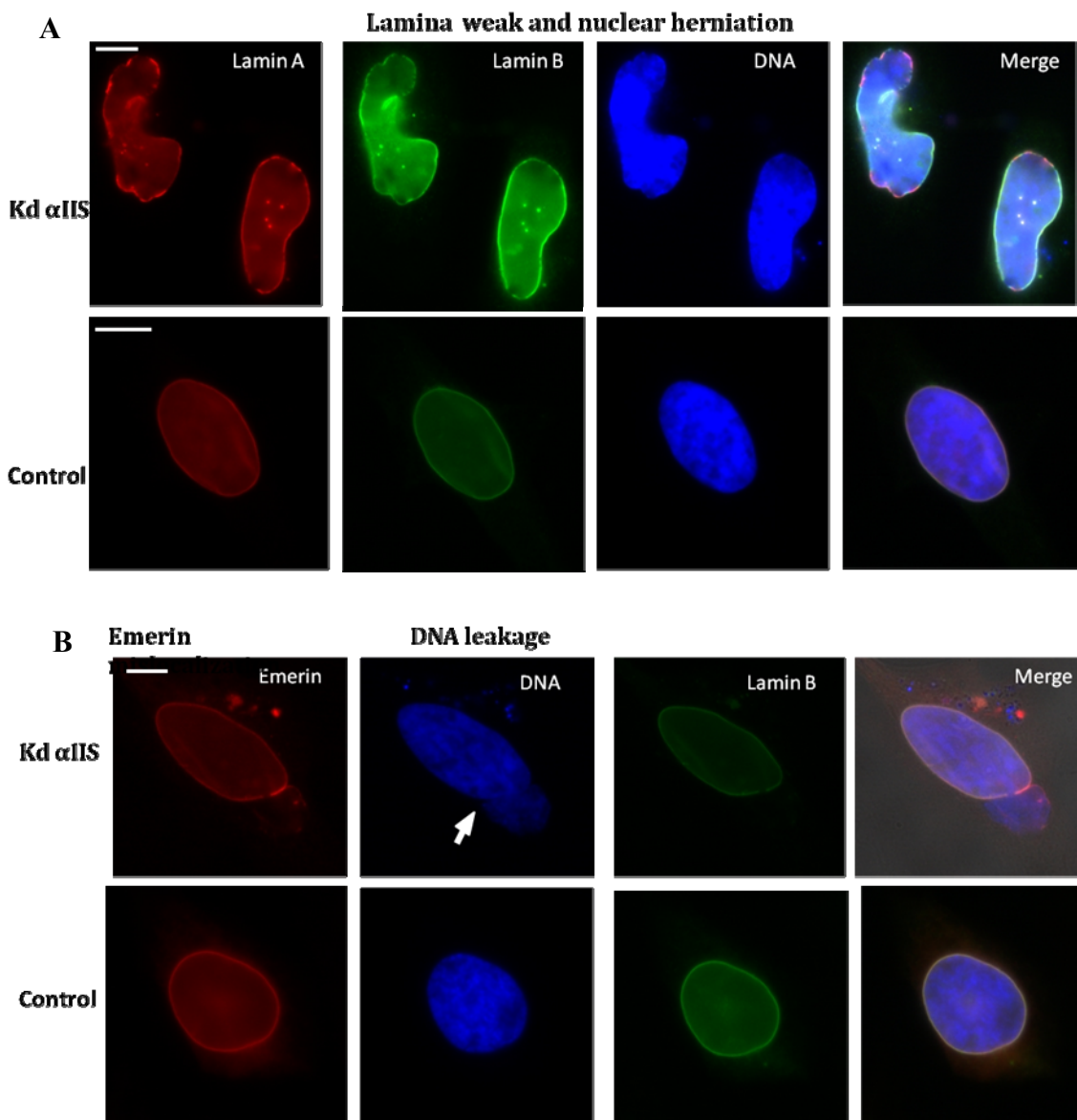


Fig.2 Cellular misbehaviors after knocking down  $\alpha$ II S. A) Nuclear herniation and lamina weakness B) DNA leakage and emerin mislocalization. Bar 10  $\mu$  m.

signal of DNA (Figure 3), suggesting loss of ‘spring-like’ structural protein  $\alpha$ II spectrin results in the expansion of both lamina network and DNA. Thus, we suggest that  $\alpha$ II spectrin is essential for the integrity of nuclear envelope and may contribute to the ‘spring-like’ properties of lamina network.

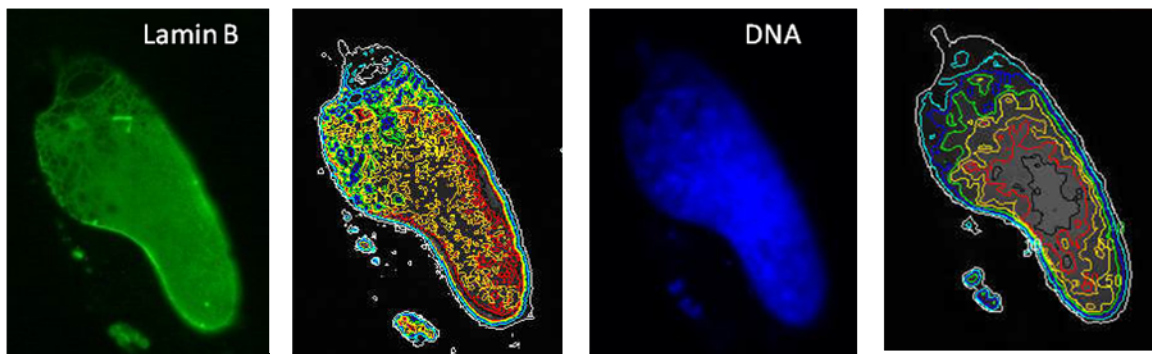


Fig. 3. Intensity analysis shows the reduced signal of local lamina is associated with DNA weakness and the nucleus expansion. From high pixel intensity to low intensity: black, red, orange, yellow, green, blue and white.

#### ***$\alpha$ II spectrin interacts with emerin and influence emerin localization***

Emerin is an INM protein that can bind to lamin A and has shown direct interactions with  $\alpha$ II-spectrin in vitro. Mutations in emerin and lamina are known to cause Emery Dreifuss Muscular Dystrophy, a typical laminopathy. Our results shown that the  $\alpha$ II spectrin also play important role in emerin localization. In our previous work, the purified emerin complex from Hela nuclear extract contains lamin A,  $\alpha$ II spectrin and other nuclear proteins. After  $\alpha$ II spectrin knockdown, most cells showed emerin mislocalization to cytoplasm and abnormal emerin aggregates in cytoplasm (Figure 2B). With increasing time after transfection of the shRNA for knockdown, the percentage of cells with emerin mislocalization increases (figure to be shown in poster). Previous studies have reported that emerin mislocalizes to ER in lamin A deficient cells. Mice lacking the A-type lamins exhibit mislocalization of emerin as

well as the appearance of muscular dystrophy. Here, we detected the emerin mislocalization by loss of  $\alpha$ II spectrin. Whether  $\alpha$ II spectrin plays roles in muscular dystrophy will also be interesting. Supporting the high affinity between  $\alpha$ II spectrin and emerin, when we overexpress GFP- $\alpha$ IIS, the mislocalization of GFP- $\alpha$ IIS in cytoplasm is correlated with weak fluorescent signal of nuclear emerin as well as emerin mislocalization to cytoplasm, while the cells with pure NE localized GFP- $\alpha$ IIS shown normal emerin distribution. All these suggest that  $\alpha$ II spectrin is an important binding partner of emerin and may play roles in emerin associated disease.

### ***$\alpha$ II spectrin influence the cell mitosis***

The nuclear envelope disassembles and reforms during mitosis. Tracking the protein dynamics during the cell mitosis is a good way to analyze the targeting and localization of nuclear envelope protein and its influence in cell proliferation. We tracked the GFP-  $\alpha$ IIS expression cell from prometaphase until the two daughter cells come to interphase (movie to be shown in poster). We can see that GFP- $\alpha$ IIS disassemble at the prometaphase, together with the nucleus condensation. The reassembly of GFP- $\alpha$ IIS start at the telophase and nearly all the cytoplasmic GFP- $\alpha$ IIS assembled at NE in the cytokinesis. Interestingly, the overexpression of this nuclear structural protein tend to influence the cell mitosis including elongate the mitosis time and generate DNA tether after cell division. Therefore, we analyze the mitosis index and the percentage of cells with multinuclei and dumbbell-shaped nuclei. The multinuclei may be caused by incomplete division of daughter cells during mitosis and the dumbbell-shaped nuclei are possible to the cells failed in

chromatin condensation or division. The results reveal that the GFP-  $\alpha$ IIS expression cells shown a slightly higher mitotic index and much higher percentage of multinuclei and dumbbell-shaped nuclei (figure to be shown in poster). The slightly higher mitotic index may be caused by the elongation of mitosis process. And the higher percentage of multinuclei and dumbbell-shaped nuclei is in agreement with the live cell imaging observation that the overexpression of GFP-  $\alpha$ IIS will cause mitosis failure or incomplete cell division.

### **Conclusion**

In this work, we analyzed the roles of spectrin repeats protein  $\alpha$ II spectrin in nucleus. Our results showed the knockdown of  $\alpha$ II spectrin by siRNA causes multiple nuclear abnormalities, including lamin A and lamin B network weakening, high percentage of cells with DNA herniation or even DNA leakage, and mislocalization of emerin. The average size of  $\alpha$ II spectrin knocked down nuclei is 40% larger than normal nuclei, suggesting the nuclei expansion due to loss of  $\alpha$ II spectrin. The overexpression of GFP- $\alpha$ II spectrin also causes emerin mislocalization especially when GFP- $\alpha$ II spectrin mislocalized to the cytoplasm. The overexpression of GFP- $\alpha$ II spectrin elongated the cell mitosis procedure and influence the cell division. All these suggest that the  $\alpha$ II spectrin is an important structure component in nuclear envelope.