Loss of nuclear compartmentalization in laminopathy patient cells

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Keywords: *nuclear lamina, nuclear ruptures, laminopathies, PML*

Abstract

The nuclear lamina structurally supports the cell nucleus and has a central role in gene regulation. A variety of mutations in the *LMNA* gene causes defects in the lamina's constituent proteins, the A-type lamins, leading to genetic diseases collectively referred to as laminopathies. There is growing evidence that laminopathies are caused by a combination of mechanical and gene regulatory cellular dysfunctions. A striking variability in disease symptoms between individual patients carrying identical *LMNA* mutations suggests that genetic screens will have limited predictive value for disease development.

Using live cell imaging, we discovered a widespread occurrence of repetitive, non-lethal ruptures of the nuclear envelope in fibroblast cultures from various laminopathy patients. These ruptures, which were absent in normal fibroblasts, could be mimicked by selective knockdown of lamin A/C and resulted in temporary loss of nuclear compartmentalization. Ruptures were accompanied by the efflux of nuclear regulatory proteins and resulted in downstream effects on stress-responsive gene expression.

Since this phenomenon was strongly correlated with disease severity, the identification of biomarkers that report on rupture events could have diagnostic relevance. One such candidate marker is the PML nuclear body (PML NB), which is normally confined to the nuclear interior, but translocates from the nucleus to the cytoplasm upon nuclear rupture. Immunofluorescence staining showed that the translocated PML NB's, were devoid of characteristic components such as DAXX and SP100, which is why these structures were coined cytoplasmic PML particles (PML CP). To test the diagnostic potential of PML CP we screened a variety of laminopathy patient cells, using a dedicated high content cytometry workflow. This way, we demonstrated a significantly increased fraction of PML CP positive cells in laminopathy patient cell cultures and showed a correlation with disease severity.

Therefore, we postulate that detection of PML CP in patient fibroblasts could become a valuable marker for diagnosis of laminopathy disease development.