SYNOPSIS

Title

Functional studies on lamin A mutants that cause laminopathies

Introduction

Lamins are the major components of the nuclear lamina that is located beneath the inner nuclear membrane, and are also present in the interior of the nucleus as constituents of the nucleoskeletal framework. Lamins play an important role in the maintenance of nuclear integrity, in the organization of replication and transcription, in gene regulation and in apoptosis. Lamins are broadly classified into A and B-type lamins. A-type lamins, A, C and C2 are differentially spliced products of the lamin A gene, LMNA. A-type lamins, A and C are developmentally regulated and are expressed in differentiated cells of many lineages, while the germ cell-specific lamin C2 is expressed only during spermatogenesis. B-type lamins, B1 and B2 are expressed in all somatic cells and are encoded by different genes. Lamin B3 is a differentially spliced product of lamin B1 and is expressed only in germ cells.

Mutations in the human LMNA gene cause a number of debilitating, inherited diseases, that affect skeletal muscle, cardiac muscle, adipose, bone and neuronal tissues, and also cause premature ageing syndromes. The majority of mutations cause Emery-Dreifuss muscular dystrophy (EDMD), familial partial lipodystrophy (FPLD), dilated cardiomyopathy (DCM) and Hutchinson-Gilford progeria syndrome (HGPS). Since 1999, a few mouse models of laminopathies have been created by gene targeting experiments and these have provided useful clues into the mechanistic aspects of laminopathies. Histological examination of tissue samples from laminopathic patients shows varying extents of abnormal nuclear morphology, nuclear membrane blebbing, disorganization of heterochromatin and mislocalization of emerin, lamina-associated polypeptides (LAPs) and lamin B1. Ectopic expression of certain disease-causing lamin mutants in cultured cells results in dominant negative effects, leading to disruption of endogenous lamins and LAPs, mislocalization of emerin and abnormal nuclear morphology. Although mutations in human LMNA gene cause a number of debilitating diseases, the cellular functions that are altered due to these mutations are not understood.
Objectives

The objectives of the present work are to examine the effects of disease-causing lamin A mutants on nuclear morphology by using known markers, and to study the effects of these mutants on cellular responses to DNA damage as well as myoblast differentiation.

Work Plan

The work plan was designed as follows:

1. To construct a wide range of disease-causing lamin A mutants as fusions with GFP, express them in HeLa and C2C12 cells, and study their assembly properties and effects on the endogenous lamin organization, intranuclear lamin speckles and emerin localization.

2. To examine the response of HeLa cells expressing disease-causing lamin A mutants to DNA damaging agents such as cisplatin and UV irradiation by analyzing formation of phosphorylated H2AX (γ-H2AX, a histone variant) foci. To optimize conditions of DNA damage so that events prior to apoptosis could be detected as lamins are proteolytically cleaved during apoptosis.

3. To study the effects of lamin A mutants on the process of muscle differentiation using C2C12 myoblasts as a model system, by detection of early markers for muscle differentiation such as myogenin and cyclin D3.

Results

The lamin A mutations studied include the missense mutations E203G, H222P, G232E, Q294P, R386K, R471C, R482L, R527C, L530P and the deletion construct lamin Adel50. H222P, G232E, Q294P and R386K have been shown to cause EDMD. E203K is mutated in DCM and the R482L mutation is known to cause FPLD. Lamin Adel50, R471C and R527C mutations cause progeroid syndromes in humans, whereas mice homozygous for the L530P mutation show strong progeroid phenotypes though this mutation causes EDMD in humans. These mutations were made by PCR-based mutagenesis of an available wild-type lamin A construct, and cloned into a GFP mammalian expression vector. GFP lamin A constructs expressed in HeLa and C2C12 cells showed similar assembly properties. Wild-type GFP-lamin A, H222P and R482L assembled normally at the nuclear periphery, whereas EDMD mutants G232E, Q294P and R386K formed intranuclear aggregates and the nuclei were distorted. HGPS mutant lamin Adel50, showed lobulation of nuclei in ~60% of cells whereas other HGPS mutants assembled at the periphery. EDMD mutants that formed aggregates disrupted
endogenous lamin A/C assembly, whereas with the other constructs endogenous lamin A/C assembled properly at the nuclear rim. The localization of the inner membrane protein, emerin was disrupted in a significant majority of cells expressing E203G, G232E, Q294P, R386K, R471C, L530P and lamin Adel50.

The formation of γ-H2AX DNA repair foci indicates the ability of cells to undergo an early response to DNA damage. HeLa cells transfected with wild-type GFP-lamin A, H222P and R482L formed γ-H2AX foci after treatment with cisplatin or UV at levels comparable to untransfected cells, whereas cells expressing E203G, G232E, Q294P, R386K, R471C, R527C, L530P or lamin Adel50 formed low levels of γ-H2AX foci after cisplatin or UV treatment. An additional marker for DNA repair sites, p53 binding protein 1 (53BP1) rapidly redistributes from a diffuse localization to foci that colocalize with γ-H2AX foci in response to DNA damage. In HeLa cells expressing E203G, G232E, Q294P, R386K, R471C, R527C, L530P or lamin Adel50 mutants, 53BP1 remained diffusely localized in the majority of cells after cisplatin treatment. To determine whether lamin mutants could affect the localization of signalling components involved in DNA damage prior to treatment, HeLa cells were cotransfected with lamin mutant constructs and either ataxia-telangiectasia-mutated (ATM) or ATM-and-Rad3-related kinase (ATR) kinase, which are key sensors of DNA damage. In HeLa cells expressing lamin mutants E203G, G232E, Q294P, R386K, R471C, R527C, L530P or lamin Adel50, ATR was either mislocalized or misexpressed, whereas there was no change in the localization or expression of ATM.

C2C12 myoblasts expressing wild-type GFP-lamin A or lamin A mutants H222P and R482L expressed early differentiation markers myogenin and cyclin D3 and were incorporated into myotubes when exposed to differentiation medium. The other lamin mutants affected the expression of differentiation markers to varying extents, and were not incorporated into myotubes. EDMD mutants which formed aggregates like G232E, Q294P and R386K showed reduced expression of MyoD, an important muscle regulatory factor.

Conclusions

2. The above mentioned set of eight lamin mutants were impaired in the formation of γ-H2AX foci after cisplatin or UV treatment, and also failed to recruit 53BP1 to foci after cisplatin treatment. These mutants mislocalized ATR but not ATM kinase. Mislocalization of ATR by lamin mutants may thus lead to impaired formation of γ-H2AX foci, which in turn reduces recruitment of 53BP1.

3. Lamin mutants which were defective in MyoD expression failed to express early differentiation markers like myogenin and cyclin D3 and thus inhibited myoblast differentiation.