7th UK Conference on the Nuclear Envelope in Disease and Chromatin Organization.
June 22nd-23rd, 2015.
Oswestry group abstracts:

Oral presentation:

The muscle-specific short isoform of nesprin-1 is highly-expressed at the nuclear rim at early stages of muscle development.

Ian Holt[a,b] , Nguyen Thuy Duong[a.c], Le Thanh Lam[a], Qiuping Zhang[d], Caroline A Sewry[a,e], Catherine M Shanahan[c], Glenn E Morris[a,b].

[a]Wolfson Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry, SY10 7AG, UK.
[b]Institute for Science and Technology in Medicine, Keele University, ST5 5BG, UK.
[c]Institute of Genome Research (IGR), Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam.
[d]Cardiovascular Division, James Black Centre, King's College, London, SE5 9NU, UK.
[e]Dubowitz Neuromuscular Centre, Institute for Child Health and Great Ormond Street Hospital, London, WC1 1EH, UK.

Although short isoforms of nesprin-1 can be seen on western blots, lack of specific antibodies has made it impossible so far to determine their localization within cells. We have produced a new monoclonal antibody specific for nesprin-1-alpha-2. Our previous qPCR study showed that alpha-2 and beta-1 are the only short forms of nesprin-1 expressed substantially in a range of human cells and tissues and that the alpha-2 form is found only in cardiac and skeletal muscle. We now show that nesprin-1-alpha-2 protein is undetectable in pre-differentiation myoblasts, but, in multinucleate myotubes, it appears at the nuclear rim with a discontinuous granular distribution, distinct from that of nesprin-1-giant. This observation was supported at the mRNA level by qPCR, which confirmed the appearance of nesprin-1-alpha 2 mRNA as myogenesis progresses. We have further shown that nesprin-1-alpha-2 protein levels decline as immature muscle (regenerating fibres) develops into adult muscle and that nesprin-1-alpha-2 mRNA levels by qPCR are much lower in adult muscle compared with fetal muscle. The results suggest that nesprin-1-alpha-2 may have a specific role during early muscle development, possibly associated with the relocation of nuclei within developing fibres.

Although human tissues express both nesprin-1 and nesprin-2, some cell lines express only nesprin-1 (vascular smooth muscle cells) or only nesprin-2 (HeLa, Ntera-2, embryonic stem cells [ESC]). Remarkably, all the nesprin-2 mRNA from ESC lacks the KASH domain and nesprin-2 protein is not localised at the nuclear rim in ESC. This shows that alternative splicing of the KASH domain can determine the intracellular localisation of nesprin-2.

Human skin fibroblasts express both nesprins 1 and 2, but when emerin is absent (Emery-Dreifuss MD), nesprin-2 no longer locates to the nuclear rim, whereas nesprin-1, SUN and lamins are unaffected. This shows that the two nesprins are not irreversibly linked at the nuclear rim in skin fibroblasts.

Supported by British Heart Foundation (PG/11/71/29091).

Poster presentation:

Two novel epsilon isoforms of nesprin-2, a protein linked to Emery-Dreifuss muscular dystrophy.

Ian Holt[a,b], Nguyen Thuy Duong[a.c], Le Thanh Lam[a], Qiuping Zhang[d], Caroline A Sewry[a,e], Catherine M Shanahan[d], and Glenn E Morris[a,b]

[a]Wolfson Centre for Inherited Neuromuscular Disease, RJAH Orthopaedic Hospital, Oswestry, SY10 7AG, UK.
[b]Institute for Science and Technology in Medicine, Keele University, ST5 5BG, UK.
[c]Institute of Genome Research (IGR), Vietnam Academy of Science and Technology (VAST), Hanoi, Vietnam.
[d]Cardiovascular Division, James Black Centre, King's College, London, SE5 9NU, UK.
[e]Dubowitz Neuromuscular Centre, Institute for Child Health and Great Ormond Street Hospital, London, WC1 1EH, UK.

Nesprin proteins have a central rod domain of spectrin repeats and are intracellular linkers and scaffolds. Full length or "Giant" nesprin-1 and nesprin-2 have N-terminal calponin homology domains that bind the actin cytoskeleton and C-terminal transmembrane KASH domains which anchor the nesprins to the outer nuclear membrane. A number of short isoforms of nesprin-1 and nesprin-2 are produced by internal promotion and by alternative splicing. Mutations in nesprin-1 and nesprin-2 can cause Emery-Dreifuss muscular dystrophy and dilated cardiomyopathy. Nesprin-1-alpha-2 is found almost exclusively in skeletal muscle and heart and all the known mutations in nesprins that are associated with EDMD or DCM lie within the alpha isoform sequence.

Two novel "epsilon" isoforms of nesprin-2 were predicted by bioinformatics and we have demonstrated the existence of corresponding proteins for the first time. Because they are similar to nesprin-1 alpha in size and structure, we evaluated the significance of the epsilon isoforms in 20 human tissues and 7 human cell lines using qPCR. N2-epsilon-1 was expressed only in ovary and early embryonic cells (Ntera-2 and ESC), while N2-epsilon-2 was expressed in several mature tissues, including cardiac, but not skeletal, muscle. Western blotting confirmed the presence of epsilon-1 protein in Ntera-2 and ESC and epsilon-2 protein in heart and brain. Total nesprin transcript in ESC was mainly nesprin-2-giant (77%) and nesprin-2-epsilon-1 (21%). PCR indicated that most of the nesprin-2 mRNA in ESC lacked the KASH domain, whereas heart and skeletal muscle appeared to contain high levels of nesprin-2 KASH. Immunofluorescence microscopy with a monoclonal antibody against nesprin-2 showed nuclear rim staining in heart and skeletal muscle sections, but in KASH-less ESC there was an intense nucleoplasmic speckle-like distribution.

Supported by British Heart Foundation (PG/11/71/29091).