Studies of YBX3 and its variants in myogenesis

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Background

The Y-box binding protein 3 (YBX3) has been described as a transcriptional regulator and translational repressor of various proteins in skeletal and heart muscle. Its functions include, among others, the gradual repression of myogenin during myogenesis[1]. It is known to have some redundant activity with its family members YBX1 and YBX2[2].

By exome sequencing in a Finnish patient with an unusual form of nemaline myopathy, we have found two rare YBX3 variants, NP_003642.3:p.(Ser34Arg) and NP_003642.3:p.(Arg129Trp).

Our previous studies have shown that YBX3 is post-translationally cleaved in non-muscle cells over-expressing YBX3. Our ultimate aim is to pinpoint the cleavage site and characterise the putatively independent roles of the cleaved and non-cleaved forms of the protein in the muscle. Due to the lack of previous studies of YBX3 in muscle tissue, we set out to define the localisation and putative cleavage of YBX3 in muscle cells.

Materials and methods

Here, we used constructs of YBX3 with a C-terminal FLAG-tag and an N-terminal myc-tag to transfect murine myoblasts (C2C12 cells). The peptides were detected with FLAG and myc primary antibodies, respectively.

We acquired two different commercial YBX3 antibodies and conducted Western blots on both cell extracts from transfected C2C12 cells and primary myoblasts from the patient and a healthy control to determine the location of the epitope the antibodies were raised against.

Results

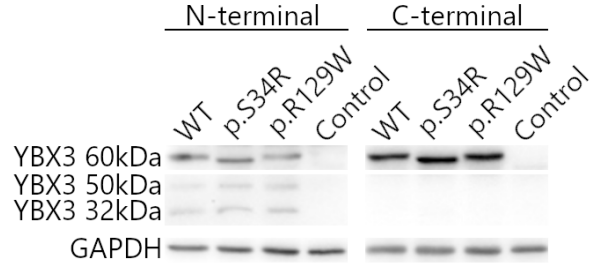


Fig 1. Western blotting of C2C12 cells transfected with double-tagged wild-type and variant YBX3 shows a similar band pattern to blots performed on extracts of other cell types, indicating that cleavage of YBX3 also happens in myoblasts.

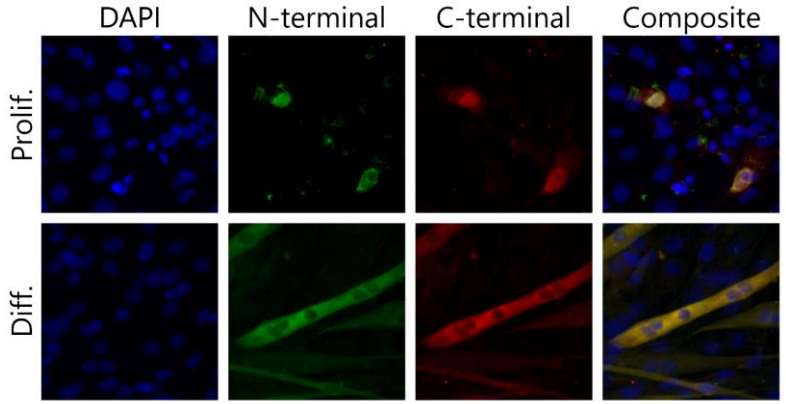
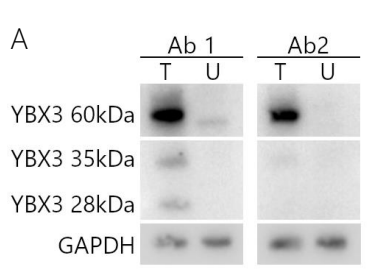


Fig 2. Immunofluorescence imaging of C2C12 cells transfected with double-tagged wild-type YBX3 constructs showed that the two terminals of the protein do indeed partly localise independently in proliferating myoblasts, as they do in other cell types. However, the distinct differential localisation is lost in differentiated cells, in which the protein is present in the cytoplasm.



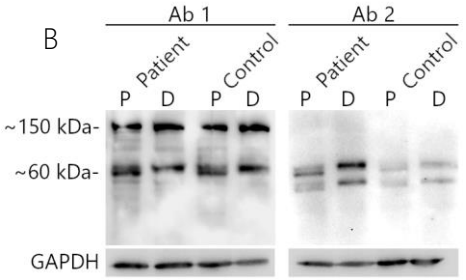


Fig 3A: Western blots of cell extracts from C2C12 cells transfected with wild-type YBX3 and detected with two different commercial anti-YBX3 antibodies indicate a significant difference in the identified fragments. Antibody 1 targets an epitope between aa 250 and 300, and antibody 2 is raised toward the complete protein, suggesting that the cleavage site is situated before the epitope. T=transfected, U=untransfected control.

Fig 3B: A Western blot of extracts from patient and control myoblasts in P=proliferation and D=differentiation using the commercial antibodies further emphasises the difference between the identified fragments. Antibody 2 seems to also recognise the shorter isoform of YBX3. The putative cleaved peptide was not identified in the Western blot.

Future and ongoing work

- Confirm cleavage site and post-translational modifications of wild-type and variant proteins
- Study the expression of the wild-type and variant proteins in the different phases of differentiation
- Study the effect of silencing of the gene on the differentiation and expression of YBX1, YBX2 and other genes with important functions in differentiation

References

For complete references, please visit lydiasagath.com/wms2020.html

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