Characterization of a MYO19 knockdown phenotype in a cultured neuron-like cell line

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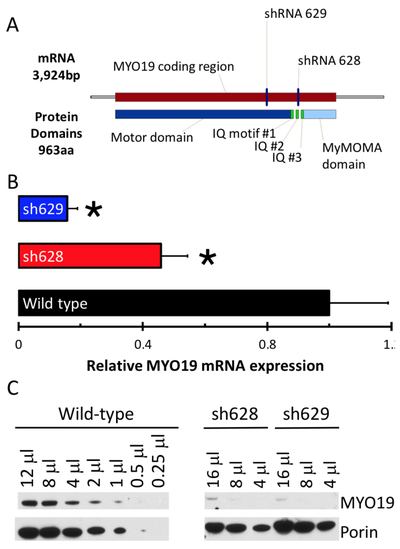
Abstract

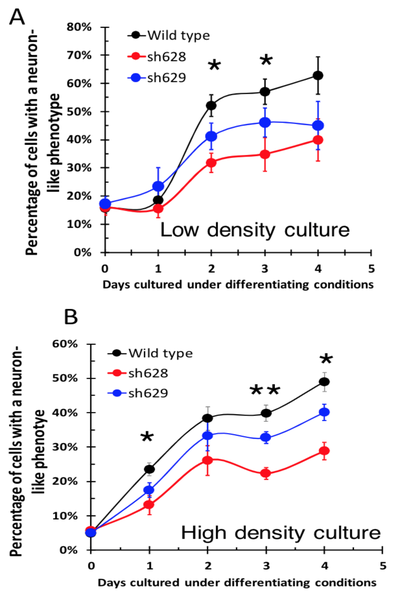
Myosin-XIX (MYO19) is an actin-based motor protein implicated in normal mitochondrial dynamics and distribution. Mitochondrial movement and positioning is thought to be essential in order for energy intensive processes (such as cell division, cell motility, neuronal growth, and morphogenesis) to occur efficiently. We hypothesized that MYO19 aids in mitochondrial dynamics and positioning during neuronal differentiation, a complex and energy intensive process resulting in long cellular extensions protruding from the cell body. MYO19 may be involved in supporting cell motility (growth cone or cell body motility) as well as overall morphogenesis (neurite establishment). We used the murine, neuron-like CAD cell line and lentiviral infection to generate new lines stably-expressing short-hairpin RNA (shRNA) that target the MYO19 message. We show that cell dynamics such as cell body motility and neurite extension velocity are decreased compared to wild type. We demonstrate that mitochondrial velocity is altered compared to wild type and can be rescued by overexpressing MYO19. These results suggest that decreased levels of MYO19 may be influencing CAD cell differentiation by altering the cell's ability to move and position mitochondria via the actin network. Preliminary rescue experiments were then performed to confirm MYO19's role in mitochondrial dynamics. It was found that mitochondrial velocity was significantly slower in the rescue motors, suggesting that the rescue was successful and confirming that the motor has a meaningful role in mitochondrial dynamics in neurites.

Experimental Approach

Lentiviral shRNA plasmid and cell lines: Plasmids were designed to deplete MYO19 expression via RNA-interference and are delivered to the cells via a lentiviral infection. Cell lines with decreased MYO19 expression can be established and compared to wild type (WT) cells. Separate low MYO19-expressing CAD cell lines were generated encoding for shRNA (A) targeted against three different regions of the MYO19 mRNA. Cultures for two shRNA targets displayed decreased MYO19 expression wither assayed by (B) qRTPCR for mRNA levels or (C) western blotting for protein levels. Based on the western blot, these lines are expressing approximately ~10%-30% of wild-type protein levels.



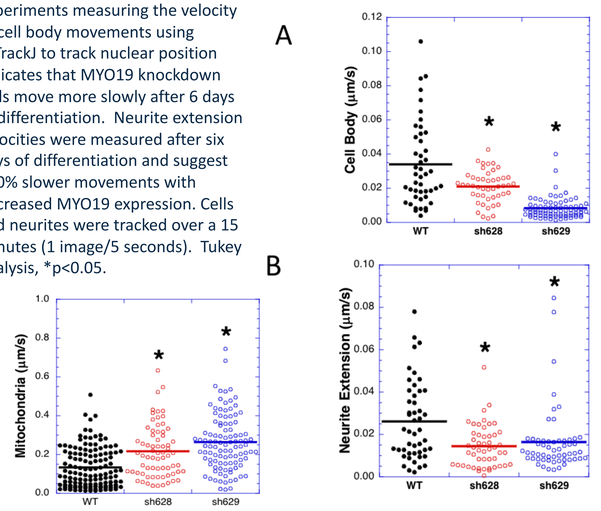
MYO19 knockdown alters differentiation profile of CAD cells



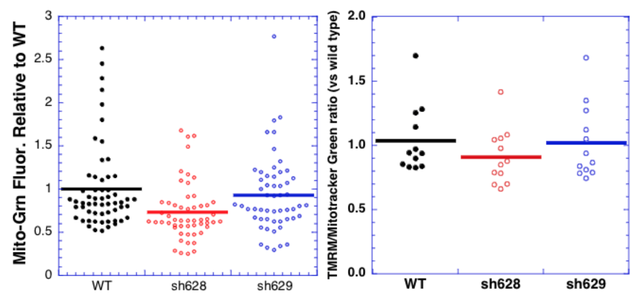
CAD (*Cath.a-differentiated*) cells are a mouse CNS cell line that can be induced to differentiate by the removal of serum from growth media. Cells were stained with DAPI, Mitotracker, and phalloidin. Compared to WT, MYO19 knockdown leads to a delay in establishment of neuron-like phenotype. This difference was apparent whether the cell populations were (A) assayed at low density where individual cells could be easily identified or (B) assayed in higher density cultures where individual cells were identified using low-concentration GFP-Lifeact transfection to identify individual cells (*p<0.05).

MYO19 knockdown alters dynamics of whole cell body, neurites, and mitochondria

Experiments measuring the velocity of cell body movements using MTrackJ to track nuclear position indicates that MYO19 knockdown cells move more slowly after 6 days of differentiation. Neurite extension velocities were measured after six days of differentiation and suggest ~50% slower movements with decreased MYO19 expression. Cells and neurites were tracked over a 15 minutes (1 image/5 seconds). Tukey analysis, *p<0.05.

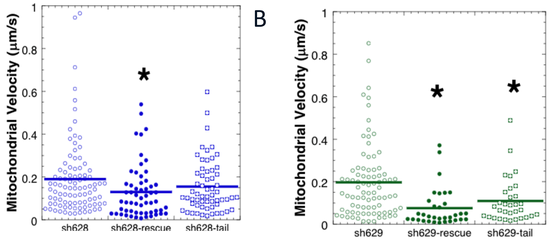


MYO19 knockdown does not drastically alter mitochondrial load or membrane potential



FACS analysis of CAD cells stained with (A) 200µM Mitotracker Green FM showed that shRNA cell lines contained similar amounts of mitochondria as wild type cells (n > 50). (B) Measures of mitochondrial membrane potential using the ψm-sensitive dye, tetramethylrhodamine methyl esther fluorescence (TMRM) also suggest similar levels of membrane potential across wild type and shRNA cell lines (n > 14). Relative fluorescence for each measure was standardized to the WT for that day's data. Spread in the WT samples indicates the variability across experimental days.

MYO19 rescue constructs restore mitochondrial dynamics within neurites



Rescue Constructs

Full-length *Homo sapiens* GFP-MYO19

MYO19 tail-GFP

Time-lapse images of differentiated CAD cells stained with Mitotracker Deep Red FM allowed us to analyze the movements of individual mitochondria within CAD cell neurites. Ectopic expression of full-length, GFP-tagged, human MYO19 constructs for about 1 day rescued the fast mitochondria phenotype in both sh628 and sh629 cells. Expression of a tail-only construct, which still localizes to mitochondria, did not decrease mitochondrial velocity in sh628 cells. (*p < 0.05, Dunnett analysis). Future rescue plasmids will include tail truncations and a W140V mutation that has a low duty ratio.

Future directions

These data suggest impaired differentiation when MYO19 is depleted, potentially due to altered mitochondrial dynamics. Preliminary rescue data suggest that MYO19 function can be rescued using MYO19 mutations. As differentiation and neurite extension are energetically costly processes, it is likely that impaired mitochondrial transport and positioning due to lack of MYO19 could be the mechanism underlying the observed whole-cell phenotype, thereby identifying a novel MYO19 cellular function.

- Continue characterizing a role for MYO19 in neuronal differentiation
- Identify other phenotypic differences
  - Antibodies against Tau and MAP2 to label axons and dendrites
  - Quantitative image analysis of whole cell and mitochondrial dynamics
- Continue MYO19 rescue experiments using human GFP-MYO19 expression to confirm phenotypic changes are results of MYO19 knockdown

The Q-lab fully supports the UR Black Student Coalition and the Protect Our Web Movement.