Localization and stress fibre formation activity in YHY FMNL2 formin isoform.

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Abstract

Temporal and spatial remodelling of actin cytoskeletons often used to establish cell shape, motility and cytokinesis is partially regulated by the nucleation of actin filaments[1]. One of the many actin nucleators found in eukaryote cells are the formin homology protein (also referred to as formins). Note that this experiment focuses primarily on formin-like 2 (FMNL2). Furthermore, the endogenous FMNL2 is subject to alternate splicing which consequently produces various isoforms that have been identified in several organisms in previous studies[2]. These isoforms may affect the subcellular localization and activity of the formin protein. The main objective of this experiment is to determine if the YHY FMNL2 isoform results in similar localization and stress fibre formation activity to that carried out by the ITM isoform and endogenous protein. In this experiment, we have found that the YHY isoform was evenly dispersed throughout the cytoplasm indicating similar localization to that of ITM FMNL2. However the nature of stress fibre activity in YHY remains unclear.

Methodology

- FMNL2 isoform FH1 FH2 domain fragments are 504-1052 amino acids in length.
- Fragment was ligated into pEFN BRSS vector
  - FMNL2 FH1 FH2 pEFN BRSS construct was tagged with cherry at the N-terminal
- Construct was transformed into DH5-alpha cells (Ecoli bacteria)
- Both isoforms were expressed in NIH 3T3 cells (mouse fibroblast) through transient transfection
- Cells were fixed with paraformaldehyde
- F-actin was stained with fluorescein phalloidin and the nucleus with DAPI
- Results were observed using laser-scanning confocal microscope

Objective and Hypothesis

The objective of this experiment is to test the hypothesis that YHY FMNL2 isoform has the same localization and stress fibre formation activity than the ITM isoform.

Introduction

- Eukaryotic cells depend on de novo nucleation of actin filaments from monomeric G-actin subunits for actin filament polymerization[3].
- The crucial rate determining step in actin polymerization is nucleation
  - Formin family proteins nucleate actin polymers and significantly accelerate polymer elongation[3].
  - Since formins remain associated with these barbed ends throughout nucleation, they prevent capping proteins (which terminate nucleation and stabilize filament) from binding to the ends[2].
- In formin protein FMNL2, two of the functional domains are formin homology domains (FH1 and FH2).
  - FH1 and FH2, which were used to build the constructs, are the domains which actively bind to the actin during nucleation (Fig.1).
  - Alternative splicing results in several isoforms (Fig. 2).
- This experiment focuses on the YHY FMNL2 isoform while comparing it to the ITM isoform (Fig.3).
- Previous studies have shown that FMNL2 is upregulated in SW620 and SW480 colorectal cancer cells.

Background information

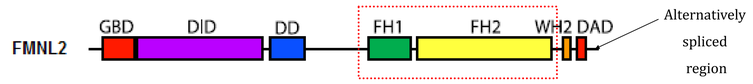

Figure 1. FMNL2 formin homology protein. The red dotted line indicates the FH1 and FH2 domains (504-1052 amino acids) which are the functional domains for FMNL2. Also indicated is the site where alternative splicing occurs - differences here result in the ITM and YHY isoforms.

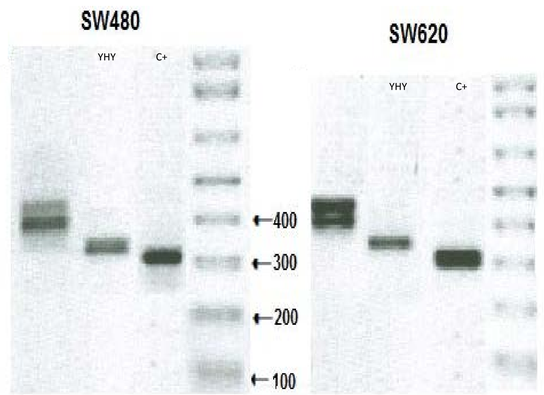

Figure 2. FMNL2 isoforms. RTPCR results from colorectal cancer cells passed though 1% agarose gel. The different bands observed under UV light indicated various FMNL2 isoforms (including YHY).

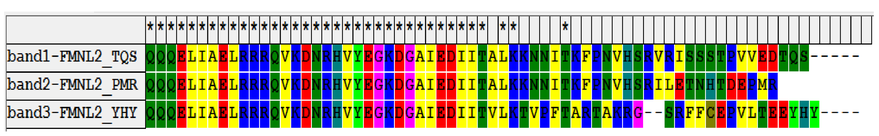

Figure 3. Sequenced FMNL2 isoforms. Sequenced FMNL2 for TQS, PMR and YHY.

Acknowledgements

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Results

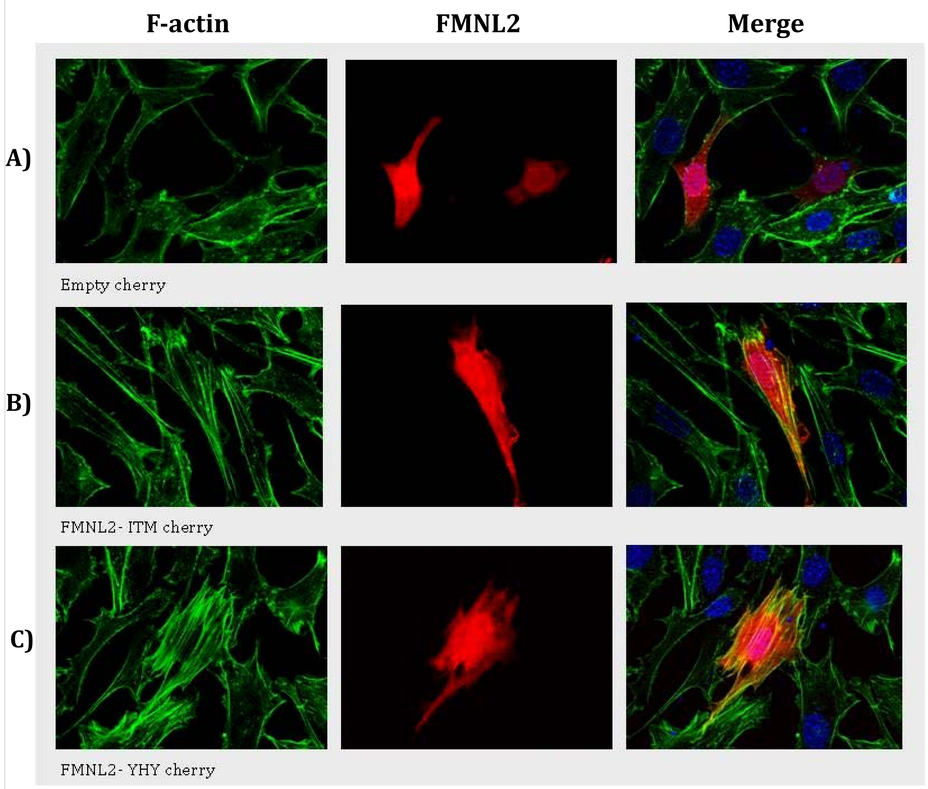

Figure 4. FMNL2 YHY localization. FMNL2 isoform (ITM and YHY) constructs (504-1052) are tagged with cherry at the N-terminal using a pEFN BRSS vector. F-actin was stained with fluorescein phalloidin (green) and nucleus with DAPI (blue). Note that NIH 3T3 cells were used and results were observed using laser-scanning confocal microscope.

Summary

- It has been shown that SW620 and SW480 colorectal cancer cells have common endogenous FMNL2 isoforms.
- Our results suggest that YHY FMNL2 evenly disperses throughout the cytoplasm. There exists a common localization between FMNL2 ITM isoform and YHY isoform.
- In cells expressing YHY FMNL2 there were some indications of what seemed to be stress fibres although there was some transfected cells that lacked stress fibre formation. Therefore, future F-actin binding assays will be performed in order to confirm the interaction of both (YHY and ITM) isoforms with actin filaments.
- These localization results contribute to our understanding of the role of FMNL2 isoforms in colorectal cancer cells.

References

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[2] Katoh et al. Identification and characterization of human FMNL1, FMNL2 and FMNL3 genes in silico. International journal of oncology 22: 1161-1168 (2003)

[3] Chesarone, Melissa et al. Unleashing formins to remodel the actin and microtubule cytoskeletons. Nature, Vol.11, Macmillan Publishers Limited. January 2011. Pp. 62-74.