ABSTRACT

Immunohistochemistry (IHC) is a useful research tool not to localize specific antigens in tissue sections with labeled antibodies based on antigen-antibody interactions. To obtain a clearer understanding of the cellular and subcellular localization of the Mind-Meld (MMD) protein during the developing Drosophila melanogaster embryo double indirect immunofluorescence was used to colocalize MMD with other proteins found in the fly. An affinity-purified antibody was used in adult fly identifying all MMD isoforms was used with the fluorophore-labeled secondary antibody Alexa Fluor 594 specific to rabbit IgG. Well-characterized murine monoclonal antibodies with known subcellular localization and function in Drosophila. Fasciclin II (FASII), Short stop (SHOT), β-Tubulin, Spaghetti squash (SQH), and Neurotactin (NRT) were used with the fluorophore-labeled secondary antibody. Alexa Fluor 488 specific to mouse IgG. All fixed embryos were stained with the nuclear marker DAPI to identify the density and arrangement of the nuclei and the F-actin marker Phalloidin. Confocal microscopy which provides a three-dimensional optical section was used to better illustrate the localization of the proteins. In the present work, it is demonstrated that MMD colocalizes with SHOT, β-Tubulin, Phalloidin and SQH suggesting MMD’s importance in cell adhesion, cell migration, and cell division as well as the cytoskeleton. The characterization of the cellular and subcellular localization of the MMD protein during the developing Drosophila will provide insight into the context in which MMD functions in human disease processes.

INTRODUCTION

In multicellular organisms, intercellular signaling and adhesion are essential functions of every cell. The nervous system, signals between neurons, as well as, neurons and glial cells play a pivotal role in nervous system homeostasis, and synaptic function (Araque, & Navarrete, 2010). The ADAMs (a disintegrin and metalloproteinase) are a family of transmembrane and secreted proteins that play an important role in regulating cell phenotype via cell adhesion, migration, proteolysis and signaling (Prymula et al, 2013). Several ADAMs function in spermogenesis, specifically in the maturation of sperm and their adhesion and migration in the uterus (Ikawa et al, 2010) while others function in the nervous system as guidance mechanisms and when disrupted are linked to cancer, cardiovascular disease, asthma and Alzheimer’s disease (Li u et al, 2009). Knowledge of protein localization often plays a critical role in characterizing the cell function of hypothetical and newly discovered proteins. The mmd gene in flies is homologous to a member of the ADAM family, specifically the ADAM23 gene. ADAM23 is predominantly expressed in the brain, suggesting that it may mediate cell adhesion through interactions with specific integrins (Cal, S., Freije, J. M. P., López, J. M., Takada, Y., & López, M., 2013). The characterization of the subcellular localization of the MMD protein during the embryogenesis of Drosophila against other proteins with well known localization and function will provide insight into how mmd and its human homolog ADAM23 function in human disease processes and further provide clues to understanding the mechanisms of human disease. This research will use immunofluorescence and confocal microscopy to map and characterize a newly expressed ADAM protein, Mind-Meld (MMD), with the specific subcellular markers: Fasciclin II (FASII), Short stop (SHOT), β-Tubulin, Spaghetti squash (SQH), Phalloidin and Neurotactin (NRT) found in the fruit fly Drosophila melanogaster, to better understand its cellular function.

MATERIAL/METHOD

Primary antibodies (Developmental Hybridoma Bank, Iowa City, IA): mouse n-Shot, mouse n-β-Tubulin, mouse n-Fasciclin II; rabbit n-MD (S. Chaise). Secondary Antibodies (ThermoFisher Scientific): goat α-mouse, Alexa Fluor Plus 488; donkey α-rabbit, Alexa Fluor 594; Phalloidin, Alexa 647.

Fixation and de-orientation

Embryos were de-chromatized in 50% bleach, washed with 1% Triton X-100 and transferred to a 20 ml vial containing buffered 4 hexane, 3 ml phosphate-buffered saline (PBS), 1 ml 1% formaldehyde (Ultropac) and shaken for 25 min. The lower phase was removed and embryos were de-orientation with graded Percoll solutions ranging from 1 ml cold MeOH to 1.5 ml (Eppendorf) tube, mixed with 3xMeOH and stored in MeOH at -20°C.

Paraffin embedding

Embryos were rinsed 2x with PBT (PBS, 0.1% Tween 20, 0.3% Triton X) and rocked for 30 min. The embryos were infiltrated with 1 ml MeOH, 1 ml xylene, and 1 ml xylene/alpha-methyl benenate (AMB) and shaken for 25 min. The lower phase was removed and the embryos were embedded by replacing the xylene with xylene-hyrocking in 1 ml cold MeOH for 1 min, transferred to a 1.5 ml (Eppendorf) tube, mixed 3x with MeOH and stored in MeOH at -20°C.

Staining

A) Embryos were incubated for 1 hr in 1 ml blotting buffer followed by incubation for 14 hr at 4°C in 0.5 ml blocking solution + first primary antibody, rinsed at room temperature in 1 ml PBT for 30 min (10x), and blocked for 1 hr in 1 ml blocking solution. Embryos were incubated with secondary antibody in 0.5 ml blocking solution for 1.5 hr at room temperature, and rinsed in 1 ml PBT 2x. Staining was repeated with the secondary antibody and the secondary antibody. The embryos were Stained with DAPI and Phalloidin in 1 ml PBT (5 μg/ml), washed in 1 ml PBT (4x) at 4°C for 2–14 hr, and mounted in Vectashield on a back glass slide.

Secondary Antibodies

• Goat anti-mouse, Alexa Fluor Plus 488; donkey α-rabbit, Alexa Fluor 594; Phalloidin, Alexa 647.

Figure 1. Expression of Mind-Meld protein and Fasciclin II protein during germ band elongation. A stage 10 embryo stained with antibodies against MMD, FASII, and the nuclear marker DAPI. The merged images are shown. 63X magnification

Figure 2. Expression of Mind-Meld protein and Short stop protein during cellularization. A stage 5 embryo stained with antibodies against MMD, SHOT and the nuclear marker DAPI. The merged images are shown. 40X magnification

Figure 3. Expression of Mind-Meld protein and β-Tubulin protein. Mind-Meld protein and Phalloidin during blastoderm cell formation. A stage 3 embryo stained with antibodies against MMD, β-Tubulin, Phalloidin and the nuclear marker DAPI. The merged images are shown. 40X magnification

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• University of Iowa. (2015). The monoclonal antibodies developed by Dr. Bruce Chase was obtained from the Developmental Studies Hybridoma Bank, Iowa City, IA 52242. http://dshb.biology.uiowa.edu/Antigen

CONCLUSION

• Although FASII is a cell membrane glycoprotein belonging to the immunoglobulin superfamily of cell adhesion molecules, no colocalization was observed between FASII and MMD. Perhaps MMD is not involved in synaptic development and plasticity.

• MMD and SHOT colocalized at the periphery during stage 5 of embryogenesis when cellularization is occurring with the elongation of the blastoderm nuclei. SHOT is important for establishing the cytoskeletal arrangement required for the axis determining maternal mRNAs. Therefore, the colocalization of SHOT and MMD at the outer periphery of the embryo suggests that MMD could play a role in the organization of microtubule that leads to the development of the cytoskeletal polarity.

• MMD colocalizes with Phalloidin with high expression during stage 3 of embryogenesis. Phalloidin labels F-actin which is important for cell motility and phagocytosis. At the beginning of cellularization, MMD and F-actin form hexagons around the nuclei suggesting their role in forming a network to aid in the fully formed cells from the underlying yolk sac.

• MMD colocalizes with SQH during stage 5 of embryogenesis especially surrounding the nuclei of pole cells. The aphp gene encodes the regulatory myosin light chain of non-muscle myosin II that is responsible for cell migration. This suggests that MMD plays a role in the migration of pole cells to the dorsal end of the embryo.

• MMD does not colocalize with NRT. However, during stage 9 with the first neuroblasts deraminating from the ecdysium, MMD appears to also form a bridge like structure between regions of contact for neuroblasts.