ABSTRACT

Immunohistochemistry (IHC) is a useful research tool used 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 raised in rabbit identifying all MMD isoforms was used with the fluorescence-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), Shortstop (SHOT), β-Tubulin, Spaghetti squash (SQH), and Neuronalin (NRT) were used with the fluorescence-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 three-dimensional optical sectioning was used to visualize 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 establishing 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. In the nervous system, signals between neurons, as well as, neurons and glial cells play a pivotal role in nervous system homeostasis, and synaptic function (Araya & Navarro 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 (Prum et al. 2015). Several ADAMs function in spermatogenesis, 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 (Lu et al. 2009). Knowledge of protein localization often plays a critical role in characterizing the cellular function of hypothetically and newly discovered proteins. The mind 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 2000). Members of this family are structurally related to the typical structure of the ADAM family members, however, the metalloproteinase domain of ADAM23 suggests that its more significant role is in cell-cell and cell-matrix interactions, including fertilization, muscle development and neurogenesis (Klein & Bischoff 2011; Hu et al. 2011). 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 mind and its human homolog ADAM23 function in human disease processes and further provide clues to understanding the mechanisms in human diseases. This research will use immunohistochemistry 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 Neuronalin (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- FASII; mouse n- SQH; mouse n- Neuronalin; mouse n- β-Tubulin; mouse n- Fasciclin II; rabbit n- MMD (B. Chase). Secondary Antibodies (ThermoFisher Scientific): goat n- mouse, Alexa Fluor Plus 488; donkey n- rabbit, Alexa 594, Phalloidin, Alexa 647.

Fixation and de-vitellination

Embryos were de-chorionated in 50% bleach, washed with 1% Triton X-100 and transferred to a 20 ml vial containing fresh 4:1 hexane, 3 ml phosphate-buffered saline (PBS), 1 ml 10% formaldehyde (UltreX) and shaken for 25 min. The lower phase was removed and embryos were de-vitellinated by fixing the embryos in 3% glutaraldehyde in 1x PBS for 1 hr, transferred to a 1.5 ml (Eppendorf) tube, mixed 3x with MeOH and stored in MeOH at –20°C.

Phalloidin

Embryos were rinsed 2x with PBS (PBS, 0.1% Tween 20, 0.3% Triton X-100), washed by rocking for 4 x 15 min in 1 ml PBS, treated for 1 hr in 1 ml PBS (250 mM imidazole), rinsed for 2 hr in 1 ml PBS and fixed as described above. After fixation, embryos were incubated in 0.1% Triton X-100 and 0.2% sodium citrate in PBS for 10 min. After fixation, embryos were incubated for 30 min in 3% glutaraldehyde in PBS. After fixation, embryos were incubated in 0.5 ml blocking solution with 1% mouse serum, 0.5% Triton X-100, and 0.01% sodium azide for 1 hr. After blocking, embryos were incubated with a primary antibody against MMD overnight at 4°C. After washing 3x in 0.5 ml blocking solution and 1% mouse serum, embryos were incubated in 0.5 ml blocking solution with 1% mouse serum, 0.5% Triton X-100, and 0.01% sodium azide for 1 hr at room temperature. After blocking, embryos were incubated with a secondary antibody against MMD in 0.5 ml blocking solution for 1 hr at room temperature, rinsed and incubated in 1 ml PBS 2x (20 min). Staining was repeated with the second primary antibody and 2x in blocking solution. After incubation with the secondary antibody, embryos were stained with DAPI and Phalloidin in 1 ml PBS (5 min), washed in 1 ml PBS (10 min for 4x), and mounted in Vectashield on a back glass-located dish.

REFERENCES

• University of Iowa. (2015). The monoclonal antibodies developed by Dr. Bruce Chase was obtained from the Developmental Studies Hybridoma Bank, maintained at the University of Iowa, Department of Biology, Iowa City, IA 52242. http://www.hybridsoma.ii.edu/ab_query.html

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CONCLUSION

Although FASII is a cell membrane glycoprotein belonging to the immunoglobin superfamily of cell adhesion molecules, no colocalization was observed between FASII and MMD. Perhaps MMD is not involved in syringe 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 nucleus. 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 cytoskelelton.

MMD colocalizes with β-Tubulin during stage 3 of embryogenesis suggesting MMD’s role in preposition of developmental cues in the cytoskelelton and cytoplasmic reorganization in the embryo.

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 tetramers around the nuclei suggesting their role in forming a network to anchor the fully formed cells from the underlying yolk sar.

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 follicle 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 delaminating from the ecdysion, MMD appears to also form a bridge like structure between regions of contact for neuroblasts.

RESEARCH

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 image is 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 Phaohiladin 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

Figure 4. Expression of Spaghetti squash protein and Mind-Meld protein during shifting of the pole cell's position. A stage 10 embryo stained with antibodies against MMD, SQH and the nuclear marker DAPI. The merged images are shown. 40X magnification

Figure 5. Expression of Neuractin protein and Mind-Meld protein during neurogenesis. A stage 9 embryo stained with antibodies against MMD, NRT, and the nuclear marker DAPI. The merged images are shown. 20X magnification