Methionine Aminopeptidase 2 Expression in Microsporidia

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Microsporidia are single-celled obligately intracellular parasites that cause opportunistic infections associated most often with persistent diarrhea and disseminated infections [16,17]. Previously considered to be protozoa, the microsporidia were recently reclassified with the fungi [2,13,14,24]. Four species, Enterocytozoon bieneusi, Encephalitozoon intestinalis, Encephalitozoon hellem, and Vittaforma corneae, are responsible for causing the majority of microsporidian infections in humans [16,17]. Fumagillin and related compounds such as TNP-470 and ovalicin appear to be among the most effective drug for treating microsporidiosis [4,5,8,9,16,17,20,21]. Fumagillin, TNP-470, and ovalicin also inhibit angiogenesis by covalently binding to methionine aminopeptidase 2 (MetAP2) in endothelial cells [7,10,18,22]. To explore whether fumagillin and TNP-470 may likewise target MetAP2 in the microsporidia, preliminary studies were performed to characterize the expression of MetAP2 in microsporidia.

MATERIALS AND METHODS

The microsporidia, Brachiola algerae, E. cuniculi, E. hellem, E. intestinalis, Trachipleistophora hominis, and Vittaforma corneae, were grown in RK-13 rabbit kidney epithelial cells (ATCC CCL 37) and harvested by sequential washes in sterile distilled water, Tris-buffered saline (pH 7.2) containing 0.3% Tween 20 (TBS-Tw), and TBS followed by centrifugation over 50% Percoll, as previously described [6]. For Western blot immunodetection, microsporidia were resuspended in electrophoresis sample buffer containing beta-mercaptoethanol, boiled 5 min at 100 °C, and centrifuged one min at 15,000 g to remove particulate material. Proteins from 1 × 10^8 organism per lane were electrophoresed in a 5–20% gradient SDS-polyacrylamide gel and transferred onto PVDF membranes as previously described [6]. For Western blot immunodetection, microsporidia were resuspended in electrophoresis sample buffer containing beta-mercaptoethanol, boiled 5 min at 100 °C, and centrifuged one min at 15,000 g to remove particulate material. Proteins from 1 × 10^8 organism per lane were electrophoresed in a 5–20% gradient SDS-polyacrylamide gel and transferred onto PVDF membranes as previously described [6]. The blots were then blocked, incubated overnight with rabbit anti-human MetAP2 (Zymed Laboratories, Inc., S. San Francisco, CA) or normal rabbit serum (NRS), incubated with alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG, and stained with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP). To determine when MetAP2 expression could be detected in culture, RK-13 cells were grown to confluency in eight-well chamber slides at 37 °C and harvested by sequential washes in sterile distilled water, Tris-buffered saline (pH 7.2) containing 0.3% Tween 20 (TBS-Tw), and TBS followed by centrifugation over 50% Percoll, as previously described [6]. For Western blot immunodetection, microsporidia were resuspended in electrophoresis sample buffer containing beta-mercaptoethanol, boiled 5 min at 100 °C, and centrifuged one min at 15,000 g to remove particulate material. Proteins from 1 × 10^8 organism per lane were electrophoresed in a 5–20% gradient SDS-polyacrylamide gel and transferred onto PVDF membranes as previously described [6]. The blots were then blocked, incubated overnight with rabbit anti-human MetAP2 (Zymed Laboratories, Inc., S. San Francisco, CA) or normal rabbit serum (NRS), incubated with alkaline phosphatase (AP)-conjugated goat anti-rabbit IgG, and stained with nitroblue tetrazolium (NBT) and 5-bromo-4-chloro-3-indolyl-phosphate (BCIP). To determine when MetAP2 expression could be detected in culture, RK-13 cells were grown to confluency in eight-well chamber slides at 37 °C, and once a day for seven days, one well per slide was inoculated with E. cuniculi (at a ratio of three spores per host cell). After one hour’s incubation, the non-adherent spores were washed off and fresh medium was replaced. On day eight, the slides were fixed in methanol for 10 min at room temperature followed by incubation with rabbit anti-human MetAP2 or normal rabbit serum for one hour followed by incubation and staining with AP-conjugated anti-rabbit IgG, NBT, and BCIP as described above.

RESULTS AND DISCUSSION

MetAP’s types 1 and 2 are metalloexopeptidases that cleave the amino terminal methionine of many newly-synthesized proteins if the second or penultimate amino acid is small and uncharged [1,19]. Most eukaryotes express both MetAP1 and MetAP2, while bacteria express MetAP1 and Archaea express MetAP2 [12,13]. Based on the E. cuniculi genome sequenced by Katinka et al., the microsporidia appear to express only MetAP2 [11]. Fumagillin appears to target MetAP2 in human endothelial cells to inhibit angiogenesis, but it is unclear if fumagillin also targets MetAP2 to inhibit microsporidia replication. To begin to address this issue, studies were performed to characterize the expression of MetAP2 in several species of microsporidia that can be grown in culture and which are known to infect humans. All the microsporidian species assayed by western blot immunos assay expressed MetAP2 as detected by the rabbit anti-human MetAP2. The results in Fig. 1 demonstrated that the strongest bands of reactivity were observed at approximately 48 kDa for E. cuniculi, 49 kDa for E. hellem, 49 kDa for E. intestinalis, 47 kDa for B. algerae, 50 kDa for T. hominis and 62 kDa for V. corneae. The MetAP2’s of two species of fungi, Saccharomyces cerevisiae and Schizosacharomyces pombe, are somewhat higher in molecular mass than those in microsporidia, composed of 421 and 426 amino acid residues, respectively [1,3,19]. These results on approximating the molecular masses of microsporidian MetAP2’s are consistent with the amino acid sequences deduced from the cloned MetAP2 sequences of the Encephalitozoon species which range from 318 to 335 residues in length [11,23]. A polylysine-rich region and an approximately 15-amino acid residue segment in the amino terminal domain appear to be absent in the Encephalitozoon MetAP2 gene [11,23] (Pandrea, I., Mittleider, D., Brindley, P.J., Didier, E.S. & Robertson, D.L.)