Molecular & Biochemical Parasitology 169 (2010) 55–58

Contents lists available at ScienceDirect

Molecular & Biochemical Parasitology

Short communication

Myristoyl-CoA:protein *N*-myristoyltransferase depletion in trypanosomes causes avirulence and endocytic defects

Helen P. Price[a,*], M. Lucia S. Güther[b], Michael A.J. Ferguson[b], Deborah F. Smith[a]

[a] *Centre for Immunology and Infection, Department of Biology/Hull York Medical School, University of York, York YO10 5YW, UK*
[b] *Division of Biological Chemistry and Drug Discovery, College of Life Sciences, University of Dundee, Dundee DD1 5EH, UK*

ARTICLE INFO

Article history:
Received 3 July 2009
Received in revised form
14 September 2009
Accepted 17 September 2009
Available online 24 September 2009

Keywords:
Myristoyl-CoA:protein
N-myristoyltransferase
N-Myristoylation
Trypanosoma brucei
Endocytosis
RNA interference
Drug target

ABSTRACT

The enzyme myristoyl-CoA:protein *N*-myristoyltransferase (NMT) catalyses the co-translational covalent attachment of the fatty acid myristate to the N-terminus of target proteins. NMT is known to be essential for viability in *Trypanosoma brucei* and *Leishmania major*. Here we describe phenotypic analysis of *T. brucei* bloodstream form cells following knockdown of NMT expression by tetracycline-inducible RNA interference. Cell death occurs from 72 h post-induction, with approximately 50% of cells displaying a defect in endocytic uptake by this time. The majority of these induced cells do not have an enlarged flagellar pocket typical of a block in endocytosis but vesicle accumulation around the flagellar pocket indicates a defect in vesicular progression following endocytic fusion. Induced parasites have a wild-type or slightly enlarged Golgi apparatus, unlike the phenotype of cells with reduced expression of a major *N*-myristoylated protein, ARL1. Critically we show that following NMT knockdown, *T. brucei* bloodstream form cells are unable to establish an infection in a mouse model, therefore providing further validation of this enzyme as a target for drug development.

N-Myristoylation is required for the function of a range of eukaryotic and viral proteins, including the ADP-ribosylation factors (Arfs), Ras, the Src tyrosine kinase and HIV gag protein [1]. The enzyme catalysing this co-translational process is myristoyl-CoA:protein *N*-myristoyltransferase (NMT). We previously reported that NMT is essential for viability in the protozoan parasites *Leishmania major* (by gene targeting via homologous recombination) and *Trypanosoma brucei* (by RNA interference, RNAi) [2] and is thus a promising target for the development of novel therapeutics [3]. In a proof-of-principle study, two compounds with activity against fungal NMTs were able to inhibit purified *T. brucei* NMT *in vitro* and severely compromise growth of the bloodstream form (BSF) of the parasite in culture [3]. Here, we present more extensive characterisation of the phenotype observed following knockdown of NMT expression in BSF *T. brucei*, both *in vitro* and in an *in vivo* infection model, providing further validation for TbNMT as a putative drug target.

RNAi knockdown in bloodstream form *T. brucei* decreases NMT-specific transcripts to 57% of the original level by 4 h post-induction, as measured by real time PCR (data not shown). No further reduction is seen by 24 h post-induction. Loss of NMT protein occurs by 72 h post-induction, at which time there is a decrease to 16% of the original level. This time correlates with the onset of cell death in both procyclic and bloodstream forms of the parasite [2]. The data presented here reveal no dominant effects on progression through the cell cycle in RNAi treated cells, with only a minor accumulation (<5%) of cells with abnormal kinetoplast: nucleus configurations by 96 h post-induction (Fig. 1A). There is a marked increase in the number of round and tear-shaped cells, peaking at 84 h post-induction (14% and 9%, respectively). However, the majority of cells show no gross morphological abnormalities during the time course studied (Fig. 1B).

The survival of BSF trypanosomes depends on the correct functioning of extremely rapid transport mechanisms, for which at least two *N*-myristoylated proteins, TbARL1 and TbARF1, are known to be required [4,5]. Therefore, specific trafficking events have been analysed in detail in the NMT-depleted cells. *T. brucei* BSF are able to internalise the lectin concanavalin A (ConA) at the flagellar pocket membrane via receptor-mediated endocytosis, trafficking the lectin through the endocytic system before it reaches the terminal lysosome. Following NMT knockdown, the proportion of cells defective in the uptake of ConA increases steadily (Fig. 1C and D) reaching approximately 50% by 72 h post-induction. In contrast,

Abbreviations: Arf, ADP-ribosylation factor; Arl, ADP-ribosylation factor-like; BSF, bloodstream form; ConA, concanavalin A; NMT, myristoyl-CoA:protein *N*-myristoyltransferase; RNAi, RNA interference; *T. brucei*, *Trypanosoma brucei*; VSG, variant surface glycoprotein.

* Corresponding author. Tel.: +44 1904 328859; fax: +44 1904 328844.
E-mail address: hp502@york.ac.uk (H.P. Price).

0166-6851 © 2009 Elsevier B.V. Open access under CC BY license.
doi:10.1016/j.molbiopara.2009.09.006

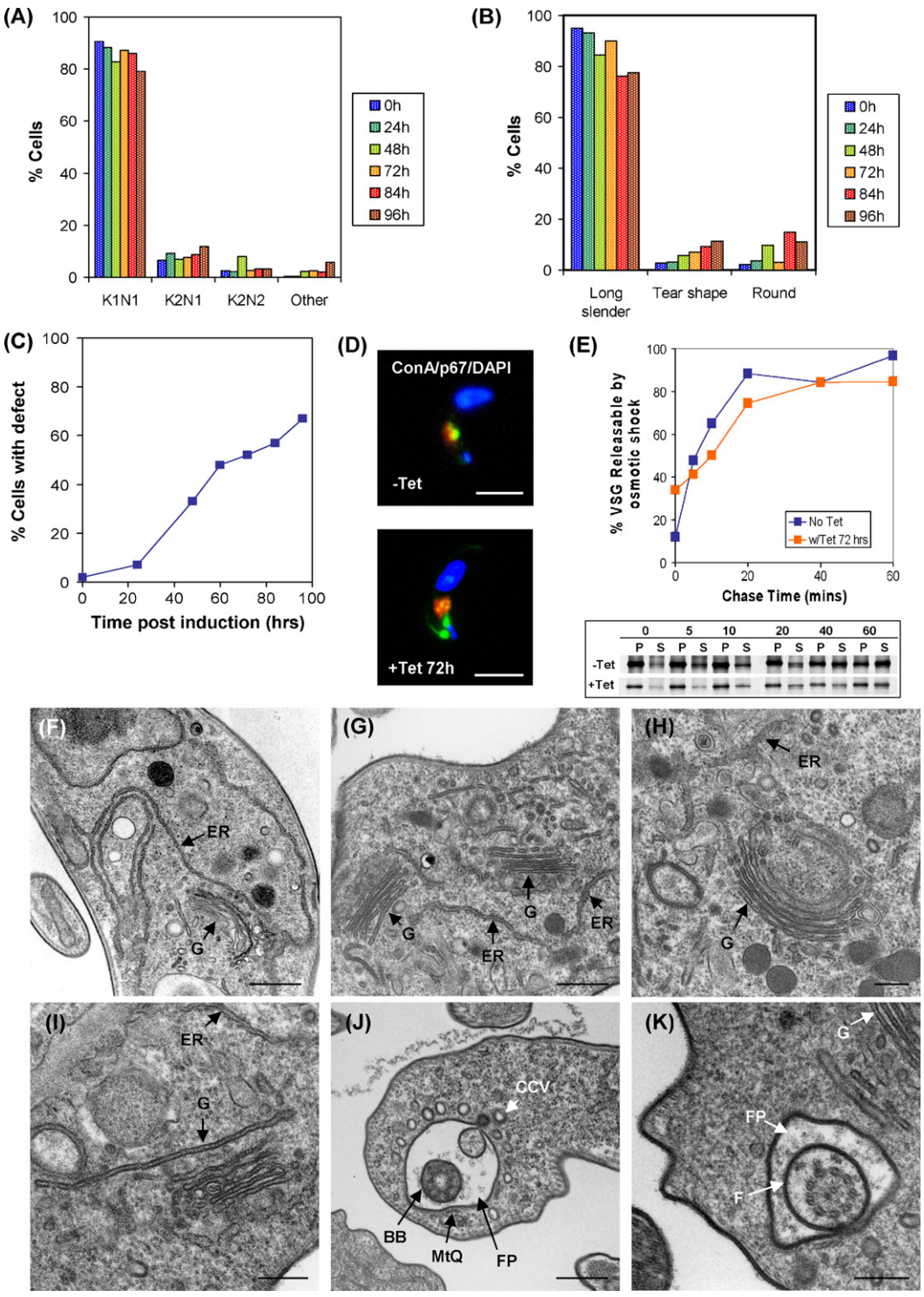

Fig. 1. Effects of NMT RNAi on cell morphology and intracellular transport. (A) Nuclei and kinetoplasts were counted in NMT RNAi parasite line Bp2T7/NMT (90-13) [2] grown in the absence or presence of tetracycline over a 96 h time course. At least 250 cells were counted per sample. (B) Abnormal cell morphology was quantified by light microscopy in the BSF line as above over a 96 h time course following addition of tetracycline. The percentage of tear-shaped and round cells are shown for each time point. At least 250 cells were scored per sample. (C) Receptor-mediated endocytosis was analysed by monitoring the uptake of FITC-labeled ConA in BSF line as above grown in the presence of tetracycline for 0–96 h. Cells labeled with FITC-ConA but with no co-localisation with p67 were classified as having an endocytic defect. >100 cells were counted per experimental group. (D) Typical uninduced and tetracycline induced (72 h) cells, the latter showing a defect in ConA uptake. Cells are labeled with FITC-ConA (green), p67 (red) and DAPI (blue). Bar, 5 μm. (E) VSG exocytosis was monitored in BSF line as above, grown in the absence and presence of tetracycline for 72 h. VSG was isolated from lysates of cells labeled by pulse-chase with [^{35}S]-labeled methionine and cysteine. Radiolabeled products were separated by SDS-PAGE, detected by autoradiography and quantified by densitometry. Results are presented as the % VSG trafficked from the endomembrane (insoluble) fraction to the plasma membrane (soluble) fraction. Data shown are representative of two independent experiments. The lower panel shows the autoradiograms from one experiment, exposed for the same length of time. P, pellet; S, soluble. Electron micrographs of parental BSF (F) and the RNAi line as above (G–K) grown in the presence of tetracycline for 72 h. ER, endoplasmic reticulum; G, Golgi apparatus; CCV, clathrin-coated vesicle; BB, basal body; MtQ, FAZ-associated microtubule quartet; FP, flagellar pocket; F, flagellum. Bar, 0.5 μm (F, G, and J) or 0.25 μm (H, I, and K).