Nature Reviews Urology **12**, 537 (2015); published online 25 August 2015; doi:10.1038/nrurol.2015.213

INFECTION

UPEC subverts endosomal recycling by modulating Rab35

New data published in *PLoS Pathogens* have revealed that intracellular uropathogenic *Escherichia coli* (UPEC) sequesters iron by modulating the Rab GTPase Rab35.

Bindu Sukumaran, corresponding author for the study, explained: "antibiotics often fail to completely eradicate UPEC, partly owing to a small fraction of bacteria persisting intracellularly within bladder cells for extended periods, resulting in recurrent infections. Intracellular bacterial pathogens need the micronutrient iron for their survival within host cells. However, as iron is also vital for host cells, the invading bacteria will inevitably have to compete to sequester iron for their own survival." Sukumaran's research group initially investigated the localization pattern 15 Rab GTPases—critical regulators of mammalian membrane trafficking pathways—during UPEC intracellular infection. Using the BEC5637-based *in vitro* infection model and overexpression of GFP-tagged or EGFP-tagged Rab GTPases, the team observed considerable localization of Rab35 with UPEC-containing vacuoles (UCV), and increased Rab35 messenger RNA and protein expression in an infection-stage-dependent manner. UPEC was also found to preferentially localize with constitutively active Rab35 over an inactive form of the protein. *In vivo* investigations in a mouse model of UPEC infection showed that ~25% of quiescent intracellular reservoirs of UPEC were associated with Rab35.

Silencing of Rab35 *in vitro* using short interfering RNA did not affect the ability of UPEC to enter BEC5637 cells but led to a significant reduction in bacterial load 24 h and 48 h after infection. No corresponding increase in bacterial expulsion from these cells was observed. Conversely, overexpression of Rab35 resulted in increased levels of UPEC, leading the team to conclude that the reduction in bacterial load was a consequence of reduced bacterial survival in these cells and that Rab35 has a role in intracellular survival of UPEC.

Rab35 is involved in the fast recycling of transferrin receptor (TfR), which is critical for the cellular uptake of iron, and UPEC is known to upregulate TfR1 levels during intracellular infection of bladder epithelial cells. TfR1 was found to colocalize with Rab35 and UCVs. To assess whether UPEC uses a Rab35-mediated pathway to meet their iron requirements via TfR, the group knocked down either Rab35 or TfR1 *in vitro*. In UPEC-infected cells, knock down of Rab35 or TfR1 resulted in a reduction in the labile iron pool and, similar to Rab35 knock down, silencing of TfR1 reduced intracellular UPEC survival. Further silencing experiments showed that Rab35 is critical for TfR recycling and, in the absence of Rab35, UPEC are trafficked to degradative lysosomes and killed.

"Our study sheds light on a unique mechanism by which intracellular UPEC acquire iron and prevent lysosomal degradation, thereby promoting survival in bladder cells," concludes Sukumaran. "The results of this study open up new avenues for therapeutic interventions."

Louise Stone

studioarz/iStock/Thinkstock

Original article Dikshit, N. *et al*. Intracellular uropathogenic *E. coli* exploits host Rab35 for iron acquisition and survival within urinary bladder cells. *PLoS Pathog*. doi:10.1371/journal.ppat.1005083