Role of Rab11 in *Leishmania* trafficking in macrophages

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By

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Dedicated to Lord Jagannath

It is all because of you….

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CERTIFICATE

This is to certify that the thesis entitled '**Role of Rab11 in *Leishmania* trafficking in macrophages**' being submitted by **Ms. Rituparna Basak** to Indian Institute of Technology, Delhi for the award of the degree of '**Doctor of Philosophy**' is a record of bonafide research carried out by her, which has been prepared under my supervision and guidance in conformity with rules and regulations of Indian Institute of Technology, Delhi, India. The results prescribed in it have not been submitted in part or full to any other University or Institute for the award of any Degree/ Diploma.

Date: 21.05.2024
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ABSTRACT

Leishmania donovani (Ld), the causative agent of visceral leishmaniasis, is an intracellular protozoan parasite which is dependent on its host cells for acquisition of various essential nutrients such as heme, purine, amino acids, fatty acids and vitamins. *L. donovani* express a specific receptor for hemoglobin uptake but how it salvages iron from the host cells is not well studied. Here, we report that *Leishmania* specifically upregulate the expression of host Rab11a and subsequently recruit it on the LAMP1+ parasitophorous vacuoles (PVs). The recruitment of Rab11a on Ld-PV is a live parasite-driven phenomenon as dead parasites are unable to recruit host Rab11a.

We have also addressed the mechanism of regulation of Rab11a expression in *Leishmania* infected macrophages. We have identified miR-181c to have a specific target site on the 3′UTR of Rab11a mRNA and overexpression of the miR-181c in macrophages results in reduced level of host Rab11a. Our results have shown that *Leishmania* infection significantly inhibits the expression of miR-181c in macrophages by degrading C/EBP-β. Thus, *Leishmania* inhibits the synthesis of miR-181c by degrading C/EBP-β and thereby enhances level of Rab11a in infected macrophages.

Leishmania infection enhances the transferrin uptake in the infected cells compared to uninfected cells and this enhanced uptake is Rab11a dependent as overexpression of miR-181c blocks the uptake of transferrin by infected cells. Interestingly, amastigotes isolated from macrophages contain significantly higher transferrin. The enhanced transferrin uptake is due to higher expression of transferrin receptor (TfR) in infected cells. Moreover, Ld-PVs also recruit and retain the transferrin receptor and thereby block the recycling of TfR to the cell surface in *Leishmania* infected macrophages. The recruitment of both Rab11a and TfR on Ld-PVs is blocked by overexpression of miR-181c indicating that their recruitment is Rab11a dependent process. Finally, we have found that intracellular growth of parasite is severely compromised in miR-181c overexpressed THP-1 cells and the inhibition of parasite growth is significantly reversed by the addition of hemin. These results demonstrate that *Leishmania* infection upregulates host Rab11a expression via downregulation of miR-181c to acquire iron from transferrin for their survival.

सार

लीशमैनिया डोनोवानी (एलडी), आंत लीशमैनियासिस का प्रेरक एजेंट, एक इंट्रासेल्युलर प्रोटोजोआ परजीवी है जो हीम, प्यूरीन, अमीनो एसिड, फैटी एसिड और विटामिन जैसे विभिन्न आवश्यक पोषक तत्वों के अधिग्रहण के लिए अपने मेजबान कोशिकाओं पर निर्भर है। एल डोनोवानी हीमोग्लोबिन ग्रहण के लिए एक विशिष्ट रिसेप्टर व्यक्त करते हैं लेकिन यह मेजबान कोशिकाओं से आयरन को कैसे बचाता है इसका अच्छी तरह से अध्ययन नहीं किया गया है। यहां, हम रिपोर्ट करते हैं कि लीशमैनिया विशेष रूप से मेजबान Rab11a की अभिव्यक्ति को बढ़ाता है और बाद में इसे LAMP1+ पैरासिटोफोरस रिक्तिका (PVs) पर भर्ती करता है। Ld-PV पर Rab11a की भर्ती एक जीवित परजीवी-प्रेरित घटना है क्योंकि मृत परजीवी मेजबान Rab11a को भर्ती करने में असमर्थ हैं।

हमने लीशमैनिया संक्रमित मैक्रोफेज में Rab11a अभिव्यक्ति के नियमन के तंत्र पर भी चर्चा की है। हमने Rab11a mRNA के 3/UTR पर एक विशिष्ट लक्ष्य साइट के लिए miR-181c की पहचान की है और मैक्रोफेज में miR-181c की अधिक अभिव्यक्ति के परिणामस्वरूप होस्ट Rab11a का स्तर कम हो जाता है। हमारे परिणामों से पता चला है कि लीशमैनिया संक्रमण C/EBP-β को ख़राब करके मैक्रोफेज में miR-181c की अभिव्यक्ति को महत्वपूर्ण रूप से रोकता है। इस प्रकार, लीशमैनिया C/EBP-β को निम्नीकृत करके miR-181c के संश्लेषण को रोकता है और इस प्रकार संक्रमित मैक्रोफेज में Rab11a के स्तर को बढ़ाता है।

लीशमैनिया संक्रमण असंक्रमित कोशिकाओं की तुलना में संक्रमित कोशिकाओं में ट्रांसफ़रिन ग्रहण को बढ़ाता है और यह बढ़ा हुआ ग्रहण Rab11a पर निर्भर है क्योंकि miR-181c की अधिक अभिव्यक्ति संक्रमित कोशिकाओं द्वारा ट्रांसफ़रिन के ग्रहण को अवरुद्ध करती है। दिलचस्प बात यह है कि मैक्रोफेज से अलग किए गए एमास्टिगोट्स में काफी अधिक ट्रांसफ़रिन होता है। ट्रांसफ़रिन ग्रहण में वृद्धि संक्रमित कोशिकाओं में ट्रांसफ़रिन रिसेप्टर (टीएफआर) की उच्च अभिव्यक्ति के कारण होती है। इसके अलावा, एलडी-पीवी ट्रांसफ़रिन रिसेप्टर को भी भर्ती करते हैं और बनाए रखते हैं और इस तरह लीशमैनिया संक्रमित मैक्रोफेज में कोशिका की सतह पर टीएफआर के पुनर्चक्रण को रोकते हैं। Ld-PVs पर Rab11a और TfR दोनों की भर्ती miR-181c के ओवरएक्सप्रेशन द्वारा अवरुद्ध है, जो दर्शाता है कि उनकी भर्ती Rab11a पर निर्भर प्रक्रिया है। अंत में, हमने पाया है कि miR-181c ओवरएक्सप्रेस्ड THP-1 कोशिकाओं में परजीवी की इंट्रासेल्युलर वृद्धि गंभीर रूप से प्रभावित होती है और हेमिन के जुड़ने से परजीवी वृद्धि का अवरोध काफी हद तक उलट जाता है। इन परिणामों से पता चलता है कि लीशमैनिया संक्रमण अपने अस्तित्व के लिए ट्रांसफ़रिन से आयरन प्राप्त करने के लिए miR-181c के डाउनरेगुलेशन के माध्यम से मेजबान Rab11a अभिव्यक्ति को अपग्रेड करता है।

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BCA	Bicinchoninic acid
bp	Base pair
BS	Bloodstream
BSA	Bovine serum albumin
B-Tf	Biotinylated transferrin
cDNA	Complementary DNA
DNA	Deoxyribonucleic acid
dNTPs	Deoxynucleotide Triphosphate
DTT	Dithiothreitol
ECL	Enhanced chemiluminescence
EEA1	Early Endosome Antigen 1
EGF	Epidermal Growth Factor
EGFR	Epidermal Growth Factor Receptor
ER	Endoplasmic Reticulum
ERC	Endocytic recycling compartment
ESAG	Expression site associated genes
ESCRT	Endosomal Sorting complex required for transport
FCS	Fetal calf serum
GAP	GTPase activating protein
GDF	GDI displacement factor
GDI	GDP dissociation inhibitor
GDP	Guanosine diphosphate
GEF	Guanine nucleotide exchange factor
GFP	Green fluorescent protein
GGT	Geranylgeranyl transferase
gp63	Glycoprotein 63
GPI	Glycosylphosphatidylinositol
GTP	Guanosine triphosphate
h	Hours
Hb	Hemoglobin
HpHbR	Haptoglobin-hemoglobin receptor

Hrg	Heme responsive gene
HRP	Horse radish peroxidase
LAMP	Lysosomal associated membrane protein
LB	Luria-Bertani
LCV	*Legionella* containing vacuoles
Ld	*Leishmania donovani*
LDL	Low density lipoprotein
LFR1	*Leishmania* ferric reductase 1
LHR1	*Leishmania* Heme Response 1
LIT1	*Leishmania* iron transporter 1
LPG	Lipophosphoglycan
M	Molar
mg	Milligram
MI99	Medium 199
mins	Minutes
miRNAs	MicroRNAs
ml	Milliliter
mM	Millimolar
mRNA	Messenger RNA
Mut	Mutated
NADPH	Nicotinamide Adenine Dinucleotide Phosphate Hydrogen
ng	Nanogram
nM	Nano molar
nm	Nano meter
O.D.600	Optical density at 600nm
PAMPs	Pathogen associated molecular patterns
PBS	Phosphate buffered saline
PBST	Phosphate buffered saline with Tween-20
PC	Procyclic
PCR	Polymerase chain reaction
PFA	Paraformaldehyde
PI3P	Phosphatidylinositol-3-phosphate
pre-miRNA	precursor miRNA
pri-miRNA	primary miRNA

PRR	Pattern recognition receptors
PV	Parasitophorous vacuole
RCV	Replicative Coxiella vacuole
RE	Recycling endosome
REP	Rab escort protein
RILP	Rab7-interacting lysosomal protein
RISC	RNA-induced silencing complex
RME	Receptor mediated endocytosis
RNA	Ribonucleic acids
ROS	Reactive oxygen species
rpm	Revolutions per minute
RT	Room Temperature
SCP	Salmonella containing phagosome
SCV	Salmonella containing vacuole
SDS	Sodium dodecyl sulphate
SDS-PAGE	SDS-Polyacrylamide gel electrophoresis
SNAP	Synaptosomal associated protein
SNAREs	Soluble N-ethylmaleimide-sensitive factor attachment protein receptors
Tf	Transferrin
TfR	Transferrin receptor
TRAPP	TRAnsport Protein Particle
Tris	Tris-hydroxy methyl- amino methyl
UTR	Untranslated regulatory region
VAMP	Vesicle associated membrane protein
v-ATPase	vacuolar ATPase
VL	Visceral leishmaniasis
WT	Wild type
µg	Microgram
µl	Microliter
µM	Micromolar