Role of Vesicular Trafficking in Infection and Cancer: A Review

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Abstract

Most of the pathogenic bacteria and viruses are capable of causing variety of infections in the respective hosts. These infectious agents enter and spread inside the host cells via various modes most notable of which is endocytosis machinery. Several pathogenic strains of bacteria like *Mycobacterium, Samlonella* and viruses like Dengue virus and HIV-1 make use of endocytic trafficking of vesicles to invade the host cells, multiply and exit. Oncogenic mutations accumulate over the time and affect many vesicular trafficking components causing aberrant distribution of cell surface proteins and downstream signalling leading to reckless proliferation of cells. In this paper, we tried to analyse how pathogens exploit vesicular trafficking machinery of cells for their advantage in infection and perturbations in the process leads to neoplasia conditions. Identification of some key proteins participating in the function may lead to development of new anaphylactic or therapeutic interventions.

Key words: *Helicobacter*, Endocytosis, Exocytosis, Metastasis, *Mycobacterium*, Neoplasia, RABs.

Introduction

There has been a surge in reports in the past two decades establishing link between pathogenesis of microbes and viruses with the vesicular trafficking operating inside the cell(1,5,6,7). In addition, reports about biology of oncogenic mutations have now started appreciating the role of compromised vesicular trafficking underlying at the heart of many neoplasias(13,10,12). To understand those links it is important that the process of vesicular trafficking is first understood properly. The cellular vesicular trafficking is divided broadly into two categories: endocytosis (directed inside the cell) and exocytosis (towards outside the cell). Fig. 1 depicts the schematic description of the two modes of vesicular trafficking operating inside the cell.

Endocytosis involves the uptake of ligand (growth factors, microbes, viruses) bound receptors via membrane invagination and ultimately pinching off the membrane vesicle called the endosome. The endosome then through a series of maturation process from early to late stages fuse with the lysosomes for degradation of the content. Right from the time of formation of endosome, there are unique class of small GTPases called Rab, associated with them for maturation, guidance and deciding their recycling or degradation fate. It is the specific demand of the internalized receptors that determine the degradation or recycle of the endosome carrying the ligand bound receptor(3,4,8,9). Another arm of the vesicular trafficking simultaneously operating inside the cell is exocytosis in which Golgi derived vesicles carrying proteins inside are delivered to the cell surface. Examples include secretion of ECM degrading matrix metalloproteinase (MMPs) in tumour metastases, hormones, gastric secretion of enzymes, and antibody secretion by B-lymphocytes in inflammation etc.(10,11).

Microbial Infection

***Salmonella typhimurium*:** *Salmlonella* an invader of human gut is notorious to gain access inside the epithelial cells of the gastric mucosa and macrophages. It is a Gram- negative facultative anaerobic pathogen, which causes gastroenteritis in humans. Infection with salmonella upsets the ratio of absorption and secretion thereby leading to diarrhoea (excessive fluid loss)(6). Direct administration of anti-diarrheals is most widely prescribed treatment method.

The bacterium gains access through the intestinal microvilli by causing membrane ruffling. After entry, the bacteria remain within a modified phagosome known as the Salmonella-containing vacuole (SCV), within which they will survive and replicate. Later on develops into filamentous structures called Salmonella induced filaments (SIFs). The transition from vacuolar to filamentous state requires participation of Rab7 and its effector molecule RILP (Rab7-interacting lysosomal protein). This transition does require the bacteria to associate with late endosomes although changing to filaments protects the bacteria from lysosomal degradation(6).

***Helicobacter pylori*:** *H. pylori* is another Gram-negative pathogenic bacterium, which colonizes pyloric area at the end of the duodenum of stomach. It is a known causative agent of gastritis and gastric carcinomas. Around 50% of world population harbours the bacterium in stomach with the infection more abundant in western countries. The bacterium corrodes pyloric mucus to gain access into underlying epithelial layer to avoid acidic pH(7).

H. pylori require the involvement of Rab7 in addition to LAMP1 (Lysosome associated membrane protein 1) and CD63 in its escape from lysosomal degradation. It however resides in a specialized kind of endosomal compartment, which is partly late endosomal and lysosomal but without degrading abilities due to which the bacterium survives inside the host cells(8).

***Mycobacterium tuberculosis*:** *Mycobacterium tuberculosis* perhaps the most dreaded disease-causing bacteria known until date in human history. First identified by Robert Koch in 1882, the bacterium is also known as Koch's bacillus. The bacterium has an outer mycolic acid layer because of which it is impervious to traditional Gram staining. It has been characterized as an obligate pathogenic bacterium which chiefly infects respiratory tract in humans and causes tuberculosis (TB). The vaccine against it is BCG (Bacillus Calmette-Guerin) which is included in routine immunization programs of infants worldwide.

Rifampicin and isoniazid are two most commonly used antibiotics used for the treatment for tuberculosis. The bacterium is known to develop resistance (MDR = Multi drug resistance) to both antibiotics due to accumulation of mutations in the gene targeted by antibiotics or changes in antibiotic dosage regime(10).

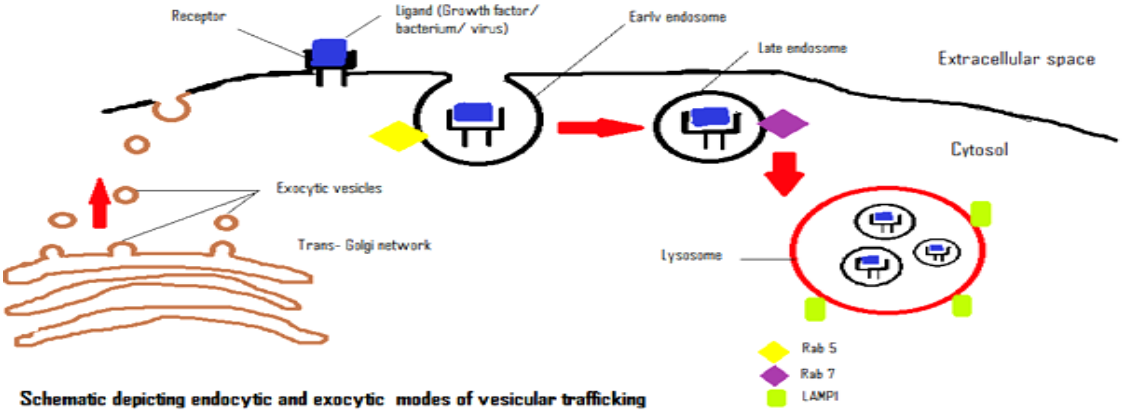
The organism also finds its route from endocytic pathway for its infection, maturation and protection from lysosomal death. Upon entry inside the host cells, bacteria rest in phagosome similar to *Salmonella*. Further maturation is however depended on Rab5 and Rab7. The bacterium recruits Rab22a on its phagosome thereby preventing phagoloysosomal conversion(9).

HIV1 (Human Immunodeficiency Virus 1): HIV 1 is a subtype of retrovirus HIV group, which causes sexually transmitted disease called AIDS (Acquired immunodeficiency syndrome). AIDS onset is highly slow and over time leads to compromised immunity and patient becomes susceptible to various kinds of infection. The virus is diagnosed mainly by PCR of HIV-RNA to ascertain the serotype of the virus. Currently there is no vaccine to safeguard against the infection. Therapeutic interventions mainly target viral reverse transcription. As of 2014, around 37 billion of world population is considered to be infected with the virus(16)

Molecular studies have shown that dominant negative expression of constitutively active and inactive Rab7 leads to significant decline in HIV1 gene expression. The viral Nef proteins traffick MHC1 and CD4to Rab7 vesicles for degradation thereby leading to escape from the host immunity. HIV infection leads to massive extracellular vesicle secretion, which has been found to contain viral coreceptors that are thought to enhance the infection in proximity(1).

DENV (Dengue Virus): Dengue virus is a mosquito borne single stranded RNA virus, which causes dengue fever. It mostly affects people in the tropical countries. The symptoms are generally observable between 3 to 14 days and include headache, vomiting, skin rashes, muscles and joint pain. Diagnosis is aimed at detecting viral proteins by antibodies or viral RNA. There is no vaccine for dengue but treatments employ the usage of anti-viral aimed at blocking viral RNA replication(1,2).

Dengue virus matures in Rab7 marked late endosomes and that mutant Rab7 expression had no effect on its ability to infect in cell culture. Rab18 primarily found on endoplasmic reticulum network and lipid droplets



Schematic depicting endocytic and exocytic modes of vesicular trafficking

Viral Infection

Viral infection involves clathrin dependent endocytosis and via late endosomal pathway, the virus reaches lysosome or leaks its content in the middle(1).

Fig. 1: Schematic description of 'Endocytic and Exocytic' modes of vesicular trafficking

was reported to regulate viral DNA replication. The viral NS3 protein redistributes fatty acid synthase (FASN) protein to sites of viral DNA replication and has been found to the co-purifying with viral RNA molecules suggesting this redistribution might be directed at viral genome packaging for egress(1,2,11).

Rab GTPases: Rabs are small GTPases entrusted with the task of correct endosomal maturation and guidance

to meet the demand of balanced vesicular trafficking. They shuttle between active GTP bound and inactive GDP bound state while executing their function. However, their overexpression is linked to various carcinomas, which indicate that vesicular trafficking pathway involving them has gone awry when their levels increase beyond the essential. Loss of Rab25 leads to abnormal integrin presentation in Caco2 cells that alters the protein composition of adherens junction and tight junctions. The depletion of Rab 25 causes decreased expression of α5 integrin gene and β1 integrin which promotes invasiveness of the tumor(5).

Metastasis: Consequence of aberrant trafficking

The property of primary neoplastic cells to invade basement membrane and colonize secondary sites is called metstasis, which in essence is spreading of tumour. Such kind of movement of cells whereby they intend to reach out of their existing location requires massive cytoskeletal rearrangements and polarized trafficking towards the "leading edge" of the cell. The primary hurdle that comes in the way is the basal lamina, which is a dense proteinaceous mixture (ECM components) which requires to be degraded. To achieve this, tumour cells give out membrane projections that are sites of trafficking of vesicles delivering membrane, ECM degrading MMPs, integrins and cytoskeletal remodelers. Rab8 and vesicle associated membrane protein 7 (VAMP7) deliver cargo containing MMP14 which help osteosarcoma cells degrade their basal lamina(12,13,14).

The following is the list (Table 1) of various key proteins participating in the vesicular trafficking process in context of infection and cancer.

Table 1: Different key proteins participating in the vesicular trafficking process and their roles in context of infection and cancer

Sl. No.	Participating protein in trafficking process	Role in infection/ cancer
1.	Rab 7	Pathogenesis of *Salmonella*, *H. pylori*, *M. tuberculosis*, HIV
2.	RILP (Rab 7 interacting lysosomal protein)	Maturation of SCV to SIF
3.	Rab 25	depletion in Caco2 leads to tumorigenesis
4.	Rab 8	Delivers MMPs at sites of invasion in tumors
5.	VAMP 7 (vesicle associated membrane protein 7)	Associates with Rab8 in its function
6.	MMP 14 (matrix metalloproteinase 14)	ECM degrading enzyme secreted at sites of invading tumor cells
7.	Nef proteins	Degrades host MHC-I to evade immune response
8.	LAMP1 (Lysosome associated membrane protein 1)	Maintains lysosomal integrity, pH and catabolism

Discussion

Trafficking is indispensable for a cell to survive. The proper functioning of any cell depends heavily on what it takes in and gives out. A pathogen is taken up inside the infected cell in fashion similar to any ligand uptake and goes through the same pathway to meet its fate. However, the virulence factors of the bacterial or viral pathogen are crucial components in hijacking the trafficking machinery for diverting it towards the survival of the infecting organism instead of degrading it inside the lysosome. A wide spectrum of molecules have been identified that work in concert with the infectious agents' proteins in channelizing the trafficking machinery towards their survival, multiplication and release.

In case of cancer, however, most of the studies pertaining to clinical samples have been focused mainly on identifying the mutations and DNA/ RNA based markers and therefore very little information is there on the trafficking status of the cancer cells.

It is now becoming clearer that vesicular trafficking is of utmost importance for onset and progression of infection and tumorigenesis. To carry out the functions, cell needs well-balanced vesicular trafficking machinery for fulfilment of demands of receptors, ligands, membranes, ion channels, cell adhesion molecules and proteins, which regulate these processes. Under conditions of infection or oncogenic stress, the balance of the trafficking tilts towards side, detrimental to cellular survival. Many key proteins like Rabs, membrane receptors and cell adhesion molecules are contemplated as diagnostic or prognostic markers because of their expression pattern associated with the diseased conditions. However, more in-depth studies are required for validation of these markers as putative diagnostic or therapeutic candidates.

Conflict of Interest: None

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