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Advances in Social Science, Education and Humanities Research, volume 196

2nd International Conference on Social Science, Public Health and Education (SSPHE 2018)

Progress in Mechanism of E.coli K1 Crossing the Blood-brain Barrier

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Abstract—Meningitis is a serious central nervous system disease, E. coli K1 is the main pathogen that causes it. E.coli K1 triggers meningitis only if it crosses the blood-brain barrier(BBB), but the detail mechanism of how E. coli K1 cross the blood-brain barrier is still unclear. This article is based on current research, according to the sequence of E. coli K1 crossing the blood-brain barrier, introduce the mechanism from three sections: severe bacteremia, adhesion and invasion to brain microvascular endothelial cells (BMEC), rearrangement of cytoskeleton.

Keywords—E. coli K1, blood-brain barrier, Meningitis

I. INTRODUCTION

Central system infection is one of the infections with high morbidity and mortality, among various pathogens, meningitis caused by *E. coli* K1 is the main type of central system infection[1]. This phenomenon not only appear in adults, but also in neonatal meningitis. Meningitis can cause neurological sequelae, such as cerebral palsy, epilepsy and so on. The damaged brain structure may cover soft meninges, arachnoid and subarachnoid.[1][2][3]

In spite of the widespread use of antibiotics in recent years, the incidence of bacterial meningitis is still stay in 5% to 40%, and the neurological sequelae rate in survivors is up to 30%[4] [5]

E. coli K1 is a kind of gram-negative bacteria, Previous studies have shown that 84% of *E.coli* strains which cause neonatal meningitis contains K1 capsule. K1 capsule can make *E. coli* survive in cerebrospinal fluid. That may owning to K1 capsule containing anti-lysosomal enzymes which can avoid the combination of internal swallowing vacuole and lysosome[2][6][7]. So this article focus on the mechanism of *E. coli* K1 crosses the blood brain barrier.

Previous researches showed that the meningitis caused by *E.coli* K1 is contributed by several vital proteins, several genetic island of them are already identified, such as sfa(S fimbriae), ksp (K1 capsule), CNF-1 and GimA(Fig.1), among them, GimA is the most unique and vital for *E.coli* K1.

This study was supported by the National undergraduate innovative experiment program of China (No. 201612121013 to X. Yang & S.-Y.Lin)

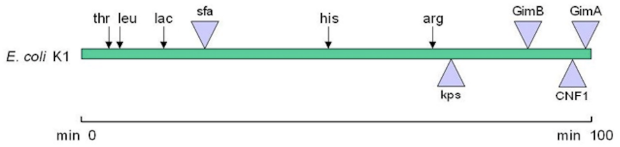

Fig. 1. Chromosomal Location of genetic islands from meningitic E. coli K1 (according to the E. coli K12 linkage system. Sfa (S fimbriae), ksp (K1 capsule), GimB, CNF1 and GimA (the genetic island carrying the invasion gene ibeA) loci in E. coli K1 strain RS218: 25 min, 66 min, 78 min, 97 min and 98 min, respectively.)

Related research showed that the mechanism of meningitis caused by *E.coli* K1 is mainly based on sever bacteremia, through adhering and invading to the brain microvascular endothelial cells (BMECs) to accomplish the rearrangement of cytoskeleton. This article will review the mechanism of *E.coli* K1 crossing the blood-brain barrier from the above three aspects.

II. THE FOUNDATION OF CROSSING: SEVERE BACTEREMIA

Severe bacteremia is the foundation of *E.coli* K1 crossing the blood-brain barrier. When pathogens enter and multiply in blood circulation, it may cause bacteremia. There are two premises to trigger severe bacteremia: one is that pathogens can effectively evade the defense mechanisms of host cells, the other is that pathogens can proliferate in host cells to reach the threshold for causing severe bacteremia.

Previous study have identified that the expression of K1 capsular polysaccharide, O-LPS (O-lipopolysaccharide), NlPI (New lipoprotein I) and Omp-A (outer membrane protein) will assist *E.coli* K1 to accomplish both premises [4][6] [8] [9] .

Among them, OMP-A was considered more important than others. Despite the expression of other virulence factors, it's a useful way to prevent meningitis by blocking Omp-A

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expression. With the help of Omp-A, *E.coli* K1 can proliferate after being phagocytized by macrophages in peripheral and cumulate to the threshold of bacteremia[4][5][9]. Besides Omp-A, K1 capsule was also considered as key for *E.coli*.K1, *E.coli* K1 was internalized within membrane-bound vacuoles during transmigrate the blood-brain barrier, vacuoles containing K1 capsule obtained endosomes without fusion with lysosomes, thereby allowing *E.coli* K1 to cross the blood-brain barrier as live bacteria[4][7]. Previous study thought *E.coli* K1 will proliferate in vacuoles during crossing as in macrophages, but recent study has cleared *E.coli* K1 stayed in vacuoles without multiplication[4][5][9].

Research shows that in the neonatal period, when the content of *E.coli* K1 in the blood is more than 10^3 CFU/mL, the probability of neonatal meningitis occurring is much higher than less than 10^3 CFU/mL[2][3]. Besides, from the comparative experiment between neonatal mouse and adult mouse, we can figure out that to stimulate same level of bacteremia, the required vaccinated amount of *E.coli* K1 is about 10^6 times for adult mouse than neonatal mouse, that may be related to the gradual improvement of the immune system throughout the growth process[4][5].

Although severe bacteremia is the foundation of *E.coli* K1 crossing the blood-brain barrier, the specific mechanism and relationship between severe bacteremia and meningitis is still unclear. But it worth noting that it's possible to reduce the incidence of meningitis by reducing the *E.coli* K1 in the blood stream especially in the neonatal meningitis[2].

III. The Initiation of Crosiing: Adhesion and Invasion to Brain Microvascular Endothelial Cells

The blood-brain barrier is formed by BMECs, pericytes and astrocytes[4]. Among them, BMECs are most essential. BMECs are tightly aligned and have poor ability to phagocytize, it forms a natural barrier that prevents exotic pathogens from entering central nervous system[4]. So the initiation of crossing the blood-brain barrier is *E.coli* K1 adhering and attacking to BMECs, which was also called transcellular penetration [2][3][4].

A. Proteins Contribute to Adhersion

Proteins which are responsible for the adhesion between *E.coli* K1 and BMECs includes Fim-H, Omp-A, NlPI and FLI-C. Among them, Omp-A is comparative more vital than others.

GP-96(glycoprotein-96) is the receptor for Omp-A[10]. The N-terminal domain of Omp-A and its surface-exposed loops contribute to binding and adhering[4][11]. GP-96 only appear on the brain microvascular endothelial cells(BMECs) cytomembrane, not on the other systemic vascular endothelial. After recognition and conjunction, the phosphatidylinositol 3-kinase (PI3K) on the surface of BMECs will be stimulated [11]. Meanwhile, Omp-A can also affect the adhesion ability of pathogens by affecting the growth of their own type-1 fimbriae[4][7][9][11]. Related research revealed that the Omp-A deletion mutant exhibited significantly lower expression of type 1 fimbriae on the bacterial surface, suggesting except recognition of GP-96, decreased binding happened in Omp-A deletion is partly related to its lower expressing of type-1 fimbriae[1][4][7].

Fim-H is demonstrated to interact with CD48, on the surface of BMECs, and the interaction can increase intracellular Ca^{2+} which is helpful for binding[12]. NlpI contribute not only expression of type 1 fimbriae but also to flagella[8][13]. FLI-C contribute to the expression of flagella, recent study found the vital role of flagella in *E.coli* K1 associated meningitis[5].

B. Proteins Contribute to Invasion

Protein CNF-1 and IBE(Invasion of brain endothelial cell protein) are responsible for the invasion of BMECs by *E.coli* K1. CNF-1 has two ways to invade BMECs, one is receptor-ligand binding approach, CNF-1 is complementary to N-terminal and full-length but not the C-terminal of 37KD LRP(laminin receptor precursor), which can further stimulate Rho-GTPases (triphosphates)[4][5][11]. After stimulation, polymerization of F-actin will rise sharply enough to change the cytoskeleton[11]. The 37KD LRP will transfer into 67KD laminin receptor(LR). It's not clear how 67KD LR transferred from 37KD LRP, but it was tested that mature 67KD LR is located on BMECs surface and function as a membrane receptor as adhesive basement protein laminin. CNF-1 up-regulate expression of 67KD LR on the surface of BMECs and it also recruit 67KD LR to the site of invasion[5][11]. On the other hand, it invade BMECs with their secretions assistance, such as α -hemolysin and β -lactamase, but the detail mechanism of secretions invade BMECs is still not clear[4][11].

One problem confused most researchers is CNF-1 as a cytoplasmic protein and its contribution to *E.coli* K1 invasion requires its secretion from bacterial cytoplasm, the mechanism of secretion is unknow, none of existing secretion systems can be applied to it. But the latest research revealed its secretion may have relationship with ferredoxin(FDX) which located in the inner membrane of bacteria and YgfZ in outer membrane.[4][5][14].

Besides CNF-1, the unique genetic about IBE is encoded in a genetic island-GimA, a genetic island located at the 98 min region of bacterial chromosome(Fig. 1). GimA consists of four operons, they are ptnIPKC, cglDTEC, gcsKRCI and ibeRAT(Fig. 2), besides IBE gene, GimA is also involves in energy metabolism and the invasion mediated by GimA is regulated by carbon source. Previous research suggested that GimA encodes 10 enzymes, 3 transporters, 1 regulatory protein and 1 invasion(IBE-A), which implicating that most of protein encoded by GimA contribute to transportation and metabolism of carbon sources, further study showed that the operons ptnIPKC(GimA1), cglDTEC(GimA2), gcxKRCI(GimA3) presumably contribute a carbohydrate transportation (PTS), glycerol metabolism and glyoxylate pathway, respectively. The last operon ibeRAT, which codes for an invasin (IBE-A) and a putative regulatory protein (IbeR), may contribute to invasion and regulation[2][14].

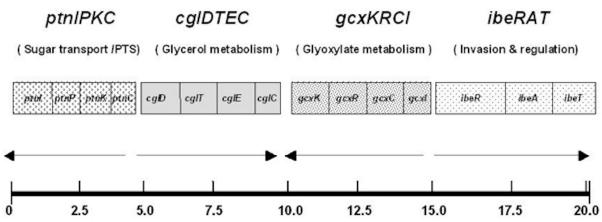

Fig. 2. Open rectangles indicate the ORFs for the GimA. (The orientation of transcription is indicated by arrows. The GimA consists of 4 operons, ptnIPKC, cglDTEC, gcxKRCI, and ibeRA.)

Related study shows that PTS-glucose is preferred carbon source for meningitis mediated by E. coli K1. GimA can affect invasion through affect the metabolism and transportation of carbon sources. PtnI and PtnP are encoded by the operon *ptnIPKC*(GimA1), they were tested have signigicant sequence homology with multiphosphoryl transfer protein(MTP), and PTS have been tested for the ability to affect *E.coli* K1 invasion of BMECs. CglY and CglC encoded by operon *cglDTEC* (GimA2) contributing to transportation of glycerol, glucarate and carnitine. Glycerol play an important role in regulation of invasion gene and virulence gene[15][14]. Glyoxylate carboligase (*gcxC*), tartronate semialdehyde reductase (*gcxR*), glycerate kinase (*gcxK*) and hydroxypyruvate isomerase (*gcxI*)] are four putative enzymes encoded by the operon *gcxKRCI* (GimA3). These four enzymes regulate the D-glycerate pathway and contribute to the metabolism of glyoxylate. They are important intermediate of the central microbial metabolism in glyoxylate bypass.

IbeA is an unique protein of *E.coli* K1, complementation of the mutant with the IBE-A locus is able to restore the effects of carbon sources on bacterial invasion, suggesting that carbon source-regulated E. coli K1 invasion be mainly dependent on the IBE-A gene locus. Taken together with former theories, these findings demonstrate that PTS-sugars and non-PTS-sugars are able to modulate IBE-A mediated *E.coli* K1 invasion of BMECs and supports that the IBE-A gene is an important determinant for E. coli K1 crossing the BBB. IBE-A has several ways to affect BMECs(Fig. 3). It can not only regulate the growth of fimbriae on E.coli K1, but also combine with VIM(vimentin). By combining, it leads to the phosphorylation of VIM and impact the activity of FAK(Focal adhesion kinase). Ultimately causing the rearrangement of the cytoskeleton[4][5]. After the ligand-receptor interaction, IBE-A can activate PI3K, then phosphorylates and activates the downstream enzyme AKT into the phosphorylated AKT(p-AKT) enhances dephosphorylation of cofilin into phosphorylated cofilin(p-cofilin, inactive form) through the negative control of LIM kinase (LIMK), which is phosphorylated and activated by ROCK. PI3K and ROCK oppositely regulate LIMK. LIMK catalyzes phosphorylation of cofilin on serine 3, which prevents its association with actin[15] [14][15][16]. Beside these, IBE-A can also binds both VIM and PSF(PTB-associated splicing factor) in concert to activate TAK1(β-activated kinase), and ultimately activate NF-kB, it will arise inflammatory in brain[8].

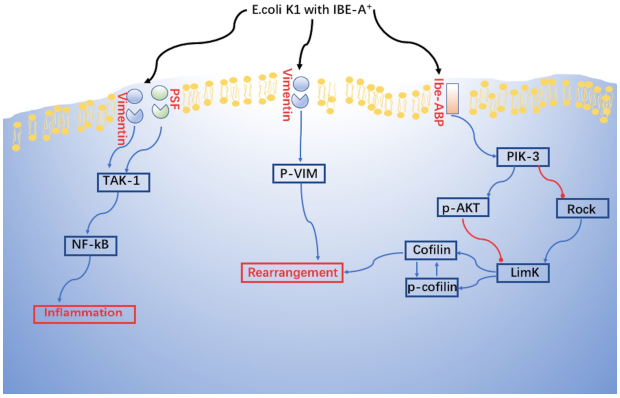

Fig. 3. The proposed model of IbeA-mediated E. coli invasion of human BMEC.(E. coli K1 invasion of human BMEC is mediated through the following major steps. IbeA binds to its receptor on human BMEC. The ligand-receptor interaction (IbeA-IbeABP) results in activation of PI3K, which phosphorylates and activates the downstream enzyme Akt. The phosphorylated Akt (p-Akt) enhances dephosphorylation of phosphorylated cofilin (p-cofilin, inactive form) through the negative control of LIM kinase (LIMK), which is phosphorylated and activated by ROCK. PI3K and ROCK oppositely regulate LIMK. LIMK catalyses phosphorylation of cofilin on serine 3, which prevents its association with actin. Activation of cofilin leads to reorganization of actin cytoskeleton. The biological activity of cofilin is regulated by a circle of phosphorylation and dephosphorylation (phosphocycle). The phosphorylated cofilin is reactivated by dephosphorylation. Cytoskeletal rearrangements eventually promote bacterial entry of human BMEC. The positively and negatively regulated signalings are indicated by different arrows.And Ibe-a can also lead to phosphorylation of VIM and impact the activity of FAK. Besides these two way, Ibe-A can bind both VIM and PSF(PTB-associated splicing factor) in concert to activate TAK1(β-activated kinase), and ultimately activate NF-kB, to arise inflammation in brain.) Positive: Blue arrow; Negative: Red arrow

In order to verify the influence of different protein on invasion of *E.coli* K1, our laboratory has knocked different genes including Omp-A, Ibe-A, CNF-1 and so on, all the knock out operation was done on E.coli K1 strain E44. And then injected them into rats as experimental group. To control groups, we injected un-knocked out E44 stain *E.coli* K1.We compared the importance of different genes on the invasion contribution by comparing the number of mice of CSF bacterial positive between the knockout group and the un-knockout group(Table I). We can figure out that on the similar degree of bacteremia, all the comparation between knock out/un-knock out has statistic value. And among them, Omp-A, IBE-A and CNF-1show the significant variation between two group.

Besides four proteins discussed above, latest experiments has reported some new pathways to stimulate adhesion and invasion to BMECs. HCP-1(Hemolysin-coregulated protein-1) is a upstream site of IQGAP-1(IQ motif containing GTPase activating protein 1), IQGAP-1 was found to stimulate apoptosis of BMECs by stimulating MAPK pathway[14]. The interaction between Omp-A and GP-96 was also found to have ability to downregulate PPAR-γ(Peroxisome proliferator-activated receptor γ) and GLUT-1(Glucose transporter 1), which inhibit BMECs glucose intake and damage barrier integrity, it can also cause the upregulate of IL-6(Interleukin-6)[15][16]. Interleukin(IL-6) and IL-8 maybe have contribute to penetration. Scientists haven't verified the role of IL-6/IL-8

in penetration, but something has been found about it, IL-8 secretion only happen in the BMECs when response to *E.coli* K1, but not in non-brain endothelial cells, and BMECs with *E.coli* K1 binding and invasion didn't affect the release of IL-8[4][5][17]. And nAChR(Alpha 7 nicotinic acetylcholine receptor) was also tested have contribution in causing meningitis by upregulate proinflammatory cytokines IL-6, TNF-a)and adhesion molecules(CD4 and ICAM-1)[1].

TABLE I. THE RELATIONSHIP BETWEEN NUMBER OF NEONATAL RAT WITH MENINGITIS POSITIVE AND NUMBER OF NEONATAL RAT INFECTED WITH E.COLI K1(E44 STRAINS) OR HOMOLOGOUS MUTANTS E44 STRAINS

	The relationship between number of neonatal rat with meningitis positive and number of neonatal rat infected with E.coli K1(E44 strains) or homologous mutants E44 strains		
Type of E44	***Number of rats***	***Degree of Bacteremia (log CFU/ml)***	***Number of CFS positive rats***
E44	19	7.18 ±0. 63	12
-omp A	22	7.05 ±0. 49	6[a]
E44	24	7.51 ±1. 25	16
-Ibe A	25	6.97 ±1. 21	4[a]
E44	27	7.01 ±1. 17	15
-Ibe B	25	7.06 ±1. 29	4[a]
E44	24	7.53 ±0. 40	18
-Ibe C	24	7.80 ±0. 67	10[a]
E44	25	6.06 ±1. 49	10
-cnf 1	28	6.07 ±1. 21	3[a]
E44	17	7.50 ±0. 32	14
-asl A	22	7.60 ±0. 49	7[a]
E44	51	7.22 ±0. 59	34
-traJ	50	7.10 ±0. 44	23[a]

[a.] – means mutuan

[b.] [a] means this figure has stastistiv value

IV. THE ESSENCE OF CROSSING:REARRANGEMENT OF CYTOSKELETON

The pattern of *E.coli* K1 crossing the blood-brain barrier was summarized as "zipper mechanism", it means *E.coli* K1 invade without affecting integrity of the blood brain barrier. When *E.coli* K1 approach to BMECs, BMECs form vesicles and phagocytize *E.coli* K1 into vesicles, the specific mechanism of phagocytosis is receptor mediated. *E.coli* K1 doesn't replicate in vesicles and whole process doesn't affect the monolayer integrity of BMECs and the transmembrane resistance[1][3][4][5].

Previously mentioned proteins as Omp-A, Ibe, Fim-H, CNF-1 and EGFR(Epidermal growth factor receptor) can stimulate or activate downstream signaling molecules like FAK, PIK3, Rho-GTPases by receptor-ligand binding. Eventually leads to the filament rearrangement, altering the cytoskeleton[4] [18].

The first step of rearrangement of filament is to activate FAK, the main ligand for activating FAK is Ibe-A, followed by Omp-A and Fim-H, all of them can activate FAK and lead FAK phosphorylation. After successful phosphorylation, FAK can combined with paxillin to further activate PIK3[4]. If the gene of FAK was knocked, the invasion ability will reduce sharply, which means FAK phosphorylation is a vital step for crossing[5].

PIK3 is one of the downstream signal of FAK, it contributes significantly in the rearrangement of cytoskeleton mediated by *E.coli* K1. PIK3 has two major subunits, one is regulator subunit, the other is catalytic subunit. Regulator can recognize FAK and bind with the complex amino acid residue of FAK to achieve the activation of PIK3. After successful combination and activation, the catalytic subunit of PIK3 will motivate second messengers like PI-3,4,5-P3 (Phosphatidyl inositol-3,4,5-triphosphate), second messengers can induce AKT(PKB, Protein kinase B, serine-threonine kinase), resulting in rearrangement of cytoskeleton[4][5]. Researcher can use inhibitor of PIK3 to reduce the invasive ability of *E.coli* K1[3][5].

Except the signal of FAK/PIK3/ART, SRC family kinase. CNF-1 and Rho GTP can rearrange cytoskeleton by different ways. SRC family kinase affect the movement of filament by affecting cofilin. Recently research showed that EGFR also plays a key role in activating SRC, it revealed that endothelial cells that were bound to *E.coli* K1 were found to produce much more S1P(Sphingosine 1 phosphate), which can bind to S1PRs(S1P receptors), resulting in releasing the EGF-related ligand, EGF-related ligand then binds to the extracellular ligand-binding domain of EGFR, activating its cytoplasmic kinase domain so that it can activate SRC[5][19]. When use PP1 or PP2 (the SRC specific inhibitor), the ability of *E.coli* K1 invasion will reduce, and it also shows a significant does dependent[4].

CNF-1 have signal transduction with two numbers of Rho-GTPases(Rho-A and Cdc-42), it accomplish rearrangement by controlling formation of stress fibers, adhesion plaque, pseudopodium and microvillus of F-actin[5][12][18]. CNF-1 also activate cPLA2(calcium-dependent phospholipase A2) and ezrin, and have some contribution in rearrangement, but the specific mechanism is not distinct[5][8]. In addition, some research illustrated the activation of Rho-GTPases can also enable BMECs to accelerate intake apoptotic bodies or induce uptake of latex beads, it may also contributes to invasion[5][18][19][20].

Details about how cytoskeleton rearrange is not clear. Experiment enunciate that after transfer gene IBE to cell lines-Hela. Cells has the strong ability of stretching out pseudopodium, all pseudopodium tries to get in touch with the substrate, if false, pseudopodia will folds to form folds. Comparing with normal Hela cell, Hela cell with IBE genes has less stress fibers and less F-actin, the ability of stretch and adhesion are improve in Hela cell with IBE. All of this characteristic changes may contribute to rearrangement[3][21]. Current research shows the whole process only appear the change of filament, the number and structure of microtubule are unchanged[2][3][4][5][22].

V. CONCLUSIONS AND DISCUSSION

The whole process and mechanism of *E.coli* K1 pass through the blood-brain barrier is still not totally clear. However, the current study can be used to achieve the initial success in clinical treating or preventing meningitis by

controlling the concentration of bacteria in the blood or blocking the proven receptors and signaling pathways [20][23][24]. In addition, latest research demonstrates that using the blockade of arachidonic acid metabolism can prevents *E.coli* penetration specifically into the brain without affecting the bacteremia level and level of nonbrain organs penetration[2][5][23][25]. So far, many genes can controversial in the role of *E.coli* K1 crossing the blood-brain barrier, and many pathways have not been discovered or confirmed yet[2][23][26]. At the same time, the effect of the intracellular environment changes on *E.coli* K1 crossing the blood-brain barrier is not considered.

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