The Role of *Escherichia coli* in the Development and Progression of Cancer

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Abstract: *Escherichia coli (E. coli) is a widely distributed microorganism in nature and normally colonize the human gastrointestinal tract. E. coli strains are part of the normal gut microbiota. In recent years, some E. coli strains producing the genotoxin colibactin have been associated with development and progression of colon cancer. However, the role of E. coli and their toxins in carcinogenesis is not entirely clear. Thus, some proteins secreted by bacteria may trigger and promote tumor development. In this paper, we focus on reviewing some proteins produced by E. coli, which have been associated with cancer. Also possible mechanisms involved on transformation of epithelial cells into tumor cells, are briefly described.*

Keywords: *Escherichia coli, colon cancer, prostate cancer, pks island.*

Abbreviations: *E. coli = Escherichia coli, PDGF= platelet-derived growth factor, CD= Crohn's disease, AIEC= Adherent-Invasive E. coli, CEACAM6= Carcinoembryonic antigen related cell adhesion molecule 6, CDT= Cytolethal distending toxin, Cif = Cycle inhibiting factor, CNF1= Cytotoxic Necrotizing Factor, EPEC= Enteropathogenic Escherichia coli, EHEC= Enterohemorrhagic Escherichia coli, UPEC= Uropathogenic Escherichia. coli, hly= α-hemolysin, HIF-1α= Hypoxia Inducible Factor-1α, VEGF= Vascular Endothelial Growth Factor, MIC-1= Macrophage Inhibitory Citokine 1, EGFR= Epidermal Growth Factor Receptor, EGF= Epidermal growth factor receptor.*

INTRODUCTION

Escherichia coli (E. coli) is a Gram-negative bacterium, commonly found as normal flora in the intestines of human, and another animal species. In addition, it is one of the most studied microorganisms worldwide.

Phylogenetically it has been classified into 5 groups (A, B1, B2, D, and E) (1). The phylogenetic distribution of these *E. coli* strains change depending of both the region and human population they colonize (2). In recent years, interest on *E. coli* has increased because *E. coli* colonization of the colon has been reported as a risk factor to development colorectal cancer (3).

In this paper, we focus on reviewing the proteins and possible mechanisms, through *E. coli* could promote the development and progression of colon cancer, as well as cancer in genitourinary tract.

Microbiota and Inflammation

Intestinal microbiota including commensal strains of *E. coli* have an important role in the homeostasis of the gastrointestinal tract (4). However, when dysbiosis take place, an imbalance on the normal microbial population into intestinal compartment is produced. Therefore, most beneficial bacteria are damaged, favoring the overgrowth of those pathogenic bacteria (5); altering the physiology and function in intestinal compartment, as well as promoting the development of intestinal diseases such as Crohn's disease and colon cancer. Dysbiosis may be induced by antibiotic exposure, diet and other factors. The local intestinal inflammation is an important factor to trigger dysbiosis (6).

In general, inflammation has been established as a risk factor for the development of several cancers including colon cancer; because different cytokines and chemokines may promote the development and progression of tumors (7). In colon cancer, alterations in cytokines pattern production have been detected (8), with increased levels of IL-8 (9), IL-6 (10), and the platelet-derived growth factor

(PDGF) (11). In addition, high levels of IL-6 and VEGF have been proposed as prognostic markers in colon cancer (10, 12).

On the other hand, chronic inflammation is a source of molecules associated to either DNA induced-damage or inhibiting DNA repair mechanisms (13), mainly by high production of reactive oxygen species (14). Also, the presence of these chronic inflammatory stimuli may turn off the expression of tumor suppressor genes (15, 16) and stimulate the production of angiogenic factors (17, 18).

The origin of chronic inflammation has been associated to bacterial and virus infections, such as *Helicobacter pylori* (19), and papillomavirus (20). Nevertheless, the precise mechanism by which chronic inflammation triggers the development of cancer is not entirely clear; but it has been suggested that inflammation is not sufficient to trigger a cancer event and bacteria are required. In this regard, bacterial agents promote the carcinogenic processes through effector proteins, which induce DNA damage in the host cell (21).

Escherichia coli and Colon Cancer

Some *E. coli* strains have been associated with inflammatory diseases of the gastrointestinal tract such as Crohn's disease (CD), where bacteria colonize the intestinal mucosa promoting an inflammatory process (22) and subsequently may trigger the development of colon cancer. Interestingly, a low percentage of patients with colon cancer, suffered an inflammatory bowel disease earlier. However, it is considered a high-risk factor for cancer development (23).

The interest to investigate *E. coli* in colon cancer began nearly two decades ago, when it was reported the presence of bacteria in colon cancer biopsies (24). Since that discovery, various studies have focused on figure out the role of *E. coli* in development and progression of colon cancer, where the chronic inflammation could be associated with dysbiosis in the gastrointestinal tract, allowing *E. coli* strains overgrowth, and it could lead to alteration of the intestinal epithelial cells (25).

The mainly *E. coli* strains associated with CD and colon cancer are member of the phylogenetic group B2. In this regard, the LF82 *E. coli* strain has been found in biopsies from patients with colon cancer (3). An important characteristic of LF82 strain is the capability to attach and invade the intestinal epithelium cells, and it has been named Adherent-Invasive *E. coli* or AIEC (26). Moreover, it was reported that adherence of AIEC to the intestinal epithelium is through the CEACAM6 receptor (carcinoembryonic antigen related cell adhesion molecule 6) (27), which is over expressed on the intestinal epithelium cells from both Crohn's disease and colon cancer patients (28). Likewise, over expression of CEACAM6 has been associated with intestine inflammation, and this mechanism is dependent of IL-6 production (29). For this reason, it is possibly than inflammatory process not only favor the overgrowth of *E. coli* but also increasing their invasiveness through over expression of CEACAM6 receptor, and this would be a first step for malignant transformation of colon cells, and after of infection the next step would be the alteration of DNA in host cell through of bacterial toxins such as cyclomodulins.

Cyclomodulins and Cancer

The term cyclomodulin was proposed in 2005 by Nougayrede J.P. et al. (30) to describe those bacterial toxins able to regulate the cell cycle by either inducing or promoting the cellular arrest. For several years, some research groups have been focused on studying the role of these toxins, as well as their mechanisms involved in cancer development; because increased production of cyclomodulins by *E. coli* from the phylogenetic group B2*,* have been detected in biopsies from colon cancer patients (3).

The most common cyclomodulins are the following:

1. **Cytolethal Distending toxin or CDT,** produced by several bacteria strains including both *E. coli* and *Salmonella typhi (S. typhi).* An important mechanism triggered by CDT is to block the cell cycle between the G2 and mitosis phases (31).

2. **Cycle Inhibiting Factor or Cif** is produced by pathogenic strains such as Entero Pathogenic *E. coli* (EPEC) and Entero Hemorrhagic *E. coli* (EHEC). Among the multiple effects induced by cif are the nuclear elongation and starting the DNA synthesis in infected cells, independently of cell division (32).

3. **Cytotoxic Necrotizing Factor 1 or CNF1** is produced by Uropathogenic *E. coli* strain (UPEC) and induces the GTPases activation, promoting both gene transcription and cell proliferation (33).

4. **Colibactin** is expressed by *E. coli* strains belonging to the intestinal microbiota and they have been associated with DNA damage (34).

To date, the role of CDT, Cif and CNF1 in development of cancer is not completely clear. Although, recently it has been reported that CDT induces malignant transformation of HCECs epithelial cells (35). On the other hand, colibactin is a ciclomodulin involved in development of colon cancer, because inflammation induces its expression througth *pks* pathogenicity island activation.

pks island and Colibactin

The *pks* pathogenicity island is a genes group highly conserved in the family of Enterobacteriaceae, including *Enterobacter aerogenes, Citrobacter koseri, Klebsiella pneumonia* and *Escherichia coli* (36-39). Interestingly, the *pks* island is present only in the *E. coli* strains from intestinal microbiota and extra-intestinal *E. coli* (ExPEC), but not in *E. coli* pathogenic strains such as EPEC and EHEC (36).

The regulation of *pks island* by inflammation still remains to be elucidated. However, using colitis murine models has allowed to known the role of *pks* island on development of colon cancer (25). In this regard, *E. coli* overgrowth was originated by intestinal dysbiosis and consequently the development of colon cancer in IL-10 -/- knockout (KO) mice after azoxymethane induced inflammation (25). Likewise, lL-10 -/- KO mice infected with *pks* island -/- *E. coli* mutant strain developed colon cancer, but in a lesser extent than lL-10 -/- KO mice infected with *E. coli* WT strain (25). Moreover, progression of gut inflammation modifies the *E. coli.* transcriptome favoring the *pks* island expression (40). All these data suggest that *E. coli* is required to promote the development of cancer, and inflammation would be important to triggering the production of genotoxic proteins by bacteria. The mechanisms by which *E. coli* strains use different proteins to regulate inflammation in the host cells has been reviewed by some researchers (41-44). So far, mechanisms about regulation of *pks* island, as well as colibactin production have not been described entirely, but it could be a bacteria adaptation mechanism to the inflammatory microenvironment.

Thereby, it is possible that synergistic action between both the inflammatory process in intestine and *pks* + *E. coli* strains, such as LF82 are required to induce colon cancer. Recent findings support this idea, because an increased tumor progression in murine xenograft model was observed after infection with *pks* + *E. coli* strains compared with those infected by *pks* - strains (45, 46). Although the mechanism by which *pks* + *E. coli* strains induced tumor progression is not completely clear, it was reported that infection with *pks* + *E. coli* strains produced DNA damage, cell cycle arrest, melanocytosis, aneuploidy and tetraploidy, thus all these effects could be associated with DNA mutations, and accordingly induce cancer (38,47). On the other hand, activation of *pks* island induces miR-20a expression, a microRNA associated with control of senescence in intestinal epithelial cells through downregulation of sumo specific protease 1 protein or SENP-1 (45). The SENP-1 controls the process of cellular senescence via SUMOylation of transcriptor factor p53 (48) (Figure 1).

Although most studies have focused on the complete *pks* island, an important product encoded inside *pks* island is the colibactin toxin; a secondary metabolite of a Non-Ribosomal Peptide Synthase-polyketide synthase (NRPS-PKS) (34). Recently, the colibactin biosynthesis pathway started to be elucidated (49), which highly support the hypothesis that colibactin induce DNA damage. Moreover, it was proposed that colibactin directly alkylates the DNA in the host cell (50). These data indicated that colibactin is essential for genotoxicity and cellular transformation. Interestingly, within the *pks* island genes there is also a gen sequence encoding for CLBs protein, which the main role is colibactin function inhibition (51). Likewise, a recent study suggests that colibactin is not involved on development and maintenance of inflammation. In contrast, the macrophages infiltration into the intestinal epithelium is induced by overgrowth of *pks* + *E. coli* strains, and inflammation dependent on COX-2 activation is established (52). Thus, once established the intestinal inflammation, *E. coli* proliferation is increased and the production of colibactin started after *pks* island activation. Therefore, it possible that above mechanisms would be associated with the development of colon cancer.

Figure1. *Alteration of Intestinal Epithelial Cells Caused by the Inflammatory Process Favor AIEC Proliferation and Malignancies Cell Transformation.*

Under normal bowel conditions commensal *E. coli* colonize intestinal epithelium, there is a low expression of CEACAM6, and there is not activation of *pks* island. Nevertheless, during

inflammation, the intestine dysbiosis favors the growth of AIEC and induces activation of the *pks* island by unknown mechanism. In adddition, the increase of IL-6 due to the inflammatory process induces CEACAM6 expression increasing invasiveness of AIEC. Once AIEC has been internalized in the epithelial cell, colibactin is produced by the bacteria may cause DNA damage. And could be associated with cellular senescence through activation of miR-20a-5p which down-regulate SENP-1, increasing the SUMOylation of p53.
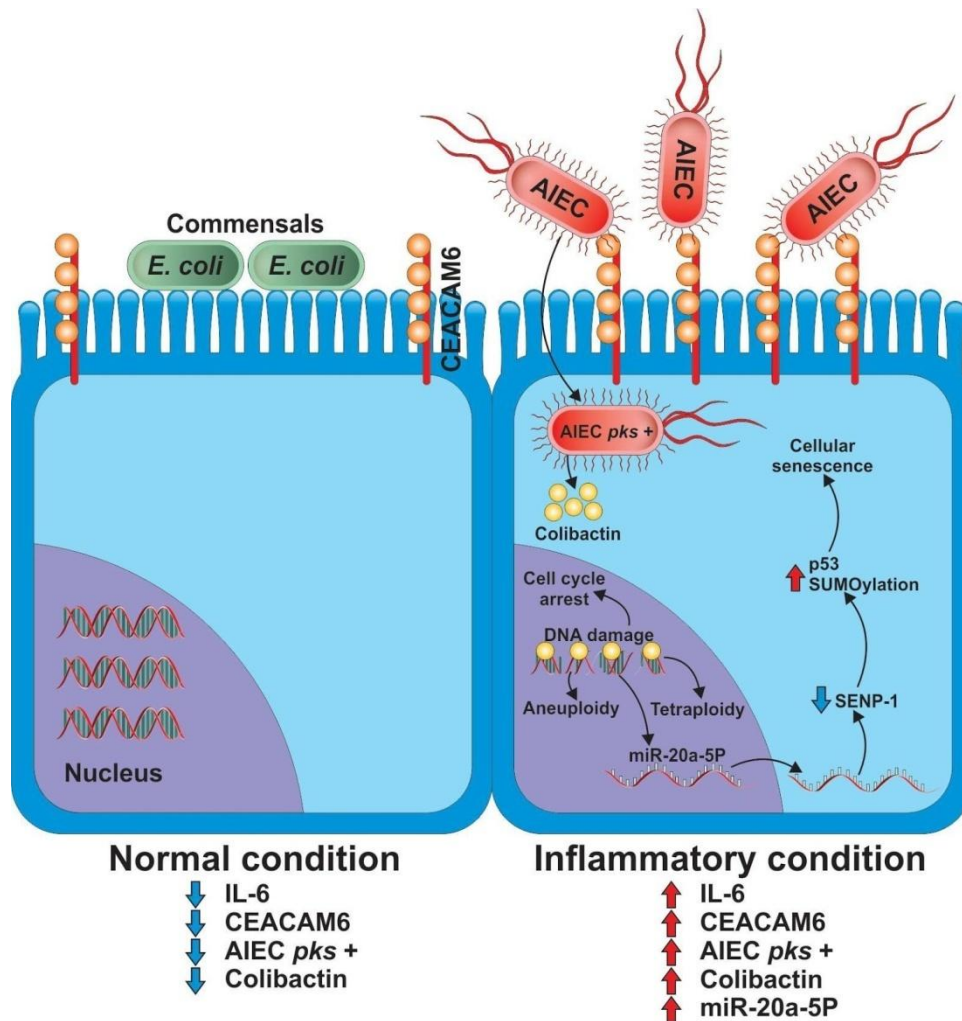

α-hemolysin and Colon Cancer

As mentioned above, different studies have suggested that development and progression of colon cancer are associated mainly to dysbiosis; where most beneficial bacteria are damaged and overgrowth of AIEC is produced; as well as activation of *pks* island and colibactin production. However, some, studies suggest that other *E. coli* pathogenic strains such as EPEC and EHEC are involved (53, 54). Therefore the actions of some other *E. coli* effector proteins could also be involved in the development of colon cancer; such as α-hemolysin (hly), because *E. coli* strains hly + have been detected in samples from colon cancer patients (53). Although the role of hly + *E. coli* strains in colon cancer is unclear, it has been suggested that hly may increase the expression of GLUT 1 receptor (Glucose transporter 1), as well as decrease the expression of tumor suppressor protein BIM, through regulation of transcription factor HIF-1α (Hypoxia Inducible factor 1α) (Figure 2). However, it is not completely clear whether regulation of HIF-1α expression is a direct effect of hly, because *E. coli* LPS in vitro stimulation induced accumulation of HIF-1α in fibroblasts, as well as increased levels of VEGF mRNA (55). In addition, when a colon cancer cell line was infected with LF82 *E. coli* strain, an important increase of both HIF-1α and VEGF was observed (56). Therefore, up-regulation of HIF-1α and VEGF by *E. coli* infection could be an early event in colon carcinogenesis. Moreover, high expression of HIF-1α is induced by hypoxic microenvironment (57, 58). Leading to down regulation of BIM (58). Interestingly, increased expression of HIF-1α induce up-regulation of CEACAM6 receptor; facilitating a secondary infection by *E. coli,* and increasing the *E. coli* survival inside the cell (56).

Figure 2. *Cellular Alteration by Pathogenics Escherichia Coli.*

Infection of the intestinal epithelium by EHEC will participate in cancer progression through effectors such as α -hemolysin, which modified the expression of BIM, GLUT 1, HIF-1α and VEGF, all related to colon cancer. Furthermore, infection by EPEC could be associated with mutations in intestinal cells through down-regulate MLH1 and MSH2 proteins by unknown mechanism. In addition, the effectors EspF and EspZ have been associated with phosphorylation of EGFR, which is related with angiogenesis and cell survival. Additionally, EPEC could favor the process of metastasis through inducing secretion of MIC-1 from infected macrophages and EspF, which disrupt the tight junction proteins favoring the migration of tumor cells.
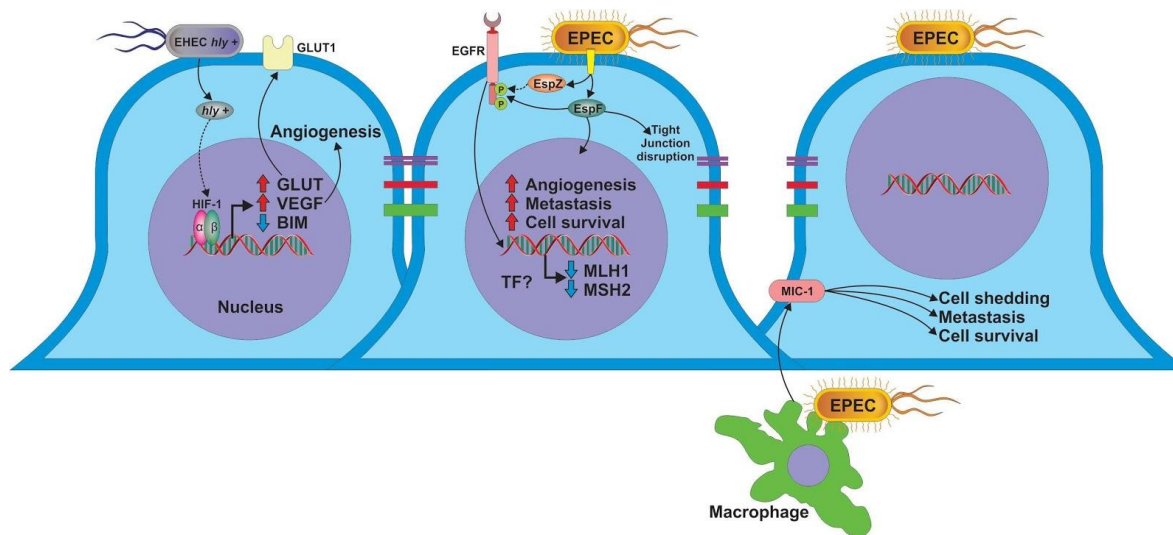
Entero Pathogenic *Escherichia coli* and Colon Cancer

As previously was mentioned, *E. coli* was found in a high percentage of biopsies from colon cancer patients (24) and the bacteria most common found were AIEC strains (3). However, Entero Pathogenic *E. coli* (EPEC) strains have been also detected in biopsies from colon cancer patients (54, 59). Although it is well known that EPEC is the main cause of diarrhea in children (60), the infection in adults is not completely clear. The presence of EPEC in biopsies from colon cancer patients could also be related to this neoplasm; because *in vitro* infection of colon cancer cell lines with EPEC causes depletion of proteins associated with DNA repair (mismatch repair MMR) such as MLH1 and MSH2, and consequently somatic mutations in the intestinal epithelial cells are produced (59). The mechanism by which EPEC infection induces DNA damage is not clear, but it has suggested that EspF protein is involved (61). EspF is an important effector protein expressed by both EPEC and EHEC (62), which is easily internalized by epithelial cell through the Type III Secretion System (63). However, it is unclear whether EspF acts directly on MLH1 and MSH2 or indirectly inducing activation of some transcription factors, which down-regulate MLH1 and MSH2 expression. This hypothesis is supported by previous *in vitro* and *in silico* assays, where some nuclear targets for *E. coli* proteins were described (64, 65). Hence, bacterial proteins could enter to cell nucleus and attach to target genes. This way important changes on host cell genes could be produced, causing DNA mutations and triggering colon carcinogenesis (66).

Additionally, EspF is capable to disrupt the tight junctions proteins in intestinal epithelial cells (67), which could facilitate the release and dissemination of tumor cells, and contributing to metastasis of colon cancer. In addition, EPEC infection increased the expression of macrophage inhibitory citokine 1 (MIC-1), this molecule has been associated with an important increase on survival and spread of colon tumor cells in a GTPase RhoA dependent pathway (68).

Also, EPEC infection induces the phosphorylation of EGFR receptor (69). This cellular mechanism is related to the development of colon cancer (70, 71), and up regulation of EGFR has been correlated with a poor prognosis in colon cancer patients (72). Although the mechanism by which EPEC induces EGFR phosphorylation is not entirely clear, it has been showed that translocation of effectors EspF and EspZ could be associated with EGFR phosphorylation (73). EPEC proteins and their possible mechanisms are summarized in Figure 2.

E. coli and Genitourinary Cancer

Currently, *E. coli* is a bacterium that has acquired relevance in colon cancer research. Nevertheless, *E. coli* not only colonizes the gastrointestinal tract but also genitourinary tract; here bacteria could disrupt the epithelial barrier favoring the development of malignancies such as cancer bladder (74). Moreover, *E. coli* could promote prostate cancer, because has been observed that prostatic secretion of patients with this neoplasm have increased number bacteria, including *E. coli*; similary as it was determined in colon cancer biopsies (75).

Additionally, in murine model of prostate infection with Uropathogenic *E. coli* (UPEC), an acute inflammatory response, epithelial cell proliferation and reactive hyperplasia were produced (76). In addition, the expression of prostate-specific antigens (PSA), the principal markers of development of prostate cancer have been identified and developed (77).

UPEC is the main pathogenic agent associated with urinary tract infections (UTIs) (78). UPEC strains share some features on pathogenicity islands with those *E. coli* strains isolated from patients with Crohn's disease or colon cancer (79). Similarly, than AIEC, UPEC attach to CEACAM receptors to adhere to the epithelial cells. Nevertheless, the molecules of AIEC involved in the interaction with CEACAM have not been identified, meanwhile it is well known that Afa/Dr adhesin expressed on UPEC interact with CEACAM receptor (80).

In addition, it has been reported that UPEC infection induced methylation of the tumor suppressor gene *CDKN2A* in uroepithelial cells (81). This mechanism has been associated with prostate cancer (82). Therefore, all these data suggest a possible role of UPEC in triggering and development of prostate cancer. For this reason, must to be important to study the role of *E. coli* on the development of genitourinary tract carcinogenesis.

CONCLUSIONS

The chronic inflammation is a major risk factor for developing cancer, recently it has been shown that the presence of infectious agents such as pathogenic bacteria, is also an important factor that participate in development of cancer by two ways: 1) contributing to the inflammatory process and 2) simulating the secretion of bacterial proteins, which modify the cellular function. The proliferation of *E. coli* during processes such as intestinal dysbiosis, and chronic inflammation, could contribute to the neoplastic process through the *pks* island activation. The *pks* island is expressed by both commensal *E. coli* strains, as well as pathogenic strains such as AIEC. Nevertheless, the regulation mechanisms of *pks* island still remain to be elucidated. Another important factor that may favor the development of cancer is the effector proteins such as α -hemolysin and EspF produced and secreted by pathogenic strains such as EPEC and EHEC. They are involved in several mechanisms that regulate the development and progression of colon cancer. Moreover, several host proteins such as VEGF, EGFR, BIM and HIF-1 α, are involved in colon cancer, but the precise molecular mechanisms triggered or regulated by *E. coli* proteins is still unknown.

The role of *E. coli* in genitourinary tract carcinogenesis is not entirely clear. Although, UPEC strains are capable to infect bladder and kidney, and they share genes with *E. coli* strains isolated from patients with colon cancer, it is unknown whether these genes are related to trigger and cancer development. Therefore, it will be interesting to study the relation between UPEC and genitourinary carcinogenesis, more studies are needed to establish the role of *E. coli* and its toxins in the development of cancer

Finally, to determine the specific role of *E. coli*, as well as their proteins in cancer development and progression further investigation need to be carrying out.

ACKNOWLEDGEMENTS

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