Cell Lines as In Vitro Model for Studying Microbial Pathogenesis

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Contents

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Abstract

To understand the effect of microorganisms on host cells, in vitro cell line studies in addition to animal studies provide translational importance in human clinical trials. For better understanding the interaction between bacteria and host cells, cultured animal cell lines and cultured human epithelial cells have extensively used for understanding the mechanisms by which bacteria induce human diseases. Cell culture models define mechanisms about the mode of interactions between bacterial virulence factors and host epithelial cell receptors. Predatory bacteria do not produce cytotoxic and inflammatory response in human cells. We described the effect of chronic exposure of *Porphyromonas gingivalis* and *Fusobacterium nucleatum* on human oral cavity squamous cell carcinoma (SCC) cell. We discussed about the bacterial virulence factors interacting with cultured epithelial cells. This may permit research leading to innovative antimicrobial therapies.

Keywords

Cell line · Bacteria · Fungi · Virus · Microbial pathogenesis · Human diseases

19.1 Introduction

Studies of microorganisms in liquid broth or agar plates are well established. This will not address the issue about the effect of microorganism on host cells. Microorganisms grow in their natural habitats effect transcriptomic alterations in host cells (Palková 2004). Biofilm formation by bacteria has been recently focused to study the growth pattern of bacteria (Azeredo et al. 2017). Bacteria primarily invades epithelial mucous membranes of host. The bacteria having invasive

property are evaluated by colony-forming unit (CFU)-enumeration on agar plates (Letourneau et al. 2011). Colonization of bacteria in epithelium of urinary bladder and intestine, and endothelial cell is quantified on the basis of fluorescence signals from green fluorescent protein (GFP) expressed bacteria. This will provide information about virulence property, immunomodulation, and antibacterial activity in the context of progression of infection (Pedersen et al. 2018). Pathogenic bacteria are used to grow on epithelial surfaces of host through irreversible attachment (Ribet and Cossart 2015). Bacteria-epithelial cell adhesion is studied in cultured cell lines at microtiter plate. Effect of bacteria on human cells in in vitro studies in addition to animal studies provides translation importance in human clinical trials. In this chapter, the application of cell line in microbial pathogenesis involved in human diseases was summarized.

19.2 Study the Effect of Shiga Toxin-Producing *Escherichia coli* on Colonization of Intestinal Cell Layers

To study the effect of Shiga toxin-producing *E. coli* O157:H7 (EDL933) on T84 cell line, formaldehyde-fixed T84 cell layers were infected with EDL933. Growth of EDL933 on the T84 cell layers was increased rapidly. STEC strain EDL933 is able to form biofilm on human intestinal epithelial layer effectively (Yu et al. 2014). Fixed cell layers are used as substratum surface for bacterial growth (Lai et al. 2013). Central virulence genes are upregulated by co-culture of STEC strain O157:H7 Sakai on adherent HeLa cells (Alsharif et al. 2015).

19.3 Colonization of *Staphylococcus aureus* on Endothelial Cell Layers

S. aureus ATCC strain 29213 was used as a bloodstream pathogen. To stimulate the opsonization, *S. aureus* ATCC 29213 was treated briefly with human blood plasma. DMEM+FBS was used as a culture medium to study the colonization of *S. aureus* in endothelial cells. Fibronectin in FBS was used for adhesion in *S. aureus* (Claes et al. 2014).

19.4 Test Susceptibility of *Chlamydia trachomatis* Serovar L2 to Antibiotics in Lymphoid Jurkat Cells

C. trachomatis L2 invasively enters lymphatic and sub epithelial layer of genital tract. It grows both in Jurkat and HeLa cells. The growth curve was analyzed by fluorescence staining and electron microscopy. To understand persistent infection of *C. trachomatis* in Jurkat cell and its susceptibility of doxycycline (DOX), azithromycin (AZM), and ofloxacin (OFLX), inclusion forming unit (IFU) assay

was performed. Antibiotics showed effectiveness against *C. trachomatis* L2 growth in both Jurkat cells and HeLa cells. Bacteria showed more sensitivity in Jurkat cells than in HeLa cells. *C. pneumonia* induces infection in lymphocytes through reduced expression of CD3 and CD25 (Kubo et al. 2012; Yamaguchi et al. 2008; Hirai et al. 2010).

19.5 Biofilm Formation by *Candida tropicalis* on Catheter by Using Human Cells

Biofilm is defined as an attached microbial community on biological or inert surfaces. Biofilms are important virulence factor for pathogenicity of *Candida* species such as *C. albicans* and *C. tropicalis*. Biofilms formation by *C. tropicalis* ATCC 750 on blastoconidia composed small catheter fragments (SCF) were investigated to its interaction with human cells (HeLa and HUVEC). *C. tropicalis* reduces virulence property of *C. albicans* through reducing of cell number, metabolic activity, and hyphal growth (Capote-Bonato et al. 2018).

19.6 Effect of *S. aureus* on Human Dendritic Cells

Methicillin-sensitive and methicillin-resistant *S. aureus* kills human monocyte-derived DCs. *S. aureus* produce toxins such as leukocidins that target cells involved in innate immunity (Berends et al. 2019).

19.7 Effect of *C. albicans* and *S. aureus* on Different Types of Endothelial Cell

Endothelial cells are involved in the pathogenesis of infections induced by *C. albicans* and *S. aureus*. To understand the pathogenesis of interaction between microbes and endothelial cell, human umbilical vein endothelial cells (HUVECs) are used in vitro model. HMEC-1 cell line was developed by transfecting of simian virus 40A gene into human microvascular endothelial cells derived from human foreskin. These cell lines are applicable to study the multiple microorganisms such as Chlamydia pneumonia, *Brucella* spp. *Bartonella henselae*, *Mycobacterium tuberculosis*, *Rickettsia rickettsia*, *C. albicans*, and *S. aureus*. Wild-type *C. albicans* showed reduced adherence and invasive property to HMEC-1 cells as compared to HUVECs. Wild-type *S. aureus* showed similar strength of adhesion and invasion to HMEC-1 cells and HUVECs cells. Secretion of IL8 from HUVECs cells becomes higher as compared to HMEC-1 cells in response to both *C. albicans* and *S. aureus* (Seidl et al. 2012).

19.8 In Vitro Human Granuloma Model of Sarcoidosis and Latent Tuberculosis Infection

To understand the pathogenesis of formation of granuloma, an in vitro model of granuloma is formed from human peripheral blood mononuclear cells (PBMCs) of patients with active sarcoidosis and latent tuberculosis (TB) infection (LTBI). Incubation of PBMCs for 7 days was performed in the presence of uncoated polystyrene beads or beads coated with purified protein derivative (PPD) or human serum albumin. PBMCs of sarcoidosis and LTBI showed the formation of granulomas in response to PPD-coated beads and secrete cytokines which are involved in inflammation. This in vitro model provides information about molecular mechanisms and biomarker discovery for sarcoidosis, latent TB infection, and granuloma biology (Crouser et al. 2017).

19.9 In Vitro Study of Adhesion, Transmigration, Invasion of *Campylobacter jejuni*

C. jejuni lives in the gut of birds and domestic animals and it causes gastroenteritis in humans (Dasti et al. 2010). *C. jejuni* interacts with porcine small intestinal epithelial cell lines such as IPEC-1 and IPEC-J2, human cell lines such as HeLa cells, HepG2 cells, chicken hepatocellular carcinoma epithelial cells, and African green monkey kidney cell lines. *C. jejuni* strains NCTC 11168, 81-176, 81116, and F38011 are commonly used in vitro infection studies (Backert and Hofreuter 2013).

19.9.1 Effect of *P. gingivalis on Oral Cancer Cell Lines*

OSCC-derived cell lines such as SCC-25, BHY and human primary oral epithelial cells express B7-homologue receptors such as B7-H1 and B7-DC. B7-H1 receptors and B7-DC receptors are overexpressed on oral cancer cell lines after infection with *P. gingivalis* strains ATCC 33277 or W83. B7-H1 receptor regulates the development of regulatory T cells whereas B7-DC receptor which is expressed mainly on dendritic cells and macrophages is involved in survival of T cell, cytokine production, and proliferation (Groeger et al. 2011). Inaba et al. (2014) examined the effect of *P. gingivalis* ATCC 33277 on metastatic potential on two OSCC cell lines such as SAS cells and Ca9–22 cells. Invasive property was measured by the activation of human matrix metalloproteinase 9 (MMP9) in OSCC cell lines. In SAS cells, *P. gingivalis* ATCC 33277 induced the expression of proMMP9 enzyme and activation of MMP9. Upregulation of proMMP9 in response to *P. gingivalis* occurs through p38, ERK1/2, and NF-kB signalling pathway (Atanasova and Yilmaz 2014) (Fig. 19.1).

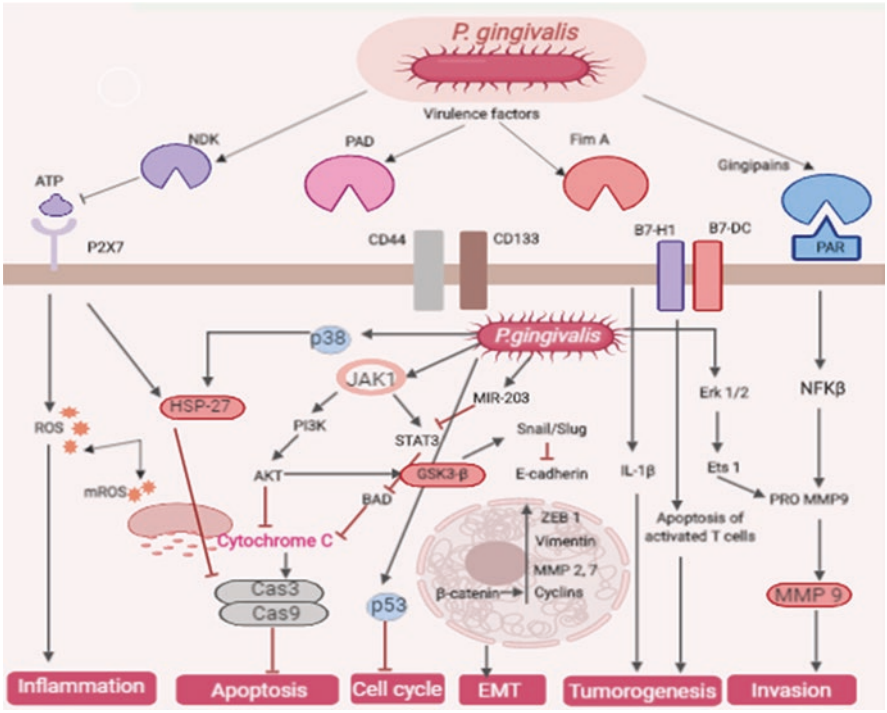

Fig. 19.1 Effect of *Porphyromonas gingivalis* on oral epithelial cell to drive oral cancer

19.9.2 Effect of *P. gingivalis* on Human Primary Oral Epithelial Cells (OEC)

P. gingivalis invades primary OECs in culture thorough β1-integrin (Yilmaz et al. 2008). *P. gingivalis* strain ATCC 33277 inhibits the apoptosis of OEC through depolarization of mitochondrial membrane, prevention of release of cytochrome-c, activation of phosphatidylinositol 3 kinase (PI3K), Akt and surviving and blocking of activation of caspase-3 and 9 (Yao et al. 2010). *P. gingivalis* also inhibits apoptosis of OEC cell through ATP coupled P2X7 signalling pathway (Yilmaz et al. 2008) (Fig. 19.1).

19.9.3 *P. gingivalis* Develop Resistance against Taxol in Oral Cancer Cells

P. gingivalis induces epithelial-mesenchymal-transition and develops resistance property against taxol in OSCC cells through overexpression of CD44 and CD133 (Woo et al. 2017).

19.9.4 Effect of *P. gingivalis* on Tumorigenic Properties of Human Immortalized Oral Epithelial Cells

Infection with *P. gingivalis* to human immortalized oral epithelial cells (HIOECs) for 5–23 weeks enhances proliferation, migration, and invasion of HIOECs. NNMT, FLI1, GAS6, lncRNA CCAT1, PDCD1LG2, and CD274 are differentially expressed in response to long-time exposure of *P. gingivalis*. These genes are considered as a potential biomarker for *P. gingivalis* induced OSCC with chronic periodontal infection (Geng et al. 2017).

19.9.5 Role of *P. gingivalis* in Epithelial-Mesenchyme-Transition of Human Primary Epithelial Cells

Infection of *P. gingivalis* induces EMT (epithelial-mesenchymal-transition) in primary oral epithelial cells (OECs) through overexpression of EMT-associated transcription factors, Slug, Snail, and Zeb1 at mRNA and protein level. It also induces overexpression of vimentin and downregulation of E-cadherin in OECs. Long-term infection of *P. gingivalis* induces the expression of matrix metalloproteinases (MMPs) 2, 7, and 9. Cellular migration of human primary OECs was enhanced in the presence of infection of *P. gingivalis* and *Fusobacterium nucleatum* (Lee et al. 2017). Epithelial-mesenchymal-transition (EMT) involves the loss of cell–cell adhesion in epithelial cells, cellular migration, invasion, and metastasis in cancer (Costa et al. 2015; Heerboth et al. 2015). MMP7, MMPs 2 and 9 are involved in metastasis of OSCCs (Hong et al. 2000) (Fig. 19.1).

19.9.6 Role of *F. nucleatum* Subspecies Animalis in Human Colorectal Tumors

F. nucleatum ssp. *animalis* ATCC 51191 strain was the most prevalent bacteria in human colorectal cancers. To identify the expression of CCL20 protein in *Fusobacterium* infected colorectal tumors, human cell–bacterium co-culture system under normoxic (optimal for human cell growth) and hypoxic (favorable for anaerobic growth of *Fusobacterium*) was established. CCL20 protein was overexpressed in *F. nucleatum* ssp. animalis infected low-passage colorectal cancer cell line HCP1 under normoxic conditions. CCL20 protein was expressed in high-passaged colorectal cancer cell lines (SW480, HT29, HCT116, and RKO) but CCL20 protein was not expressed in a non-cancerous colonic cell line CCD841 infected with *F. nucleatum* ssp. *animalis*. CCL20 protein was also overexpressed in *F. nucleatum* ssp. *animalis* infected THP-1 monocytes. Hypoxic condition enhanced basal level expression of CCL20 protein in HCP1 and THP-1 cells which showed synergistic expression of CCL20 protein due to infection with *F. nucleatum* ssp. *animalis*. Infection of Fusobacterium and hypoxic

microenvironment contribute the overexpression of CCL20 protein in colorectal cancer cells (Ye et al. 2017).

19.10 Effect of *P. gingivalis* on Human Monocyte-Derived Dendritic Cells (DCs)

P. gingivalis invades myeloid DCs through glycoprotein Mfa1 fimbriae. *P. gingivalis* with *Streptococcus gordonii* and *F. nucleatum* induces biofilm formation and invasion of DC cells through the expression of mfa-1. It has been reported that DC of periodontitis patients having abundant load of *F. nucleatum* and *P. gingivalis* showed increased expression of mfa-1 (El-Awady et al. 2019).

19.11 Study of Inflammatory Responses Induced by *Bdellovibrio bacteriovorus* Strains 109J and HD100, and *Micavibrio aeruginosavorus* Strain ARL-13 in Human Cell Lines

Some multi-drug resistant gram-negative bacteria are considered as predatory bacteria because they prey on other gram-negative bacteria. They are cytotoxic to human cell lines. *B. bacteriovorus* belonging to delta-proteobacterium and *M. aeruginosavorus* belonging to an alpha-proteobacterium are considered as predatory bacteria. High doses of these bacteria were challenged to five different human cell lines (HaCaT—human keratinocytes, HepG2—human liver epithelial cells, HK-2—human kidney epithelial cells, MD—loosely adherent human spleen monocytes, and THP-1—human blood monocytes) to study the inflammatory response. *B. bacteriovorus strains* 109J (ATCC® 43826™) and *M. aeruginosavorus* strain ARL-13 were used for the cytotoxicity and cytokine assay (GM-CSF, IFN-γ, IL-10, IL-12p70, IL-1β, IL-2, IL-6, IL-8, and TNF-α) to study the inflammatory response against lipopolysaccharide (LPS) and flagellum of these predatory gram-negative bacteria. *P. aeruginosa* strain PA14 and *E. coli* ATCC strain 43888 (serotype O157:H7) were used as positive controls for these studies. Cell viability was significantly higher for predatory bacterial strain in all five cell lines. *P. aeruginosa* strain PA14 was cytotoxic to all human cell lines. *P. aeruginosa* showed the reduction of cell viability of HaCaT cells, HepG2 cells, and HK-2 cells by 93.5%, 78.5%, and 90.5%, respectively. Levels of IL-1β and IL-6 were elevated in macrophages exposed to *B. bacteriovorus*. Levels of GMCSF, IL-10, and IL-6 were elevated in *M. aeruginosavorus* exposed macrophages. Levels of IL-1β, IL-6, GMCSF, IL-10, IL-12p70, and TNF-α were elevated in predatory bacteria exposed activated macrophages (THP-1). *B. bacteriovorus* and *M. aeruginosavorus* are not responsible for the significant elevation of the cytokines in four of the five human cell lines. Predatory bacteria did not produce cytotoxic effect and inflammatory response in different cell lines (Gupta et al. 2016).

19.12 Effect *Lactobacillus paracasei* on Human Intestinal Caco-2 Cell Line

Cell wall proteins of *L. paracasei* induce programmed cell death of Caco-2 cancer cell line and are considered as a chemotherapeutic agent. *L. paracasei* enhances annexin V and propidium iodide staining in Caco-2 cancer cell line (Nozari et al. 2019).

19.13 Effect of Metabiotic Derived from Probiotic *L. rhamnosus* MD 14 on Caco-2 and HT-29 Human Colon Cancer Cells

Metabiotic of probiotic *L. rhamnosus* MD 14 showed antitumorigenic property in colon cancer Caco-2 and HT-29 cells (Sharma et al. 2019).

19.14 Role of Proteases from Opportunistic Pathogen *Scedosporium aurantiacum* on Human Epithelial Cells

Peptidases of *S. aurantiacum* isolate (strain WM 06.482; CBS 136046) reduce cell viability of A549 human lung epithelial cells (Han et al. 2019).

19.15 Effect of Fungal Pathogen *S. aurantiacum* on Human Lung Epithelial Cells

S. aurantiacum strain WM 06.482 showed adherence property to human lung epithelial A549 cells which induced transcriptomic upregulation of genes involved in cell repair and inflammatory processes. Most of the differentially expressed genes induced by *S. aurantiacum* strain WM 06.482 in human lung epithelial A549 cells are involved in NF-kB pathway that drive the secretion of pro-inflammatory cytokines. *Scedosporium* species are involved in colonization in the lung of cystic fibrosis (CF) patients (Kaur et al. 2019).

19.16 Effect of Conidia of *Aspergillus fumigatus* on Human Bronchial Epithelial Cells

A. fumigatus is responsible for the development of allergic bronchopulmonary aspergillosis, aspergilloma, and invasive aspergillosis. *A. fumigatus* conidia infection induces transcriptomic alterations in human bronchial epithelial cell line (16HBE14o-). Genes involved in DNA repair (glutathione S-transferase) and inflammation (e.g., matrix metalloproteinases and chemokines) are significantly upregulated. Gene set enrichment analysis revealed that genes involved in

chromatin assembly, G-protein-coupled receptor binding, chemokine activity, and glutathione metabolic process are overexpressed (Gomez et al. 2010).

19.17 Effect of *P. aeruginosa* on Alveolar Epithelial Cells to Understand Molecular Pathogenesis of Cystic Fibrosis

P. aeruginosa is responsible to develop cystic fibrosis (CF). *P. aeruginosa* induces genetic alterations in A549 cells. It shows high adherence to A549 cells. It induces reactive oxygen species (ROS) formation, production of pro-inflammatory cytokine and apoptosis in A549 cells (Hawdon et al. 2010).

19.18 Effect of *A. fumigatus* on Lung Epithelial Cells

In vitro cell line model was applied to study the interaction between lung epithelial cells such as BEAS-2B and HBE and *A. fumigatus*. Epithelial cells are incubated with culture filtrate (CF) secreted by mature mycelium of dormant *A. fumigatus* conidia. Adherent *A. fumigatus* conidia undergo internalization into endosomes through polymerization of actin surrounding the endosome. The adherent *A. fumigatus* conidia induce the activation of MyD-dependent NF-kB, PI3 kinase, and MAP kinase signalling pathway (MAPKs ERK1/2, JNK, and p38) that drive secretion of chemokine and cytokine. IL-6, IL-8, TNF-α, GM-CSF, and MCP1 are produced in A549 cells in response to fragments of mycelia (Osherov 2012).

19.19 Effect of Zika Virus Infections in Human Embryonic Kidney (HEK293) Cells

Zika virus (ZIKV) is responsible to develop neurological abnormalities in fetal stage. To understand the molecular pathogenesis in ZIKV-infected neuronal developmental, human embryonic kidney (HEK293) cell line was used. HEK293 cells were infected with ZIKV MR766 strain, PRVABC59 strain (PRV), and FLR strain. American PRV and FLR ZIKV isolates are able to infect human epithelial HEK293 cell lines derived from human embryonic kidney cells. PRV and FLR ZIKV strains induced cytopathic effects and cytolysis of HEK293 cells. ZIKV v-RNA genome is produced in ZIKV-infected HEK293 cell lines. ZIKV-infected HEK293 cells grow slowly. The persistently ZIKV-infected human cell lines will be helpful to study signal transduction pathway in host cell due to infection (Liu et al. 2019).

19.20 Effect of *S. epidermidis* in Human Alveolar Epithelial Cells

S. epidermidis is responsible to develop sepsis and bronchopulmonary dysplasia. To understand the molecular pathogenesis in neonatal inflammatory morbidities, A549 cells were infected by biofilm-positive and biofilm-negative strain of *S. epidermidis* for 24 h with infection ratio of 10. Both *S. epidermidis* strains are responsible to develop pro-inflammatory responses through the overexpression of tumor necrosis factor (TNF)-α, IL-1β, interleukin (IL)-6, IL-8, monocyte chemoattractant protein (MCP)-1, interferon γ-induced protein 10 (IP-10), and intercellular adhesion molecule (ICAM)-1in lung epithelial cells. Biofilm-positive *S. epidermidis* strains are more active to produce pro-inflammation than biofilm-negative strain (Dong et al. 2019).

19.21 Study of Viral Infection in Human Cell Lines

EBV, HTLV-1, HBV, B19V, HHV-6, and HHV-7 viruses were detected in 43 human cell lines. Virus-positive cell lines will be helpful to identify the viral integration site in host cell lines (Shioda et al. 2018).

19.22 Conclusion

The cell line study will provide information that may not be easily accessible clinically. Interactions between fungal pathogen and human lung epithelial cells provide the molecular mechanisms about the hyphal invasion of the human airway epithelial cells. In vitro study will provide molecular mechanisms of adaptive mutations of viruses and effect of persistence infection of viruses on host cells. In vitro study of effect of non-pathogenic predatory bacteria on host cell will provide information that can be helpful to develop live antibiotics or alternative to traditional antibiotics.

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