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Ito, Naofumi
Division of Developmental Neurobiology, Faculty of Life Sciences, Kumamoto University

Ohta, Kunimasa
Division of Developmental Neurobiology, Faculty of Life Sciences, Kumamoto University

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Naofumi Ito and Kunimasa Ohta*

Division of Developmental Neurobiology, Faculty of Life Sciences, Kumamoto University, 1-1-1 Honjo, Chuo-ku, Kumamoto 860-8556, Japan

*Correspondence should be addressed to: Kunimasa Ohta

Phone: (81)-96-373-5293

E-mail: ohta9203@gpo.kumamoto-u.ac.jp

Abstract

In general, it had been believed that the cell fate restriction of terminally differentiated somatic cells was irreversible. In 1952, somatic cell nuclear transfer (SCNT) was introduced to study early embryonic development in frogs. So far, various mammalian species have been successfully cloned using the SCNT technique, though its efficiency is very low. Embryonic stem (ES) cells were the first pluripotent cells to be isolated from an embryo and have a powerful potential to differentiate into more than 260 types of cells. The generation of induced pluripotent stem (iPS) cells was a breakthrough in stem cell research, and the use of these iPS cells has solved problems such as low efficiency and cell fate restriction. These cells have since been utilized for clinical application, disease investigation, and drug selection.

As it is widely accepted that the endosymbiosis of Archaea into eukaryotic ancestors resulted in the generation of eukaryotic cells, we examined whether bacterial infection could alter host cell fate. We previously showed that when human dermal fibroblast (HDF) cells were incorporated with lactic acid bacteria (LAB), the LAB-incorporated HDF cells formed clusters and expressed a subset of common pluripotent markers. Moreover, LAB-incorporated cell clusters could differentiate into cells derived from each of the three germinal layers both *in vivo* and *in vitro*, indicating successful reprogramming of host HDF cells by LAB. In the current review, we introduce the existing examples of cellular reprogramming by bacteria and discuss their nuclear reprogramming mechanisms.

Key Words: Reprogramming, Multipotency, Bacteria, Eukaryotic cells

Introduction

The most undifferentiated cell is the zygote, and this cell is totipotent. While receiving a number of stimuli through the modulation of cell-intrinsic and exogenous factors, fertilized eggs repeat cell division to eventually construct the whole body of an organism. In the case of humans, the cells continue to proliferate and differentiate from the fertilized egg, resulting in a cellular diversity of over 260 distinguishable cell types belonging to various developmental stages (Alberts, 2008). Though cell fate was thought to be restricted during development, the generation of animals by somatic cell nuclear transfer (SCNT) into ova has demonstrated that the epigenome of differentiated cells can be reset to a pluripotent state (Briggs & King, 1952; Gurdon, 1962).

Pluripotent stem cells are undifferentiated cells that have the ability to proliferate for an indefinite period of time, divide to generate daughter cells by self-renewal, and differentiate into a various types of specialized cell to satisfy developmental requirements. Stem cells are categorized into three groups; somatic stem cells, embryonic stem (ES) cells, and the induced pluripotent stem (iPS) cells. In the nervous system, for example, the retinal neural stem cells, a type of somatic cell, can generate not only six types of neuronal cells but also one glial cell, Mullar glia, during the development of the eye (Ohta *et al.*, 2008). Furthermore, ES cells derived from the inner cell mass of preimplantation blastocyst stage embryos can be propagated *in vitro*. Thomson *et al.* (1998) were the first to succeed in establishing human ES cells from human blastocysts *in vitro*. In 2006, Takahashi and Yamanaka first reported the generation of iPS cells in mice by the retroviral transduction of Yamanaka's Factors (*OCT4*, *SOX2*, *Klf4*, and *c-Myc*) and they went on to successfully generate human iPS cells with this breakthrough method (Takahashi & Yamanaka, 2006; Takahashi *et al.*, 2007). Dezawa's group exploited the unique pluripotent cells, multilineage-differentiating stress-enduring (muse) cells, isolated from human fibroblasts or bone marrow stromal cells, that are tolerant to stress conditions including long-term trypsin incubation (Kuroda *et al.*, 2010).

The fetus exists in a sterile state inside the mother's body and is exposed to bacteria for the first time at birth (Aagaard *et al.*, 2014). After birth, and until death, humans and bacteria continuously interact in the gut and/or at the body surface. The human body is protected from pathogens by its immune system, although in total over 500 kinds of bacteria live in the human body without being excluded by the immune system (Kawamoto *et al.*, 2014). Whilst much remains unclear about the influence of microorganisms on human cells, recent studies have provided great discoveries regarding their effects in the fields of immunology, ecology, and

cancer therapy (Kawashima *et al.*, 2013; Collins, 2014; Louis *et al.*, 2014). The results of these studies have shown that human–microbial interactions are not only involved in pathogenesis but also have maintenance effects in homeostasis.

In recent years, the phenomenon of somatic cell reprograming by bacteria has been reported (Fujii *et al.*, 2012; Ohta *et al.*, 2012; Masaki *et al.*, 2013). In this review, we discuss this newly discovered reprogramming technique to generate stem cell-like cells by human cell-bacteria interactions.

Microbial communities

In nature, host-microbial communities exist in all kinds of organisms (Matsuura *et al.*, 2012; Kleiner *et al.*, 2012). When multiple species live in sympatry, their interaction is called as symbiosis. The effects of symbiosis can be classified into groups such as being mutually beneficial (mutualism), benefiting only one organism (commensalism), or benefitting one organism to the detriment of another (antagonism). In the case of mutualism, the bacteria-host interactions are strictly limited by the signal molecule cascade in their specific inducted organs (Kawaguchi & Minamisawa, 2010). Alternatively, host-symbiont relationships have been moderately observed in the metabolite attainment process without the involvement of specific organs (Saito *et al.*, 2008; Behie *et al.*, 2012). In these examples, the endosymbiotic bacteria act like an additional organ and play the role of the host's metabolic system.

In humans, the presence of microbial communities has been identified as being either beneficial or harmful. Studies have been conducted on various parts of the human body in relation to the homeostasis and metabolic relationships occurring with microbial communities (The Human Microbiome Project Consortium, 2012). It has been found that the host-microbe balance may be restricted by factors such as host Body Mass Index and/or age (Costello *et al.*, 2009), and it has also been reported that abnormalities in microbial species in the gut of individuals may be linked with disease pathogenesis (Holmes *et al.*, 2012).

Surprisingly, microbial communities also affect their host's epigenetic status via injected material produced by bacteria. *Shigella flexneri* (*S. flexneri*) is a pathogenic bacterium that causes fever and diarrhea in humans by circulating its toxic proteins in the blood (Ashida *et al.*, 2011). *S. flexneri* infect the host and disseminate through repressing the host innate immunity by injecting the bacterial protein OspF, which in turn involves in interacting with NF-κB-responsive genes (Arbibe *et al.*, 2007). The pathogenic bacteria, *Chlamydia trachomatis* (*C. trachomatis*), also stimulate host epigenetics via their injected protein (Lad *et al.*, 2007; Pennini *et al.*, 2010). *Listeria monocytogenes*, a pathogenic bacteria found in food, also acts by

interacting with human epigenetics (Lebreton *et al.*, 2011). Virulence factor LntA, produced by *L. monocytogenes*, binds chromatin repressor BAHD1 in the host nucleus and activates interferon stimulated genes with chromatin remodeling. The anaerobic commensal class of bacteria, *Clostridia*, also stimulate host cell epigenetic regulation by butyrate secretion (Furusawa *et al.*, 2013).

Cellular reprogramming by *Helicobacter pylori*

Helicobacter pylori (*H. pylori*) is a gram-negative spiral-shaped bacterium that grows in the epithelial cells of the gastric mucosa. To be able to tolerate the acidic environment in the stomach, *H. pylori* creates a habitable environment via neutralization of the gastric acid present locally by utilizing urease to convert urea to ammonia, thus increasing the pH. It is reported that *H. pylori* is associated with the pathogenesis of various gastric problems including ulcers, MALT lymphoma, and cancer (McColl, 2010). *H. pylori* cause host genome methylation accompanied with cancer risk accumulation (Maekita *et al.*, 2006: Schneider *et al.*, 2013).

H. pylori attached to the host cell surface, which has not infected the cells, can cause gastric cancer in the human gut via injection of bacterial products (Hatakeyama & Higashi, 2005). *H. pylori* encodes a major virulence factor, CagA, and also protein secretion apparatus called the Type IV secretion system (T4SS) that works to develop a state of infection. CagA (135kDa) is unique, and no homologue has been found in any other genomic sequence, including in *Mycobacterium* spp. or lactic acid bacteria (LAB). CagA is injected by the T4SS, it then binds to the tyrosine phosphatase, SHP2, and cell polarity protein Par-1, to destroy the polarity of epithelial cells, causing abnormal cell growth (Saadat *et al.*, 2007). CagA injected human cells are at increased risk of cancer due to their development of abnormal intracellular signaling.

Intestinal metaplasia, which transforms stomach cells to intestine-like cells, is one of the signs of gastric cancer (Correa *et al.*, 2010). Recently, it was reported that intestinal metaplasia is caused by CagA activation of caudal-related homeobox 1 (CDX1) transcription factor (Murata-Kamiya *et al.*, 2007). CDX1 induces KLF5 and SALL4 expression (Fujii *et al.*, 2012). KLF5 and SALL4 are involved in the maintenance of pluripotent markers of stem cells (Zhang *et al.*, 2006). *H. pylori* infected gastric epithelial cells generate epithelial–mesenchymal transition (EMT) cells (Bessede *et al.*, 2014), that possess stemness properties (Mani *et al.*, 2008; Nakaya & Sheng, 2014). To summarize this process, it is hypothesized that *H. pylori* induces CDX1 expression, which in turn causes dedifferentiation of epithelial cells, resulting in intestinal metaplasia that converts the gastric epithelial cells into intestine-like cells.

Cell reprogramming by lactic acid bacteria (LAB)

LAB is the generic name for the gram-positive bacteria that produce lactic acid. Since ancient times, LAB has been widely used for the production of fermented foods, and it is deemed safe as it does not produce any harmful substances in humans. LAB are frequently detected in the human gastrointestinal tract and provide metabolic support (Vaughan *et al.*, 2005).

LAB has been empirically awarded the status of GRAS: "generally recognized as safe" (Rodrigues da Cunha *et al.*, 2012). GRAS bacteria are used in a variety of therapeutic strategies (Kruger *et al.*, 2002; Taniguchi *et al.*, 2010). Despite microbial species being stable over a long period of time when established in a host's gut, differences are often detected in the species of flora between human individuals (Schloissnig *et al.*, 2013). LAB in the gut benefits human health by eliminating pathogenic bacteria through the production of antibacterial substances, such as polycyclic peptide antibiotic, nisin, that are harmful to other pathogenic bacteria (Martin *et al.*, 2013).

Numerous studies have provided evidence that pathogenic bacteria activate the host's immune system, stimulate epigenetic regulation, and change the differentiation stage of the cells via secretion of bacterial molecules. Our group conducted an experiment whereby human cells were artificially infected with the non-pathogenic and non-infectious bacterium, LAB, and observed changes in stemness characteristics (Ohta *et al.*, 2012). LAB reprograming takes place in a completely different way to the conventional methods, such those for iPS cell production; although both methods do employ the addition of exogenous materials to host cells (Table 1).

LAB has been successfully incorporated into human dermal fibroblast (HDF) cells by trypsinization and co-cultivation. This effect was achieved without the use of genetic modification or pluripotency inducing chemicals (Figure 1). The LAB-treated HDFs were observed to be clustered like embryoid spheres and had lost their self-renewal ability. In the cells that were clustered, LAB was found in the host cell's internal membrane, as is seen for host organelles. LAB-incorporated cell clusters also expressed a subset of pluripotent stem cell marker genes, such as *NANOG*, *OCT3/4*, and *SOX2*. *HOX* gene expression, which controls the body plan of an embryo, was notably decreased. LAB-incorporated cell clusters could transform into any of the derivatives of the three germ layers *in vivo* and *in vitro*. When these cells were implanted into immune-deficient mice testis, after three months the cells had survived without forming a teratoma. Among these cells, anti-α1-fetoprotein (endoderm),

-neurofilament (ectoderm), or -smooth muscle actin (mesoderm) antibody immunoreactive cells existed, indicating that implanted LAB-incorporated cells had a capacity to differentiate into any of the derivatives of the three germ layers. In an *in vitro* experiment, LAB-incorporated cell clusters could differentiate into cells derived from the three germ layers by culturing with lineage specific culture media. LAB-induced cell clusters did not divide, although differentiated cells proliferated.

LAB possesses no homologue of the *H. pylori cagA* gene, thus acquisition of stemness is induced by LAB via a mechanism differing to that of *H. pylori*-induced intestinal metaplasia. Activation of innate immunity accelerates nuclear reprogramming in pluripotent stem cells (Lee *et al.*, 2012; Hong & Carmichael, 2013). Innate immune activation stimulated by bacterial contact might be pertinent in bacterial reprogramming. Abad *et al.* (2013) reported that *in vivo* reprogramming by iPS cells causes the occurrence of many teratomas in the tissue. LAB-induced cell masses show no self-renewal activity. Self-renewal is an important ability for tissue regeneration; therefore LAB-induced spheres hold promise for the possibility of an *in vivo* cell-based therapy without the risk of teratoma formation.

Further study of the reprogramming process activated by LAB and the comparison of dedifferentiated cells will elucidate new knowledge about the effects of the process as well as mechanisms of action. Finding the LAB-derived reprogramming factor (s) will open a new avenue for the acquisition of pluripotency at the molecular level.

Cell reprogramming by *Mycobacterium leprae*

Mycobacterium leprae (*M. leprae*) is an acid-fast and gram-positive bacteria belonging to the *Mycobacterium* genus, which includes *M. tuberculosis* (Lienhardt *et al.*, 2012). *M. leprae* is an obligate parasite that in nature has only been identified in humans and armadillos and can grow only in infected cells, i.e. it cannot grow in laboratory medium (Cole *et al.*, 2001). *M. leprae* mainly infects macrophages and Schwann cells, and results in the peripheral neuropathy designated as Hansen's disease (Moura *et al.*, 2013). *M. leprae* infects macrophages and/or Schwann cells- nerve-fiber sheath cells of the peripheral nervous system. *M. leprae* cause chronic lesions with inflammation in the peripheral nerves and skin, resulting in sensory impairment, numbness, and muscle paralysis. *M. leprae* is taken up by macrophages through the reorganization of bacterial surface layer PGL-1 (Tabouret *et al.*, 2010). *M. leprae* cell wall proteins, Laminin binding protein 21 (LBP21), and Phenolic glycolipid 1 (PGL-1) bind to Schwann cells (Shimoji *et al.*, 1999).

In the infection dissimilation process, *M. leprae* expand their infection by utilizing the

reprogramming system that transforms infected Schwann cells into stem cell-like cells (Masaki *et al.*, 2013; Masaki *et al.*, 2014). Schwann cells that have been infected with *M. leprae* show suppressed *SOX10*, *MPZ*, and *P75* expression, genes involved in the maintenance of the myelin sheath structure and of homeostasis. Infected Schwann cells were shown to express the mesenchymal stem cell markers: *CD73*, *CD44*, *Sca-1*, and *CD29*, and also highly expressed EMT master regulator genes such as Twist/Snail. These results suggest that the loss of the conventional properties of Schwann cells transformed these characteristics into those of mesenchymal stem cells. In addition, cells that were transformed into mesenchymal-like stem cells differentiated into muscle cells after relocation to smooth muscle and skeletal muscle. *M. leprae* expand their infection by using a system that transforms Schwann cells into stem cell-like cells via a reprogramming mechanism. *M. leprae* infected cells also demonstrate epigenetic regulation by binding with host ErbB2 receptor tyrosine kinase and extracellular bacteria (Tapinos *et al.*, 2006).

Conclusions

The bacteria that facilitate cellular reprogramming share no phylogenetic or phenotypical commonality such as the presence or absence of pathogenic nature or gram positive or negative status. It is suggested that cell reprogramming by bacteria is a general aspect of human-microbe interaction. The types of bacterial reprogramming are summarized in Figure 2.

It is considered that dedifferentiated human cells have a distinct state dependent on the individual method of reprogramming (Gafni *et al.*, 2013; Takashima *et al.*, 2014). In human reprogrammed cells, naïve state cells are well-dedifferentiated cells compared to primed state cells, and naïve state cells are capable of differentiating into a variety of cell types. Cells reprogrammed by bacteria cannot be classified into any of these states because of their pluripotency marker expression complexity (Tanabe, 2013). This means that if one naïve state-maintenance gene undergoes change due to, for example, mutation or environmental stress conditions, the enduring gene expression in the cell can support this loss of gene expression, counterbalancing the event, in order to protect against collapse of the reprogramming state. Further analysis in needed to reveal the hallmarks of epigenetic regulation and to achieve elucidation of the mechanics and effects of the bacterial reprogramming process.

Bacterial reprogramming describes one mechanism for inducing cellular reprogramming that categorically differs from well-known reprograming methods such as

those for ES cell or iPS cell states. The bacterial reprogramming phenomenon is one of the recently discovered aspects of human-microbe interaction. Interestingly, bacteria can have a therapeutic application and can be used as a vector to transfer therapeutic gene sequences into the target cells of intestinal tissues, by providing them with a plasmid carrying reprogramming genes encoding pluripotency factors (Wagnerova & Gardlik, 2013). The mechanisms and/or signaling that are involved in cell reprogramming by bacteria remain unclear. After several comparisons and much debate, bacterial cell-reprogramming has been established as a mechanism through which we can better understand human-microbial interaction and somatic cell lineage reprograming.

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Figure Legends

Figure 1. Schematic drawing of the *in vitro* cell cluster formation by lactic acid bacteria (LAB). Human dermal fibroblast (HDF) cells in confluent states are repelled by trypsin and dissociated into single cells. LAB in logarithmic phase of cell culture is collected by centrifugation and mixed with HDF cells. LAB-incorporated cell clusters are able to differentiate into other cell types in appropriate differentiation medium. Note that cell clusters were never formed by the addition of LAB into the culture dish directly.

Figure 2. Proposed models of bacterial reprogramming. Bacteria inject their own protein or invade into the host cells. In the intracellular area, bacteria down-regulate the gene expression of the host cells and up-regulate their stemness related genes and/or genes which have different lineage. Arrows indicate the defined processes and dashed arrows indicate ambiguous processes during the bacterial reprogramming process.