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Ellagic acid and its fermentative derivative urolithin A show reverse effects on the gp91-phox gene expression, resulting in opposite alterations in all-*trans* retinoic acid-induced superoxide generating activity of U937 cells

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ABSTRACT

Ellagitannins (esters composed of glucose and ellagic acid) are hydrolyzed to generate ellagic acid in gut followed by conversion of ellagic acid to urolithins such as urolithin A by intestinal bacteria. Since urolithins are absorbed by gut easier than ellagitannins and ellagic acid, and show various physiological activities (e.g. anti-cancer, anti-cardiovascular disease, anti-diabetes mellitus, anti-obesity and anti-Alzheimer disease activities), they are expected as excellent health-promoting phytochemicals. Here, using human monoblast U937 cells, we investigated the effect of ellagic acid and urolithin A on the superoxide anion (O_2^-)-generating system of phagocytes, which is consisted of five specific protein factors (membrane proteins: p22-phox and gp91-phox, cytosolic proteins: p40-phox, p47-phox and p67-phox). Twenty micromolar of urolithin A enhanced the all-*trans* retinoic acid (ATRA)-induced O_2^--generating activity (to ~175%) while 20 μM ellagic acid inhibited the ATRA-induced O_2^--generating activity (to ~70%). Semiquantitative RT-PCR showed that transcription level of gp91-phox was certainly decreased (to ~70%) in ATRA plus ellagic acid-treated cells, while that of gp91-phox was significantly increased (to ~160%) in ATRA plus urolithin A-treated cells. Chromatin immunoprecipitation assay suggested that urolithin A enhanced acetylations of Lys-9 residues of histone H3 within chromatin surrounding the promoter region of gp91-phox gene, but ellagic acid suppressed the acetylations. Immunoblotting also revealed that ATRA plus urolithin A-treatment up-regulated protein levels of p22-phox (to ~160%) and gp91-phox (to ~170%) although ATRA plus ellagic acid-treatment down-regulated protein levels of p22-phox (to ~70%) and gp91-phox (to ~60%). These results suggested that conversion of ellagic acid to urolithin A in gut may bring about reverse effects on the gp91-phox gene expression, resulting in opposite alterations in O_2^--generating activity of intestinal macrophages.

1. Introduction

Ellagic acid (2,3,7,8-tetrahydroxy [1]-benzopyrano [5,4,3-cde] [1] benzopyran-5, 10-dione) exists in various vegetables and fruits (e.g. raspberry, strawberry, walnuts, pomegranate *etc.*) as ellagitannins that are esters composed of glucose and ellagic acid [1]. After hydrolysis reactions of ellagitannins, ellagic acid is generated. Furthermore, urolithins (dibenzo [*b*,*d*]pyran-6-one derivatives) such as urolithin A with anti-oxidant and anti-inflammatory effects are synthesized by the microbial conversion of ellagic acid and residual ellagitannins in human gut [1-9]. Urolithins are absorbed by gut easier than ellagitannins and ellagic acid, and exert various physiological activities: anti-cancer, anti-cardiovascular disease, anti-diabetes mellitus, anti-obesity, anti--Alzheimer disease and so on [2-8]. Even at physiological concentration, urolithins can show effects on various chronic degenerative diseases [2]. Therefore, urolithins and their related compounds are expected as health-promoting phytochemicals. However, molecular mechanisms of physiological functions of urolithins remain unclear.

Here, we investigated the effect of ellagic acid and urolithin A on the superoxide anion (O_2^-)-generating system of phagocytes (e.g. neutrophil

Abbreviations: ATRA, all-*trans* retinoic acid; ChIP, chromatin immunoprecipitation; H3K9, Lys-9 residues of histone H3; H3K14, Lys-14 residues of histone H3; O_2^-, superoxide anion; PMA, phorbol 12-myristate 13-acetate.

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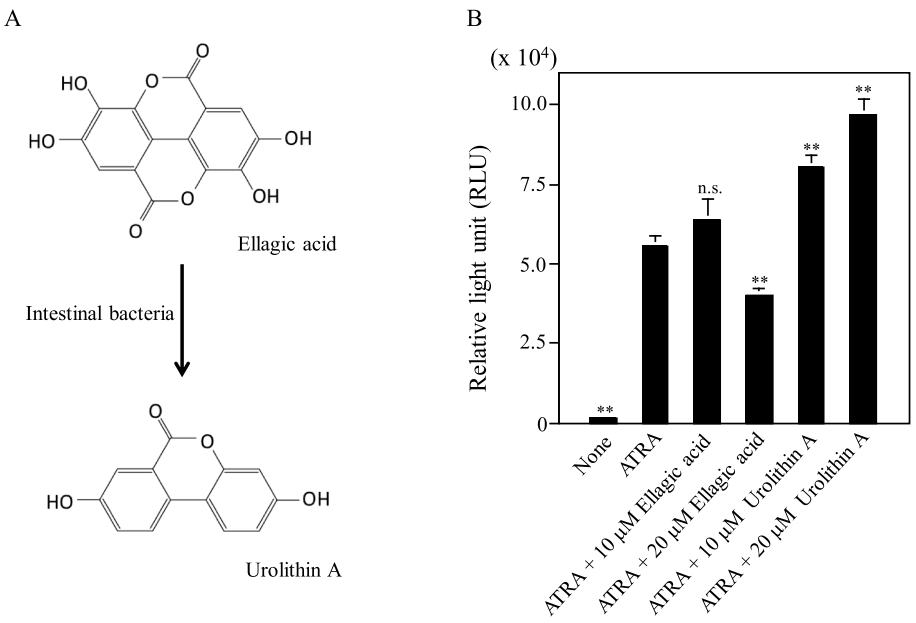

Fig. 1. Influences of ellagic acid and urolithin A on the ATRA-induced O_2^--generating activity in U937 cells. (A) Conversion scheme of ellagic acid to urolithin A by intestinal bacteria. (B) Influences of ATRA plus ellagic acid or urolithin A on induction of the O_2^--generating activity. O_2^- generation was determined after culture of the cells at 37 °C for 48 h in the absence (None) or presence of each agent (ATRA, ATRA plus ellagic acid or ATRA plus urolithin A) as in "Materials and methods." Cells (1×10^6 cells/ml) were stimulated with 200 ng/ml PMA at 37 °C. PMA-induced chemiluminescences were measured at 10 min after stimulation. Data represent the averages of three separate experiments and error bars indicate standard deviation. **, $P < 0.01$ compared with the data of ATRA-treated cells; n. s., not significant.

and macrophage) for killing the invading bacteria [10]. This system is consisted of five specific protein factors (membrane proteins p22-phox and gp91-phox, cytosolic proteins p40-phox, p47-phox and p67-phox), and small G-protein Rac [11]. Cytochrome b_{558}, a heme-binding membrane protein, is a heterodimer protein formed by p22-phox and gp91-phox proteins, and mediates the final steps of electron transfer to molecular oxygen resulting in the O_2^- generation. In a response to infectious stimuli, phagocytes release O_2^- outside of the cells or inside of phagosomes where various stronger reactive oxygen species are formed from O_2^- step by step. These reactive oxygen species are used to kill the invading bacteria. U937, a human monoblastic leukemia cell line, has been used as an excellent model to study mechanisms of leukocyte differentiation [12-14]. Although U937 cells lack the O_2^--generating activity, they are differentiated to macrophage-like cells and produce O_2^- by various differentiation-inducing agents such as interferon-γ and all-*trans* retinoic acid (ATRA) [12-14]. Of course, gene expressions of the five specific protein factors of the O_2^--generating system are remarkably up-regulated by ATRA during differentiation [15-18]. Moreover, various phytochemicals can affect gene expressions of the five specific protein factors of the O_2^--generating system, resulting in alteration of its activity. For example, in recent year, we reported that curcumin [15], resveratrol [16], chalcone [17], hydroxychalcones [17] and sulforaphane [18] regulated the ATRA-induced O_2^--generating activity via controlling gene expressions of these five specific protein factors in U937 cells. Our data in this study suggested that conversion of ellagic acid to urolithin A in gut may bring about reverse effects on the gp91-phox gene expression followed by opposite alterations in the O_2^--generating activity of intestinal macrophages.

2. Materials and methods

2.1. Materials

Ellagic acid, PMSF (FUJIFILM Wako, Osaka, Japan), urolithin A (Cayman Chemical, Ann Arbor, MI, USA), phorbol 12-myristate 13-acetate (PMA), ATRA, luminol (Sigma, St Louis, MO, USA), plasmocin (InvivoGene, CA, USA) and Diogenes (National Diagnostics, Atlanta, GA, USA) were purchased. Monoclonal anti-gp91-phox antibody, monoclonal anti-p47-phox antibody (BD Biosciences, San Jose, CA, USA), monoclonal anti-p67-phox antibody (Santa Cruz Biotechnology, Santa Cruz, CA, USA), anti-p40-phox antibody (GeneTex, Irvine, CA, USA), monoclonal anti-β-actin antibody, monoclonal anti-Na^+/K^+-ATPase antibody (Abcam, Cambridge, UK), and horseradish peroxidase-conjugated anti-mouse or anti-rabbit immunoglobulin (Promega, Madison, WI, USA) were obtained. Monoclonal anti-human p22-phox antibody (449) was kindly provided by Dr. Roos and Dr. Verhoeven (Sanquin Research, and Landsteiner Laboratory, Academic Medical Centre, University of Amsterdam, The Netherlands).

2.2. ATRA-induced monocytic differentiation of U937 cells

Human monoblastic leukemia cell line, U937 cells (RCB0435) were provided by the RIKEN BRC through the National Bio-Resource Project of the MEXT, Japan [16]. Cells were grown in RPMI-1640 culture medium containing 10% fetal bovine serum and 5 μg/mL plasmocin [16-18]. Cells (2.0×10^6) in 5 ml of the culture medium were incubated with 1 μM ATRA in the absence or presence (10 μM or 20 μM) of ellagic acid or urolithin A at 37 °C for 48 h.

2.3. Measurement of O_2^- generation

Measurement of O_2^- generation was carried out by Lumat^3 LB9508 luminometer (Berthold Technologies, Bad Wildbad, Germany) using Diogenes-luminol chemiluminescence probes as described previously [16-18].

2.4. Semiquantitative RT-PCR

Cells (2.0×10^6) in 5 ml of the culture medium were incubated with 1 μM ATRA in the absence or presence of 20 μM ellagic acid or 20 μM urolithin A at 37 °C for 48 h. Semiquantitative RT-PCR was performed using specific sense and antisense primers of five human genes essential for the O_2^--generation system (p22-phox, gp91-phox, p40-phox, p47-phox and p67-phox) as described previously [14-18]. Human GAPDH gene was used as internal controls. Semiquantitative RT-PCR data were obtained using a luminescent image analyzer STAGE-5100 (AMZ System Science, Osaka, Japan), analyzed by Quant-AMZ software (TotalLab., Newcastle upon Tyne, UK) as described [16-18].

2.5. Chromatin immunoprecipitation (ChIP) assay

Cells (2.0×10^6) in 5 ml of the culture medium were incubated with

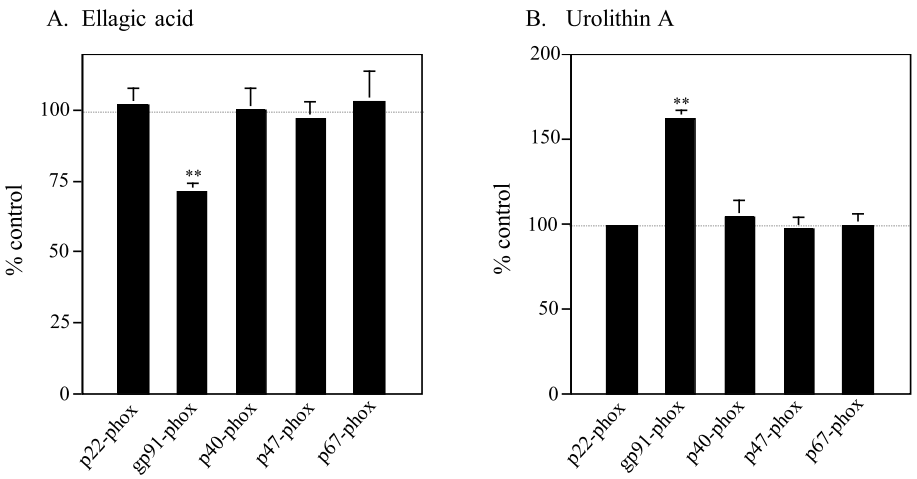

Fig. 2. Influences of co-treatment with ATRA and ellagic acid or urolithin A on transcription of the O_2^--generating system-related genes. Total RNAs were extracted from ATRA-treated, ATRA plus ellagic acid-treated (A), and ATRA plus urolithin A-treated (B) U937 cells, and mRNA levels of p22-phox, gp91-phox, p40-phox, p47-phox and p67-phox were determined by semiquantitative RT-PCR as in "Materials and methods." Data calibrated with the internal controls are indicated as percentages of control values (100%) obtained from ATRA-treated U937 cells and represent the averages of three separate experiments. Error bars indicate standard deviation. **, $P < 0.01$ compared with the data of ATRA-treated cells.

1 μM ATRA in the absence or presence of 20 μM ellagic acid or 20 μM urolithin A at 37 °C for 48 h. ChIP assay was carried out using a ChIP assay kit (Merck, Darmstadt, Germany) according to the instruction manual as described previously [16,17]. To confirm the presence of human gp91-phox promoter sequences containing critical *cis*-element (and also Hox/Pbx consensus-like *cis*-element) and inverted PU.1 binding site [14,19-21], the immunoprecipitated DNA samples were analyzed by PCR technique. PCRs were performed at 96 °C for 20 s, 55 °C for 30 s and 72 °C for 30 s, for 36–40 cycles, using forward primer (5′-TCAGTTGACCAATGATTATTAGCCAATT-3′) and reverse primer (5′-CTATGCTTCTTCTTCCAATGACCAAAT-3′). PCRs were stopped before reaching the plateau. PCR products were analyzed using a luminescent image analyzer STAGE-5100 with Quant-AMZ software as described [16,17].

2.6. Immunoblotting

Immunoblotting was performed as described previously [14-18]. In brief, cells (2.0×10^6) in 5 ml of the culture medium were incubated with 1 μM ATRA in the absence or presence of 20 μM ellagic acid or 20 μM urolithin A at 37 °C for 48 h. Cells (5×10^6) were sonicated in 100 μl of 50 mM Tris-HCl buffer, pH 7.5, containing 0.25 M sucrose, 2 mM EDTA and 1 mM PMSF. Disrupted cells were divided into cytosolic fractions and membrane fractions by centrifugation. These protein samples were subjected to SDS-PAGE followed by immunoblotting. Data analyses were carried out using a luminescent image analyzer STAGE-5100. Human β-actin (for cytosolic fractions) and Na^+/K^+-ATPase (for membrane fractions) were used as controls [17,18].

2.7. Statistical analysis

Quantitative data are presented as averages of three separate experiments. Error bars indicate standard deviation. Statistical differences were calculated with Student's *t*-test.

3. Results and discussion

3.1. Urolithin A significantly enhances the ATRA-induced O_2^--generating activity while ellagic acid inhibits the ATRA-induced O_2^--generating activity

As well known phenomenon, ellagic acid is converted to urolithin A by intestinal bacteria and schematic is represented in Fig. 1A. To investigate the physiological functions of ellagic acid and urolithin A in monocytic differentiation, first, we examined the influences of both of them, on the ATRA-induced O_2^--generating activity in U937 cells (Fig. 1B). As with our previous reports [15-18], ATRA-treated U937 cells showed remarkable O_2^--generating activity by PMA treatment while untreated U937 cells only generated a negligible level of O_2^- by PMA treatment. Although 10 μM of ellagic acid showed no effect on the ATRA-induced O_2^--generating activity, 20 μM of ellagic acid significantly down-regulated the activity (to ~70%). In contrast, very interestingly, urolithin A could enhance the O_2^--generating activity in a dose-dependent manner (10 μM: to ~150%, 20 μM: to ~175%). On the other hand, both ellagic acid and urolithin A showed little effect on the O_2^--generating activity in the absence of ATRA (Supplementary Fig. 1). These results revealed that ellagic acid and urolithin A would play opposite roles in the regulation of the ATRA-induced O_2^--generating activity in U937 cells.

3.2. Opposite effects of ellagic acid and urolithin A on transcription levels of the gp91-phox genes essential for the O_2^--generating system during ATRA-induced monocytic differentiation

As shown in Fig. 1B, urolithin A significantly up-regulated the ATRA-induced O_2^--generating activity while ellagic acid definitely down-regulated the activity in U937 cells. These findings suggested that ellagic acid and urolithin A may affect transcription of five genes of the essential components (p22-phox, gp91-phox, p40-phox, p47-phox and p67-phox) for the O_2^--generation in the presence of ATRA. To confirm the effects of ellagic acid and urolithin A on transcription of these five genes in the presence of ATRA, we carried out semiquantitative RT-PCR and compared transcription levels of these five genes in ATRA plus ellagic acid or urolithin A-treated cells with those in ATRA-treated cells (Fig. 2). Total RNAs were prepared from ATRA-treated, ATRA plus ellagic acid-treated and ATRA plus urolithin A-treated U937 cells. Quantitative data of semiquantitative RT-PCR were indicated as percentages of control values obtained from ATRA-treated U937 cells. In ATRA plus ellagic acid-treated U937 cells, just transcription level of gp91-phox was certainly decreased (to ~70%) (Fig. 2A). In contrast, as expected, only transcription level of gp91-phox was significantly increased (to ~160%) in ATRA plus urolithin A-treated U937 cells (Fig. 2B). These results suggested that such reverse effects of ellagic acid and urolithin A on transcription of gp91-phox gene in the presence of ATRA resulted in their opposite influences on the ATRA-induced O_2^--generating activity (see Fig. 1B).

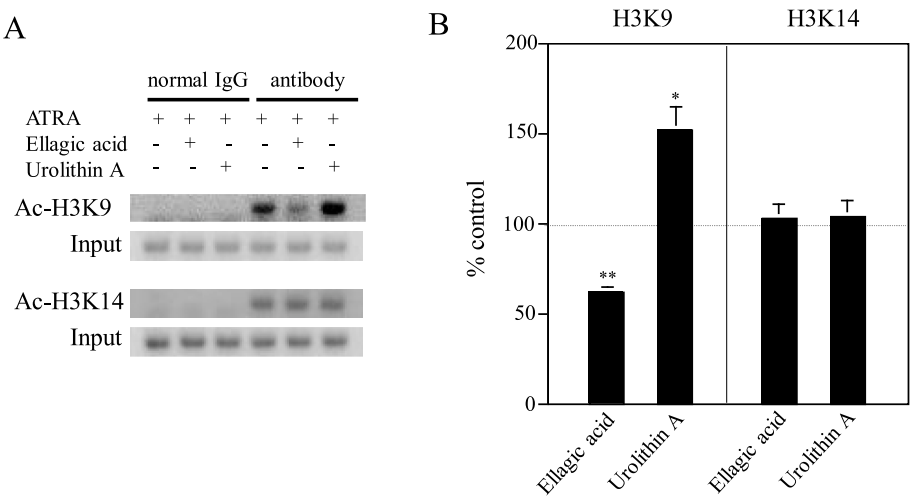

Fig. 3. Influences of co-treatment with ATRA and ellagic acid or urolithin A on acetylation levels of H3K9 and H3K14 residues within chromatin surrounding the promoter regions of gp91-phox gene. (A) Typical patterns of PCR. ChIP assay was performed as in "Materials and Methods". PCR products were subjected to 2% agarose gel electrophoresis and analyzed using a luminescent image analyzer as described [16,17]. (B) Quantitative analysis. Cross-linked chromatins of ATRA-treated, ATRA plus ellagic acid-treated and ATRA plus urolithin A-treated U937 cells were co-precipitated by antibodies specific for acetylated H3K9 and H3K14 residues. PCRs were performed as in "Materials and methods." Data are indicated as percentages of control values (100%) obtained from ATRA-treated U937 cells and represent the averages of three separate experiments. Error bars indicate standard deviation. *, $P < 0.05$; **, $P < 0.01$ compared with the data of ATRA-treated cells.

3.3. Urolithin A enhanced acetylations of Lys-9 residues of histone H3 within chromatin surrounding the promoter region of gp91-phox gene, but ellagic acid suppressed these acetylations

As mentioned above, our findings suggested that ellagic acid suppresses transcription of gp91-phox and urolithin A enhances it. To determine whether or not ellagic acid and urolithin A participate in acetylations of histone H3 within chromatin surrounding the promoter region of gp91-phox gene, we carried out ChIP assay using anti-acetylated Lys-9 and Lys-14 residues of histone H3 (H3K9 and H3K14) antibodies (Fig. 3). These two Lys residues of histone H3 are the typical acetylation sites that are involved in transcriptional activation [22-24]. As shown in our previous reports, ATRA remarkably accelerated acetylation levels of H3K9 residues within chromatin surrounding the promoter region of gp91-phox gene, but not H3K14 residues [16,17]. On the other hand, acetylation levels of H3K14 residues were enhanced by resveratrol and butein in the presence of ATRA [16,17]. In this study, we compared ATRA plus ellagic acid- or urolithin A-treated U937 cells with ATRA-treated U937 cells. Typical electrophoresis patterns are shown in Fig. 3A. As expected, ellagic acid caused remarkable down-regulation of acetylation levels of H3K9 residues within chromatin surrounding the promoter region of gp91-phox gene (to ~60% of control value obtained from ATRA-treated U937 cells) while urolithin A brought about significant up-regulation of them (to ~150% of control value obtained from ATRA-treated U937 cells) (Fig. 3B). In contrast, both ellagic acid and urolithin A did not affect acetylation levels of H3K14 residues within chromatin surrounding the promoter region of gp91-phox gene (Fig. 3B). These data revealed that ellagic acid down-regulates the ATRA-induced O_2^--generating activity via suppressing acetylations of H3K9 residues within chromatin around the promoter regions of gp91-phox gene while urolithin An up-regulates the ATRA-induced O_2^--generating activity through enhancing them. In other words, ellagic acid and urolithin A may regulate the ATRA-induced O_2^--generating activity in opposite epigenetic manners. Further studies are required for exploring what kinds of histone acetylation-related enzymes participate in acetylations of H3K9 residues within chromatin around the promoter regions of gp91-phox gene, and how these two phytochemicals are involved in the histone acetylations.

3.4. Opposite effects of ellagic acid and urolithin A on protein levels of cytochrome b_{558} composed of p22-phox and gp91-phox proteins during ATRA-induced monocytic differentiation

To investigate the effects of ellagic acid and urolithin A on amounts of five proteins (p22-phox, gp91-phox, p40-phox, p47-phox and p67-phox proteins) essential for the O_2^--generation in phagocytes, immunoblotting was performed for these proteins using antibody specific for each protein. Quantitative data obtained from ATRA plus ellagic acid- or urolithin A-treated U937 cells were indicated as percentages of control values obtained from ATRA-treated U937 cells (Fig. 4). Both ellagic acid and urolithin A showed insignificant effect on protein level of three cytosolic factors (p40-phox, p47-phox and p67-phox proteins) as with amounts of their mRNA (see Fig. 2). On the other hand, ATRA plus ellagic acid-treatment down-regulated protein levels of p22-phox (to

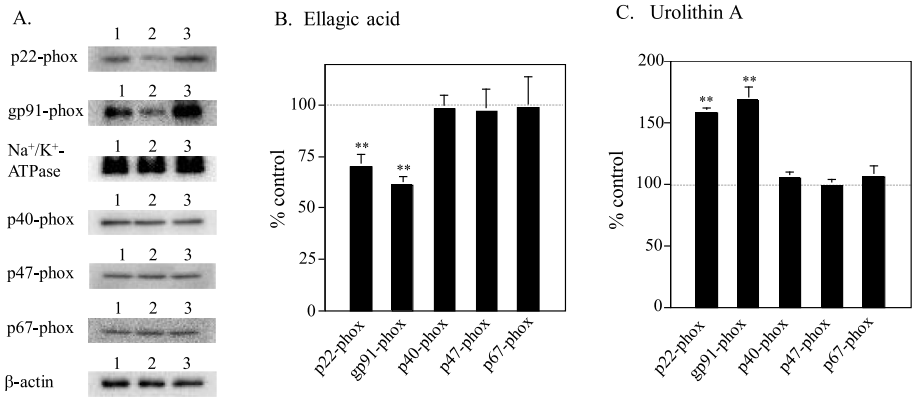

Fig. 4. Influences of co-treatment with ATRA and ellagic acid or urolithin A on protein levels of the O_2^--generating system-related factors. (A) Typical immunoblot profiles. Cytosolic (for p40-phox, p47-phox and p67-phox) and membrane (for p22-phox and gp91-phox) fractions were prepared from ATRA-treated (lane 1), ATRA plus ellagic acid-treated (lane 2) and ATRA plus urolithin A-treated (lane 3) U937 cells, and protein levels were determined by immunoblotting as in "Materials and methods." Human β-actin (for cytosolic fractions) and Na^+/K^+-ATPase (for membrane fractions) were used as controls. Quantitative data of ATRA plus ellagic acid-treated (B) and ATRA plus urolithin A-treated (C) U937 cells are indicated as percentages of control values (100%) obtained from ATRA-treated U937 cells, and represent the averages of three separate experiments. Error bars indicate standard deviation. **, $P < 0.01$ compared with the data of ATRA-treated cells.

~70%) and gp91-phox (to ~60%) (Fig. 4A). In contrast, as expected, ATRA plus urolithin A-treatment up-regulated protein levels of p22-phox (to ~160%) and gp91-phox (to ~170%) (Fig. 4B). Expression of gp91-phox protein limits the O_2^--generating activity in U937 cells [13]. It is thought that the amount of p22-phox protein also was increased according to enhancement of gp91-phox protein since cytochrome b_{558} is a heterodimer protein composed of gp91-phox and p22-phox proteins. These findings suggested that protein levels of cytochrome b_{558} would reflect the changes of amount of gp91-phox mRNA caused by ellagic acid or urolithin A in the presence of ATRA, resulting in their opposite influences on the ATRA-induced O_2^--generating activity (see Fig. 1B).

3.5. Concluding remarks

Both ellagic acid and urolithin A influenced protein level of only cytochrome b_{558} composed of p22-phox and gp91-phox among the four essential proteins (cytochrome b_{558}, p40-phox, p47-phox and p67-phox) for the O_2^--generating system. Although resveratrol enhanced the ATRA-induced O_2^--generating activity via increase of cytochrome b_{558} protein in U937 cells, it also caused the accumulation of p47-phox protein [16]. The accumulation mechanisms of p47-phox protein caused by resveratrol are unresolved yet. In addition, both ellagic acid and urolithin A showed little cytotoxicity up to 20 μM (Supplementary Fig. 2). Therefore, ellagic acid and urolithin A may be used as modifiers having slight side effects for cytochrome b_{558} functions. Interferon-γ, one of trans activators for gp91-phox gene [14,21], improves the splicing efficiency of the gp91-phox gene primary transcripts in a particular group of X-linked chronic granulomatous disease patients [25,26]. For example, combination of interferon-γ and urolithin A may rescue a certain kind of X-linked chronic granulomatous disease patients with a mutation in gp91-phox gene from infectious diseases.

Moreover, in this study, we demonstrated using U937 cells that urolithin A epigenetically enhances the ATRA-induced O_2^--generating activity via activating gp91-phox transcription while ellagic acid epigenetically suppresses the ATRA-induced O_2^--generating activity via inhibiting gp91-phox transcription. As is well known, U937 cells have been used as an *in vitro* model for studying differentiation mechanisms and functions of macrophages [12]. Macrophages also exist in intestine and play important roles to maintain the intestinal homeostasis including intestinal immunity [27,28]. In addition, interestingly, urolithin A is generated from ellagic acid by intestinal bacteria [1-9]. As is well known, a large number of bacteria live in human gut. It is believed that they act as key factors of health and disease through participating in various metabolic pathways in human gut [29]. Taken together, these findings showed the possibility that conversion of ellagic acid to urolithin A by intestinal bacteria may affect the intestinal homeostasis via controlling intestinal macrophage functions. Unfortunately, we have no data how much concentration of urolithin A affects human intestinal macrophages in human gut under physiological conditions. Although localization of urolithin A is especially difficult to be elucidated in human gut, these constraints will be resolved in the future.

Author statement

Hidehiko Kikuchi: Data curation, Investigation, Methodology, Project administration, Supervision, Writing – original draft, Writing – review & editing. Kaori Harata: Data curation, Writing – review & editing. Harishkumar Madhyastha: Writing – review & editing. Futoshi Kuribayashi: Writing – review & editing.

Declaration of competing interest

The authors declare the following financial interests/personal relationships which may be considered as potential competing interests: This work was supported in part by JSPS KAKENHI Grant Number 19K02329 (to H. K.) and 18K07804 (to F. K.).

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.bbrep.2020.100891.

References

[1] B. Cerda, P. Periago, J.C. Espin, F.A. Tomas-Barberan, Identification of urolithin A as a metabolite produced by human colon microflora from ellagic acid and related compounds, J. Agric. Food Chem. 53 (2005) 5571–5576.
[2] C. Garcia-Munoz, F. Vaillant, Metabolic fate of ellagitannins: implications for health, and research perspectives for innovative functional foods, Crit. Rev. Food Sci. Nutr. 54 (2014) 1584–1598.
[3] C. Sanchez-Gonzalez, C.J. Ciudad, V. Noe, M. Izquierdo-Pulido, Health benefits of walnut polyphenols: an exploration beyond their lipid profile, Crit. Rev. Food Sci. Nutr. 57 (2017) 3373–3383.
[4] B.M. Burton-Freeman, A.K. Sandhu, I. Edirisinghe, Red raspberries and their bioactive polyphenols: cardiometabolic and neuronal health links, Adv. Nutr. 7 (2016) 44–65.
[5] F.A. Tomas-Barberan, A. Gonzalez-Sarrias, R. Garcia-Villalba, M.A. Nunez-Sanchez, M.V. Selma, M.T. Garcia-Conesa, J.C. Espin, Urolithins, the rescue of "old" metabolites to understand a "new" concept: metabotypes as a nexus among phenolic metabolism, microbiota dysbiosis, and host health status, Mol. Nutr. Food Res. 61 (2017), https://doi.org/10.1002/mnfr.201500901.
[6] S. Wu, L. Tian, Diverse phytochemicals and bioactivities in the ancient fruit and modern functional food pomegranate (*Punica granatum*), Molecules 22 (2017) E1606, https://doi.org/10.3390/molecules22101606, pii.
[7] G.E. Muke, I.A. Murray, J.C. Espin, G.H. Perdew, Urolithin, A is a dietary microbiota-derived human aryl hydrocarbon receptor antagonist, Metabolites 8 (2018) E86, https://doi.org/10.3390/metabo8040086, pii.
[8] P. Dey, Gut microbiota in phytopharmacology: a comprehensive overview of concept, reciprocal interactions, biotransformations and mode of actions, Pharmacol. Res. 147 (2019) 104367, https://doi.org/10.1016/j.phrs.2019.104367.
[9] A. Cortes-Martin, M.V. Selma, F.A. Tomas-Barberan, A. Gonzalez-Sarrias, J. C. Espin, Where to look into the puzzle of polyphenols and health? The postbiotics and gut microbiota associated with human metabotypes, Mol. Nutr. Food Res. 64 (2020), e1900952, https://doi.org/10.1002/mnfr.201900952.
[10] F. Morel, J. Doussiere, P.V. Vignais, The superoxide-generating oxidase of phagocytic cells. Physiological, molecular and pathological aspects, Eur. J. Biochem. 201 (1991) 523–546.
[11] M.C. Dagher, E. Pick, Opening the black box: lessons from cell-free systems on the phagocyte NADPH-oxidase, Biochimie 89 (2007) 1123–1132.
[12] P. Harris, P. Ralph, Human leukemic models of myelomonocytic development: a review of the HL60 and U937 cell lines, J. Leukoc. Biol. 37 (1985) 407–422.
[13] H. Kikuchi, T. Fujinawa, F. Kuribayashi, A. Nakanishi, S. Imajoh-Ohmi, M. Goto, S. Kanegasaki, Induction of essential components of the superoxide generating system in human monoblastic leukemia U937 cells, J. Biochem. 116 (1994) 742–746.
[14] H. Kikuchi, F. Kuribayashi, N. Kiwaki, Y. Takami, T. Nakayama, GCN5 regulates the superoxide-generating system in leukocytes via controlling gp91-phox gene expression, J. Immunol. 186 (2011) 3015–3022.
[15] H. Kikuchi, F. Kuribayashi, N. Kiwaki, T. Nakayama, Curcumin dramatically enhances retinoic acid-induced superoxide generating activity via accumulation of p47-phox and p67-phox proteins in U937 cells, Biochem. Biophys. Res. Commun. 395 (2010) 61–65.
[16] H. Kikuchi, H. Mimuro, F. Kuribayashi, Resveratrol strongly enhances the retinoic acid-induced superoxide generating activity via up-regulation of gp91-phox gene expression in U937 cells, Biochem. Biophys. Res. Commun. 495 (2018) 1196–1200.
[17] H. Kikuchi, H. Mimuro, H. Madhyastha, F. Kuribayashi, Chalcone skeleton promotes transcription of gp91-phox gene but inhibits expression of gp91-phox protein, and hydroxyl groups in hydroxychalcones participate in the stable expression of gp91-phox protein, Microbiol. Immunol. 63 (2019) 438–443.
[18] S. Akiyoshi, H. Kikuchi, F. Kuribayashi, H. Madhyastha, H. Minami, Sulforaphane displays the growth inhibition, cytotoxicity and enhancement of retinoic acid-induced superoxide-generating activity in human monoblastic U937 cells, Fundam. Toxicol. Sci. 8 (2019) 319–325.
[19] S. Suzuki, A. Kumatori, I.A. Haagen, Y. Fujii, M.A. Sadat, H.L. Jun, Y. Tsuji, D. Roos, M. Nakamura, PU.1 as an essential activator for the expression of $gp91^{phox}$ gene in human peripheral neutrophils, monocytes, and B lymphocytes, Proc. Natl. Acad. Sci. U.S.A. 95 (1998) 6085–6090.
[20] L. Bei, Y. Lu, E.A. Eklund, HOXA9 activates transcription of the gene coding $gp91^{Phox}$ during myeloid differentiation, J. Biol. Chem. 280 (2005) 12359–12370.

[21] A. Kumatori, D. Yang, S. Suzuki, M. Nakamura, Cooperation of STAT-1 and IRF-1 in interferon-γ-induced transcription of the gp91^{phox} gene, J. Biol. Chem. 277 (2002) 9103–9111.
[22] C.D. Allis, S.L. Berger, J. Cote, S. Dent, T. Jenuwien, T. Kouzarides, L. Pillus, D. Reinberg, Y. Shi, R. Shiekhatter, A. Shilatifard, J. Workman, Y. Zhang, New nomenclature for chromatin-modifying enzymes, Cell 131 (2007) 633–636.
[23] H. Kikuchi, F. Kuribayashi, S. Imajoh-Ohmi, H. Nishitoh, Y. Takami, T. Nakayama, GCN5 protects vertebrate cells against UV-irradiation via controlling gene expression of DNA polymerase η, J. Biol. Chem. 287 (2012) 39842–39849.
[24] K. Karmodiya, A.R. Krebs, M. Oulad-Abdelghani, H. Kimura, L. Tora, H3K9 and H3K14 acetylation co-occur at many gene regulatory elements, while H3K14ac marks a subset of inactive inducible promoters in mouse embryonic stem cells, BMC Genom. 13 (2012) 424.
[25] F. Ishibashi, T. Mizukami, S. Kanegasaki, L. Motoda, R. Kakinuma, F. Endo, H. Nunoi, Improved superoxide-generating ability by interferon γ due to splicing pattern change of transcripts in neutrophils from patients with a splice site mutation in *CYBB* gene, Blood 98 (2001) 436–441.
[26] P.R. Errante, J.B. Frazao, A. Condino-Neto, The use of interferon-gamma therapy in chronic granulomatous disease, Recent Pat. Anti-Infect. Drug Discov. 3 (2008) 225–230.
[27] C.C. Bain, A. Schridde, Origin, differentiation, and function of instetinal macrophages, Front. Immunol. 9 (2018) 2733, https://doi.org/10.3389/fimmu.2018.02733.
[28] P.A. Muller, F. Matheis, D. Mucida, Gut macrophages: key players in intestinal immunity and tissue physiology, Curr. Opin. Immunol. 62 (2020) 54–61.
[29] P.D. Cani, Human gut microbiome: hopes, threats and promises, Gut 67 (2018) 1716–1725.