Supplementary materials

Title

The surprising effect of phenformin on cutaneous darkening and characterization of its underlying mechanism by a forward chemical genetics approach

Journal

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Supplemental Figure Legends

Supplemental Figure 1. Phenformin darkens skin samples from a Hispanic donor and a Caucasian donor in tissue culture.

(a) Skin tissues obtained from a Hispanic subject (48y) were subjected to tissue culture with or without 300 μM phenformin for 8 days. The photographs shown are representative samples. Scale bars = 5 mm. (b) Treated skins were subjected to Fontana-Masson staining. Scale bars = 100 μm. The areas indicated by the squares are shown at higher magnification under each image. (c) Skin tissues obtained from a Caucasian subject (18y) were subjected to tissue culture with or without 300 μM phenformin for 6 days. The photographs shown are representative samples. Scale bars = 5 mm.

Supplemental Figure 2. Phenformin does not affect DOPA oxidase activity and the expression levels of melanogenesis-related proteins in melanocytes.

(a) After treatment of NHEMs with or without phenformin for 3 days (at the indicated concentrations), DOPA oxidase activity was measured. Values represent means ± SD of 6 independent samples (***$P < 0.001$ versus control (DMSO treatment) by Dunnett's test). (b) Microscopic images of NHEMs treated with DMSO (control) or phenformin (300 μM or 1 mM). (c) After incubation with or without phenformin for 4 days (at the indicated concentrations), NHEMs were lysed and subjected to western blot analysis of Tyrosinase and TRP1 proteins. β-Actin = internal control.

Supplemental Figure 3. Evaluation of the potential effects of biguanide compounds on the regulation of skin color.

Skin tissues obtained from a Japanese subject (age unknown) were subjected to tissue culture with

or without (a) phenformin, (b) phenylbiguanide, (c) metformin, (d) buformin or (e) N-[amino(imino)methyl]piperidine-1-carboximidamide for 7 days. The photographs shown are representative samples. Scale bars = 5 mm.

Supplemental Figure 4. SAR (structure-activity relationship) analysis of biguanide compounds to evaluate their potential skin darkening effects.

Chemical structures of (a) a skin darkening compound (phenformin), and (b) non-skin darkening compounds (phenylbiguanide, metformin, buformin and N-[amino(imino)methyl]- piperidine-1-carboximidamide) are shown. We speculate that amino groups of phenformin are not involved in the darkening activity in contrast to the phenyl group.

Supplemental Figure 5. Preparation of phenformin-immobilized FG-beads.

The reaction scheme shows the immobilization of phenformin on magnetic FG-beads. The experimental procedure is described in “Materials and Methods”.

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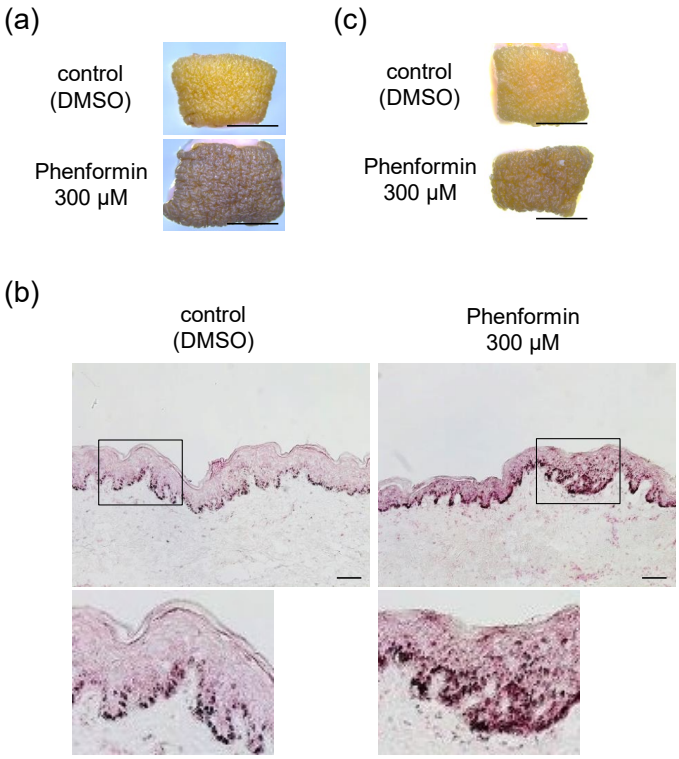
Supplemental Figure 1
Takano *et al*.



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Supplemental Figure 2
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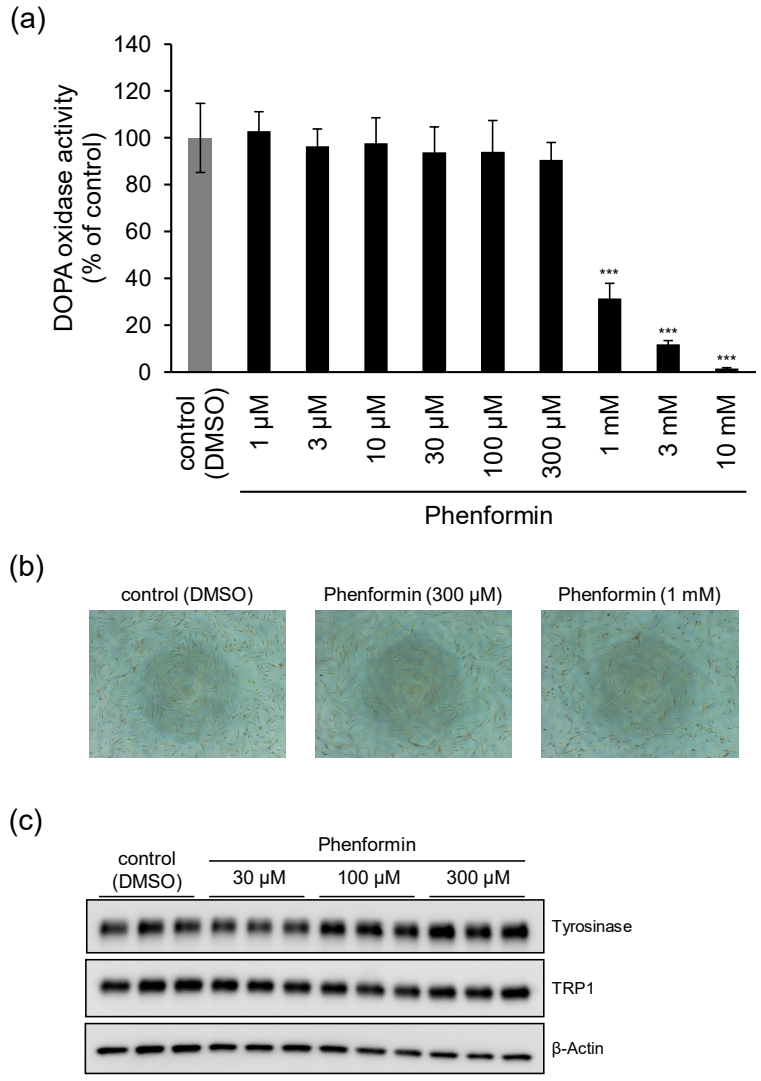

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Supplemental Figure 3
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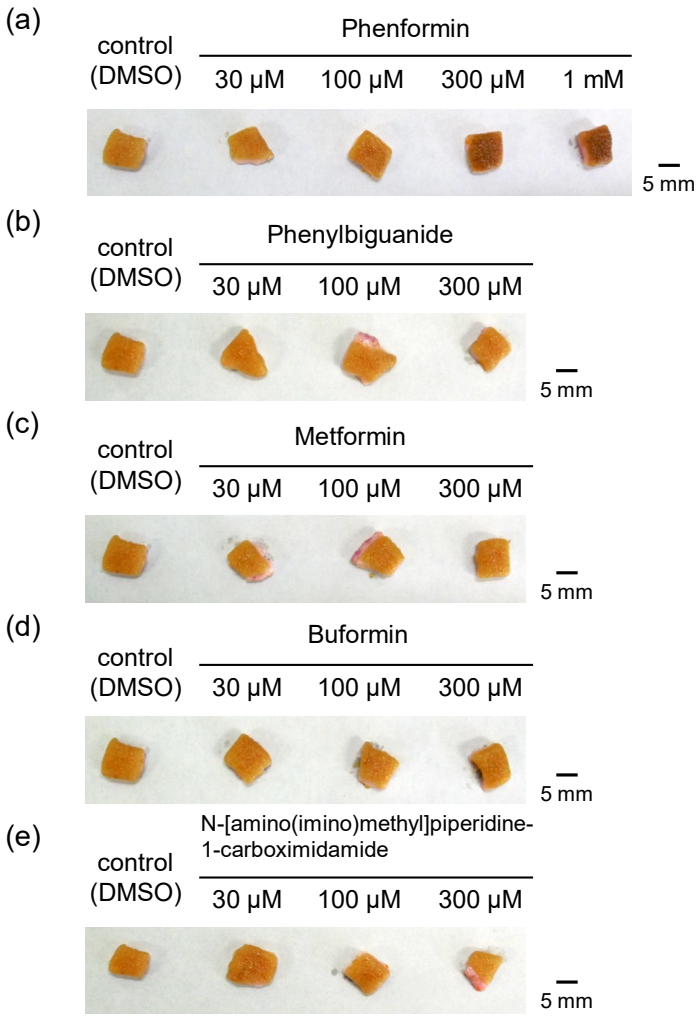

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Supplemental Figure 4
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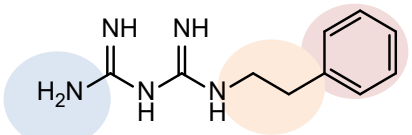
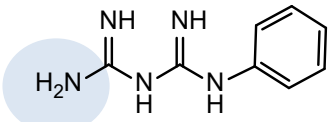
(a) Skin darkening compound

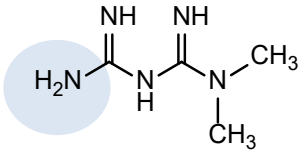


Phenformin

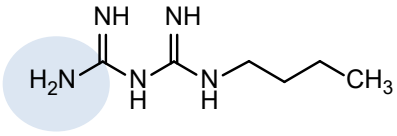
(b) non-skin darkening compounds



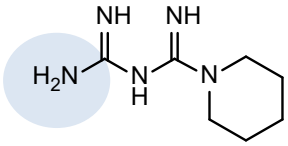
Phenylbiguanide



Metformin



Buformin



N-[amino(imino)methyl]piperidine-
1-carboximidamide

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Supplemental Figure 5
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