Gene		Primer Sequences 5'-3'
TRP-1	Forward	CGTTCGACAAGGGTGACTAT
	Reverse	TCGATTGCCACCAAAAGTGC
TRP-2	Forward	CCCTGCATGTGCTGGTTCTT
	Reverse	ATTGTGAACCAATAGGGGGCCAG
MITF	Forward	GGGAGCTCACAGCGTGTATT
	Reverse	ATGGTTCCCTTGTTCCAGCG
P65	Forward	CTTCCAAGAAGAGCAGCGTG
	Reverse	AGTTTCGGTTCACTCGGCAG
Ι-κΒα	Forward	CAAGCACCCGGATACAGCA
	Reverse	AGTCATCATAGGGCAGCTCG
β-Trcp	Forward	TGAGAGGTAAGAGAGGGCGG
	Reverse	ACCTGGGCATAGAGCACATAA
JNK1	Forward	CCGCGTCTCTGTTACTCAGC
	Reverse	AATTCACCAAGAAGCCGGCAG
β-actin	Forward	TGGCACCCAGCACAATGAA
	Reverse	CTAAGTCATAGTCCGCCTAGAAGCA

Table 1 Sequences of primers for target genes of amplified fragment



Fig. 1 The effect of UVB irradiation dose on viability of the PIG1 cells. Human Epidermal Melanocyte (PIG1) cells were exposed to UVB at doses ranging from 0 to 120mJ/cm², and the cell viability was examined at 24h post-irradiation. Values are presented as the mean \pm SD (n=3). *p<0.05, **p<0.01, compared with the 0 UVB irradiation dose.



Fig. 2 The effect of UVB irradiation dose on the melanin content in PIG1 cells. Human Epidermal Melanocyte (PIG1) cells were exposed to UVB at doses ranging from 50 to 90mJ/cm² to measure melanin content with the NaOH method. Values are presented as the mean \pm SD (n=3). *p<0.05, **p<0.01, compared with the 0 UVB irradiation dose.



Fig. 3 The effect of RPH on the melanin content in PIG1 cells. The Human Epidermal Melanocyte (PIG1) cells were treated without UVB (Control), with 100mJ/cm² UVB (Model), with 70mJ/cm² UVB in the presence of 10µg/mLβ-arbutin (Positive), with 100mJ/cm² UVB in the presence of Rice Protein Hydrolysate (RPH) at the concentration of 50, 100 or 200 µg/mL. Values are presented as the mean \pm SD (n=3). **p*<0.05, ***p*<0.01, compared with Control, # *p*<0.05, ##*p*<0.01, compared with Model.



Fig. 4 The effect of RPH on the tyrosinase activity in PIG1 cells. The tyrosinase activity of Human Epidermal Melanocyte (PIG1) cells was detected by L-DOPA. The PIG1 cells were treated without UVB (Control), with 70mJ/cm² UVB (Model), with 100mJ/cm² UVB in the presence of 10µg/mLβ-arbutin (Positive), with 100mJ/cm² UVB in the presence of Rice Protein Hydrolysate (RPH) at the concentration of 50, 100 or 200 µg/mL. Values are presented as the mean \pm SD (n=3). **p*<0.05, ***p*<0.01, compared with Control, #*p*<0.05, ##*p*<0.01, compared with Model.



Fig. 5 The effect of RPH on the ROS levels in PIG1 cells. Iinhibition of intracellular reactive oxygen species (ROS) generation in Human Epidermal Melanocyte (PIG1) cells was measured by DCFH-DA assay. The PIG1 cells were treated without UVB (Control), with 100mJ/cm² UVB (Model), with 70mJ/cm² UVB in the presence of $10\mu g/mL\beta$ -arbutin (Positive), with $100mJ/cm^2$ UVB in the presence of Rice Protein Hydrolysate (RPH) at the concentration of 50, 100 and 200 $\mu g/mL$. Values are presented as the mean \pm SD (n=3). *p<0.05, **p<0.01, compared with Control, #p<0.05, ##p<0.01, compared with Model



Fig. 6 Contents of exopeptidase-resistant peptides from Rice Protein Hydrolysate (RPH). One-letter abbreviations were used for amino acid residues. pE represents pyroglutamic acid (pyroGlu) residue. Values are presented as the mean \pm SD (n=3).



А



В



Fig. 7 HPLC–MS/MS analysis to reveal the structure of RPH with a lysine residue at the C-terminus. Total ion chromatograms (TIC) and ions intensity (Inten.) of Q3, and product ion scan were showed. (A) the map of sequence Leu-Leu-Lys, (B) the map of sequence Leu-Pro-Lys, (C) the map of sequence pyroGlu-Lys.



Fig. 8 The effect of LLK, LPK and pEK on melanin content and tyrosinase activity in PIG1 cells. The Human Epidermal Melanocyte (PIG1) cells were treated without UVB (Control), with 100mJ/cm² UVB (Model), with 70mJ/cm² UVB in the presence of 10µg/mL β-arbutin (Positive), with 100mJ/cm² UVB in the presence of 10 µg/mL Leu-Leu-Lys (LLK), Leu-Pro-Lys (LPK), and pyroGlu-Lys (pEK).Values are presented as the mean \pm SD (n=3). **p*<0.05, ***p*<0.01, compared with Control, #*p*<0.05, ##*p*<0.01, compared with Model



В

С

Fig. 9 Effects of LLK, LPK, and pEK on the mRNA level of UVB-induced PIG1 cells. The Human Epidermal Melanocyte (PIG1) cells were treated without UVB (Control), with 100mJ/cm² UVB (Model), with 70mJ/cm² UVB in the presence of 10μg/mL β-arbutin (Positive), with 100mJ/cm² UVB in the presence of 10 µg/mL Leu-Leu-Lys (LLK), Leu-Pro-Lys (LPK), and pyroGlu-Lys (pEK). The mRNA expression levels were detected by specific primers for (A) tyrosinaserelatedprotein 1 (TRP-1), tyrosinase-relatedprotein 2 (TRP-2) (B) microphthalmia-associated transcription factor(MITF), nuclear factor kappa-B-p65/ nuclear factor kappa B inhibitor alpha (NFκB-p65/IκB-α) (C) Beta-tansducin repeats containing proteins (β-Trcp), c-Jun N-terminal kinase (JNK) and β-actin as the internal control. Values are presented as the mean \pm SD (n=3). *p<0.05, **p<0.01, compared with Control, p < 0.05, p < 0.01, compared with Model.



Fig. 10 Effects of LLK, LPK, and pEK on the protein level of UVB-induced PIG1 cells. The Human Epidermal Melanocyte (PIG1) cells were treated without UVB (Control), with 100mJ/cm² UVB (Model), with 70mJ/cm² UVB in the presence of 10µg/mL β-arbutin (Positive), with 100mJ/cm² UVB in the presence of 10 µg/mL Leu-Leu-Lys (LLK), Leu-Pro-Lys (LPK), and pyroGlu-Lys (pEK). The protein expression levels were detected by specific antibodies for microphthalmia-associated transcription factor(MITF), phospho nuclear factor kappa-B-p65 (p-NFκB-p65), nuclear factor -kappa-B inhibitor alpha (IκB-α), Beta-tansducin repeats containing proteins (β-Trcp), phospho c-Jun N-terminal kinase (JNK) and β-actin as the internal control. Values are presented as the mean ± SD (n=3). **p*<0.05, ***p*<0.01, compared with Control, #*p*<0.05, ##*p*<0.01, compared with Model.



Fig. 11 Intracellular signaling pathways of the UVB-induced melanin production in Human Epidermal Melanocyte (PIG1) cells (Dotted area).