Abstract

Project Code : TRG5880209

Project Title : การผันแปรของสภาวะเหนือพันธุกรรมในความชราของเซลล์ภายใต้ภาวะพร่องเอนไซม์จีซิกพีดี
(Epigenomic alterations in aging influenced by G6PD deficiency)

Investigator : ผู้ช่วยศาสตราจารย์ ดร.ชาลิสา หลุยเจริญ ชีพสุนทร
ภาควิชาชีวเคมี คณะแพทยศาสตร์ จุฬาลงกรณ์มหาวิทยาลัย

E-mail Address : chalisa_loui@yahoo.com, chalisa.l@chula.ac.th

Project Period : 1 กรกฎาคม 2558 ถึง 30 มิถุนายน 2561

Global methylation (Alu, LINE-1) plays a role in the development of aging. Both aging & epigenomic alterations are associated with increased oxidative stress, which is counteracted by glucose 6-phosphate dehydrogenase (G6PD). G6PD activity decreases with age. Therefore, it is hypothesized that deficiency in G6PD activity could lead to the disruption of redox homeostasis and subsequently increased susceptibility to epigenomic alteration. Here, we demonstrated the association of G6PD activity & age-related methylation levels of Alu, LINE-1 in G6PD deficient elders & *in vitro* model. Alu and LINE-1 methylation were not changed between G6PD deficient patients and controls. Methylation in patients with neurodegenerative diseases (NDs) was also significantly increased in mC and uCmC of Alu (p=0.001 and 0.017) and decreased in uCuC of LINE-1 (p=0.001). After adjusting OR with age, gender and G6PD deficiency status, only a decrease of uCuC of LINE-1 was associated with NDs. *In vitro* system, we found a significant correlation between reduction of G6PD activity and LINE-1 hypermethylation in SK-N-SH neuronal cells. Cellular senescence was also significantly increased in G6PD knockdown neurons. As mentioned earlier, G6PD deficiency causes an imbalance of cellular redox state, which might lead to DNA damage and subsequently genomic integrity. Therefore, G6PD deficiency might be involved in cellular senescence by inducing genomic instability via alteration of DNA methylation during oxidation.

Keywords : G6PD deficiency, epigenomic alteration, LINE-1 methylation, aging

Objectives

1. To study the correlation between the levels of Alu, LINE-1 methylation and blood G6PD activity in elders

2. To examine the role of G6PD in regulation of cellular aging via methylation of Alu and LINE-1 in aged human neuronal cell line

Methodology

***IN VIVO* STUDY:** To study the correlation between the levels of Alu, LINE-1 methylation and blood G6PD activity in elders

Subjects

Ethical permission was approved by Research Ethics Committee of the National Blood Centre of Thai Red Cross Society, Bangkok (COA No. NBC 7/2016) and by Research Ethic committee of the Faculty of Medicine, Ramathibodi Hospital. One hundred and fourteen elders (60-90 years old) consisted of 66 volunteers (28 males and 38 females) from Division of Neurology, Department of Medicine, Ramathibodi Hospital and 48 volunteers (30 males and 18 females) from Thai Red Cross were enrolled in this study. All subjects agreed to be assessed for socio-demographic variables and lifestyle risk factor such as smoking and alcohol consumption.

Blood collection and G6PD activity assay

Peripheral blood samples were collected in ethylenediaminetetraacetic acid (EDTA) tube and stored at 2-8 $^{\circ}$C. Hemoglobin (Hb) concentrations were measured using CBC machine Mindray BC-5150 (Mindray, CPR). Quality controls (QC) of Hb measurement were daily measured and recorded. Hb concentrations were used to calculate U/gHb for all G6PD tests. All bloods samples were then analysed for G6PD activity within 7 days using quantitative spectrophotometric method (Trinity Biotech, Ireland), according to the manufacturer's instructions (Procedure No.345-UV). The kinetic of G6PD activity at 30°C was monitored using spectrophotometer (Shimadzu Corp., Japan) at 340 nm with 5 min of time intervals from 10 to 15 min. Lyophilized hemolysates of normal and deficient G6PD controls were run with every test of samples (G6888, G5888) (Trinity Biotech, Ireland). The result was expressed as U/g Hb. The results were valid, only if G6PD activities of the controls were within the reference range.