Cytoplasm and nuclear crude protein proportion observed in peripheral blood mono nuclear cells under senescence inducing stress exposure

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Abstract. Premature cellular senescence can be induced by exposing H2O2 in concentration-dependent manner. There is protein that contribute to changes driven early alteration in intracellular dynamic. Crude protein proportion in nuclear and cytoplasm were evaluated in this study for capturing acute stress response after 48 hours H2O2 exposure. Viability experiment were performed in isolated human PMBNCs which seeded in 6 plate well. After percentage of the surface of a culture dish covered by adherent cells >60%, cell was treated with 200 µm and 400 µm H2O2 for 48 hours incubate in 37 °C. Harvesting cell with tripsin EDTA and nanodrop were performed to visualization crude protein proportion in nuclear and cytoplasm. Results and discussion. mean the cytoplasm crude protein were found to increased significantly in H2O2-treated cell (7.2 mg/ml ± SD 0.1 in 200 µM and 7.6 mg/ml ± SD 0.6 in 400 µM) than non-treated cell (6.5 mg/ml ± SD 0.8). H2O2 treatment led to dose-dependent increase cytoplasm crude protein but not in nuclear proportion rate. These results demonstrate that hydrogen peroxide triggers cellular senescence program with up regulated major cytoplasm crude protein. Elevation nuclear crude protein may contribute to another information related cellular response.

Keyword: H2O2 exposure, PBMNCs, crude protein.

1. Introduction
Intracellular proteins shiftiness, such as transcription factors, are a key mechanism in regulating the transcription process at the time when basal conditions or as a result of stimulus induction [1].

Various types of signals both phosphorylation / dephosphorylation, acetylation / deacetylation, and oxidative modification, can cause changes in protein ratios in the cytoplasm and nucleus, this is necessary to regulate subcellular protein interactions in maintaining the homeostatic response [2-7]. Such signaling is important for activating cellular defense mechanisms to protect against oxidative injury [4, 11]. Signaling occurrence after Reactive Oxygen Species (ROS) exposure have been widely discussed in various published studies [8-11].

As a reaction to oxidative stress with hydrogen peroxide (H2O2) treatment (20 mM, 1 h), it causes damage to the nucleus of HeLa cells which is characterized by increased levels of the enzyme beta-galactosidase [12]. The increase in the enzyme is a manifestation produced by the interaction of various
transcription factor regulatory mechanisms and intracellular protein distribution. The activated transcription factor is translocated to the nucleus to regulate some gene expression as a form of cellular response. Disruption in the distribution of intracellular protein signals, prompting the development of several diseases such as neurodegenerative [13].

Hydrogen peroxide is known to induce aging in vitro. Cell aging can be confirmed by impaired primary cell proliferation ability or cell line in vitro [14-16]. On the other hand, Bono and Yang [17] showed that endothelial cells treated with 100 micromolar H_2O_2 underwent early apoptosis. This suggests that cells exposed to H_2O_2 will undergo a temporary shock state characterized by different morphological changes depending on the cell type eg endothelial cells will tend to round. In these circumstances, CIP1 will be induced to stop cells in the G0-G1 phase, on the other hand, BCL-2 levels tend to increase in response and markers of oxidative damage to delay apoptosis and allow a longer time for cells to repair themselves. If the damage caused is extensive and the cells are unable to repair themselves, the cells will enter the path of aging or apoptosis [18].

Here we using primary pheripheral blood mononuclear cells (PBMNC), we investigate the occurrence diversity of intracellular protein as a function of the amount of H_2O_2.

2. Methods

2.1. Human PBMNC collection and Isolation

Five ml of heparinized whole blood was collect from each participant at the resting time of physycal exercise. Whole blood was diluted with an equal volume of Axisxhield’s Lymphoperm as previously described [18] and placed in 15 ml Falcon tube to isolated using centrifugation for 30 min at 6000 rpm. The mononuclear cell layer (buffy coat) were collected with a pippette and placed in sterile 15 ml falcon tube. The cell pellet was resuspended in approximately 100-200 µl medium complete.

2.2. H_2O_2 treatment

PBMNC cells that had been isolated were seeded on a 6 well plate. To assess the effect of oxidative stress, the treatment cells were divided into 3 groups (i) no treatment, (ii) exposed to 200 micromolar H_2O_2 and (iii) exposed to 400 micromolar H_2O_2, then incubated for 24 hours at 37°C [25-26, 33].

2.3. Nanodrop assay

The cell suspension is then quantified with a spectrophotometer or nanodrop and by electrophoresis in the absorbance range of 280 nm as well as describe before.

2.4. Statistical Analysis

The statistical software SPSS version 25 was used to analyze the data. All data were presented as mean value ± standard deviation. Comparison between the tree groups were analyzed by T-test. Statistical significance was assumed if hypothesis could be accepted at p<0.05.

3. Result

3.1. 24-hour H_2O_2 induction causes the effect of increasing the proportion of crude protein in the cytoplasm to be more dominant than the cell nucleus

H_2O_2 induction induces increased protein in the cytoplasm as compared to nucleus. Based on this data, it can be concluded that the identification of the type of protein in the cytoplasm provides more information on cellular responses to stress exposure (Figure 1).
3.2. Increasing the amount of protein in the cytoplasm linearly with increasing doses of H$_2$O$_2$ induction but not with the amount of protein in the nucleus

The mean crude protein in the cytoplasm with H$_2$O$_2$ induction at a dose of 400 µM was 7.6 mg/ml ± 0.6, which was higher than that of 200 µM induction which ranged from 7.2 mg/ml ± 0.1.

Meanwhile, the amount of cell nucleus crude protein actually shows a value of 1.11 mg/ml ± 0.49 after giving H$_2$O$_2$ induction at a dose of 400 µM. This value was actually lower than the amount of crude protein that was not given treatment, namely 1.23 mg/ml ± 0.34. This suggests that the identification of intracellular proteins lacks information on cellular responses to H$_2$O$_2$ induction.

Treatment with H$_2$O$_2$ induction at a dose of 200 µM showed an increase in the mean amount of crude protein in the nucleus 1.9 mg/ml ± 0.5 compared to untreated cells. However, if given a higher dose of treatment, (400 µM H$_2$O$_2$), the amount of crude protein in the nucleus actually decreased with an average of 1.11 mg/ml ± 0.5.

4. Discussion
This time we observed crude protein ratios in both the cytoplasm and nucleus via short-term H$_2$O$_2$ induction, to demonstrate the complexity of regulation of protein expression as a display of intracellular stress response. As noted earlier that the proportion of protein subcellular changes signify a form of response to the stimulus induced stress [19].
This time the study results indicate that there are differences in the ratio of crude protein both in the cytoplasm and in the nucleus. H2O2 induction caused a significant effect on changes in the amount of protein in the cytoplasm that did not occur in the nucleus. Increasing the induction dose is also followed by an increase in the amount of crude protein in the cytoplasm. Based on these results, cytoplasmic protein measurements provide more information about the cellular response to H2O2 induction.

Systematic studies measuring transcription factors and proteins at the genome scale reveal that there are several processes beyond transcription concentration that need to be studied because they affect the level of protein expression [20]. Most of the changes in protein content in the nucleus are not linear with changes in the amount of mRNA [21]. The study was carried out by differentiating the proportion of protein in three cellular fractions including cytoplasm, nucleus, and nucleolus and differences in the amount of protein degradation [22-23].

Maintaining homeostatic conditions is the key to cellular maintenance physiology. Stress markers will always be activated in response to metabolic stress. Adaptation of the endothelial reticulum system to various metabolic conditions including stress response is important for cell function, cell survival, and organism well being [27]. Subcellular protein distribution takes place is very dynamic, not least due to the induction of ROS [28-32].

5. Conclusion
Taken together, these finding suggest that elevation protein product in cytoplasm may regulate cellular response viability and acute responses in opposing way during in vitro models of oxidative stress.

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