Expression of cytoglobin and PGC-1α is high during tail tissue regeneration of the house gecko (Hemidactylus platyurus)

Titta Novianti
Universitas INDONUSA Esa Unggul  https://orcid.org/0000-0002-0058-7222

Vetnizah Juniantito
Department of Pathology Faculty of animal Medicine, agriculture Institute of Bogor ,

Ahmad Aulia Jusuf
Departement of Histology, Faculty of Medicine, University of Indonesia

Evy Ayu Arida
Indonesian Institute of Sciences (LIPI)

Mohamad Sadikin
Centre of Hypoxia, Department of Biochemistry, Faculty of Biology Medicine,

Sri Widia A. Jusman (✉ sriwidiaaj@gmail.com )
https://orcid.org/0000-0002-8169-6825

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Abstract

Background Tissue regeneration is a process that need a high demand of oxygen and energy supply. Cytooglobin (Cygb) is a hexacoordinate globin superfamily that possesses strong oxygen-binding ability. Cygb also has a role in preventing cells from oxidative stress and carrying oxygen into the mitochondria. The production of energy for regeneration is associated with mitochondria, especially mitochondrial biogenesis. The peroxisome proliferator-activated receptor gamma coactivator (PGC-1α) is a protein that plays an important role in regulating mitochondrial biogenesis. The house gecko (Hemidactylus platyurus) is a reptile with high ability for tissue regeneration in its tail. The house gecko was selected as the animal model for this research to analyse the role of Cygb which is associated with oxygen supply and PGC-1α that is related to energy production in tissue regeneration. Results The curve for the tail growth showed three different phases. Cygb mRNA was highly expressed during tissue regeneration. PGC-1α mRNA was expressed earlier than Cygb, with a lower expression but still higher than the control. Conclusions During the tail tissue regeneration process of the house gecko, the expression of Cygb and PGC-1α were dynamic and relatively higher than their expression observed in the control. Cygb and PGC-1α were suspected to have a significant role in the tissue regeneration process.

Background

Tissue regeneration is a complex process in attempt to restore organ morphology back to its functional level after an injury. This process involves cell proliferation, migration, differentiation and extracellular matrix synthesis. [1] [2][3] [4] The tissue regeneration process aims to restore tissue morphologies and physiologies and clearly requires a large amount of energy. The high energy used for the tissue regeneration process requires aerobic metabolism to produce a high number of ATP. The high number of ATP used in this process could be fulfilled only when the O₂ supply was sufficient. [5] [6],[7]

Increased aerobic metabolism occurs in parallel with an increase in oxygen demand. The tissue will enter a relative hypoxic state. Relative hypoxia is the state when tissue is oxygen deficient because of high metabolism; however, the oxygen supply remains unchanged. [8][9] In the hypoxic state, the organisms strive to fulfil the oxygen demand to maintain metabolism for cellular activities. Therefore, the oxygen obtained must be transferred to the mitochondria by the cytoglobin (Cygb) protein. [10] [11] [12]

The extremely high affinity of Cygb for oxygen leads to the assumption that Cygb might have a significant role as an oxygen diffusion factor in the mitochondria. This function is similar to the myoglobin in muscle cells, which contributes to the maintenance of the oxidative phosphorylation process. Cygb is able to store oxygen and will release it in hypoxic conditions. Therefore, we suspect that Cygb is involved in adaptive responses to hypoxia-mediated injuries. [12] [13]

The role of the Cygb protein in tissue regeneration remains unclear.[12] Previous studies have acknowledged how Cygb contributes to maintaining oxygen diffusion in the mitochondrial respiratory
chain during hypoxia. [10] The Cygb protein simultaneously acts in oxygen storage and as an oxygen sensor. [14]

In certain cellular events, such as cell proliferation and differentiation, that require high energy, mitochondria, as the energy-producing organelle, plays an important role. When cells are in high demand of energy, the process of mitochondrial biogenesis meets this energy demand. [5] [15] [16] PGC-1α is a well-known mitochondrial biogenesis biomarker during tissue regeneration. PGC-1α protein in the cell nucleus regulates nuclear respiratory factor (Nrf-1) gene transcription and mitochondria Transcription Factor A (mtTFA). Both genes play a role in regulating mitochondrial biogenesis. [15][16] [17]

Studies on the gene expression, protein synthesis, and cell structure involved in tissue regeneration during hypoxia are limited. This research is important as an initial research in medicine to analyse aspects of hypoxia during the tissue regeneration process. In this study, the house gecko (Hemidactylus platyurus) was used as a model animal because this reptile has a high regeneration ability in its post-autotomy tail, and among the other vertebrate animal groups that show autotomy, it also has the closest taxonomy to mammals. [18] [19]

This research was designed to analyse the expression of Cygb and PGC-1α in tail regeneration of the house gecko (Hemidactylus platyurus). The expression of Cygb and PGC-1α was hypothesized to increase significantly during the tail regeneration process due to the high O₂ and energy demand. Cygb and PGC-1α were also hypothesized to have a role in the tissue regeneration process.

**Methods**

This study utilized a healthy house gecko, indicated by constant active movement, as a model animal. This suitable physical characteristic was recommended to be observed by a herpetology expert from Indonesian Institute of Sciences (LIPI) Zoology, Dr. rer nat Evy Ayu Arida.

This research used descriptive and analytical experimental and cohort design to study tissue regeneration of the house gecko (Hemidactylus platyurus) tail. The source of animal that used in our research is Zoology Laboratory of Indonesian Institute of Sciences (LIPI), Cibinong -Indonesia. This study included animal adaptation, autotomized procedure, DNA primer design, RNA isolation, qPCR, haematoxylin and eosin staining, western blot, immunohistochemistry, data collection, and data analysis. Ethical permission for research was obtained from the Faculty of Medicine University of Indonesia (FKUI) Research Ethics Committee with no. 672/UN2.F1/ETIK/VII /2, as relevant licenses to Animal Scientific Procedures.

The research was performed at the Zoology Laboratory of the Indonesian Institute of Sciences (LIPI), Cibinong; laboratory of Histology Department, Faculty of Medicine Universitas Indonesia; and at Center of Hypoxia and Oxidative Stress Studies Department of Biochemistry & Molecular Biology, Faculty of Medicine Universitas Indonesia from January 2015 until February 2018.
**Materials and Reagents**

The materials and reagents included antibodies against the Cytoglobin (Cygb) and horseradish peroxidase-conjugated donkey anti-rabbit and goat anti-mouse IgG secondary antibodies from Jackson Immuno-Research Laboratories (West Grove, Penn., USA).

**Animal Model**

The research is a descriptive experimental study and cohort study design on tissue regeneration of a house gecko (*Hemidactylus platyurus*) tail that undergoes autotomy. The number of house gecko samples used in this study was thirty three, which include experimental and control group based on Federer's formula, with every group consisted of three geckos.

Federer's formula: \((t - 1)(r - 1) \geq 15\)

\[ t = \text{experiment groups and control (11 groups)} \]

\[ r = \text{the number of animals in each experimental group} \]

\[(11 - 1)(r - 1) \geq 15, \quad r = 3 \text{ animals/group} \]

The thirty three adult male house geckos were obtained from the LIPI Zoology laboratory. The house geckos were maintained in a glass cage with a size of 40 x 20 x 30 cm³ at the Zoological Herpetology Laboratory, LIPI, for one week to allow them to adapt. The house geckos used in this research weighed 5 ± 0.5 grams, 10-13 cm in body length, and had never undergone autotomy. International classification of *Hemidactylus platyurus* are the class of Reptilia, ordo of Squamata, the family is Gekkonidae, and the genus is *Hemidactylus*. [20]

House gecko were kept in a cage which exposed to sunlight through the room, with variation of 12 hours of light and 12 hours of darkness. Geckos were fed with small active insects, such as mosquitoes, cockroaches, and grasshoppers and were given water ad libitum in a small bowl stored in the middle of the cage. Healthy subjects were identified by their ability to catch their prey actively.

**Autotomization Procedure**

All house geckos were chosen randomly to be autotomized (Fig. 8) by simultaneously gripping the body part and rocking their tail tip slowly. The house gecko would then release its tail as easily and naturally as
in accordance with the protocol of the Herpetology Laboratory-LIPI. These autotomized house geckos were returned to the cage to allow the regeneration process to take place, with observations being made on day 1, 3, 5, 8, 10, 13, 17, 21, 25, 30, and control group by randomization with blind method.

**Tissue Collection and Analyses**

The newly grown post-autotomy house gecko tail was incised with a scalpel on the autotomy area. Following this procedure, the house geckos were euthanized using ketamine (100 mg/kg) by Intra Peritoneal (IP) surgical injection, after which their bodies were buried.

The regenerated house gecko tail tissue was then collected and stored at -30°C, and its gene expression and protein expression were analysed. The tissue was kept for RNA analysis and haematoxylin and eosin staining. The Cygb and PGC-1α gene expression was analysed using qPCR. The Cygb protein was analysed using immunohistochemistry and western blot. The tissue regenerated was used to analyse the histology using haematoxylin eosin staining.

**Design of Primer DNA**

Tracking of the Cygb, PGC-1α, and 18S ribosome (housekeeping gene) genes of the house gecko (*Hemidactylus platyurus*) began with phylogenetic studies of species which closest taxonomic kinship to *H. platyurus*. *Gecko japonicus* taxonomically is closest species to *H. Platyurus*. The *Gecko japonicus* genome has been described and is on deposit with the National Center for Biotechnology Information (NCBI) at https://www.ncbi.nlm.nih.gov/. Furthermore, these genes were analysed by BLAST method to find the sequences of genes. A determination of DNA conserved sequences was made using multiple alignment by ClustalX in Mega7 program and using Primer 3 program to design primer DNA.

**Isolation of total RNAs**

The frozen regenerated tissue tail was crushed using micro-homogenizer. RNA was isolated using the Illumina Company's Epicentre MasterPure™ RNA Purification Kit and its protocol (www.epibio.com/applications/nucleic-acid.kits/rna/masterpure-rna-purification-kit). The isolated RNA was pipetted into a 35 μL solution of TE buffer and stored at -80°C.

**Quantification PCR (qPCR)**
The mRNA expression of Cygb and PGC-1α was determined using qPCR, with 18S being used as an internal control. The relative differences in gene expression between groups were determined using the Livak formula. The qPCR yielded gene expression values for comparison with the 18S gene expression and with the gene expression of the tissue control.

**Haematoxylin and Eosin (HE) Staining**

Regenerated tail tissues from days 1, 3, 5, 8, 10, 13, 17, 21, 25, 30, and control after autotomy were fixed overnight in formalin. The tissue was dehydrated with alcohol for 24 hours and then purified with xylol for 24 hours. After that, the tail tissue was embedded in liquid paraffin and left to solidify in block paraffin to be cut by machine. The slices obtained were mounted on glass objects and incubated for 24 hours, and the slices were ready to be stained by Haematoxylin-Eosin. The protocol of Histology Department, Faculty of Medicine UI was used as the procedure of staining in this study.

**Immunohistochemistry**

Immunohistochemistry was performed on paraformaldehyde-fixed, paraffin-embedded samples based on the manufacturer’s instructions (My BioSource and LifeSpan BioScience). As primary antibodies, rabbit antibody anti-Cygb 1:1000 was used. In immunostaining to bind the primary antibody, 4 drops of Trekkie Universal Link (BioCare Medical; https://biocare.net/wp-content/uploads/STUHRP700.pdf) was used as the secondary antibody. HRP streptavidin molecules were used as the marker molecules that bind to secondary antibodies. HRP molecules would be detected by DAB Chromogen dye and visualized by ImageQuant.

**Data collection**

Data were collected as Cygb and PGC-1α (mRNA) gene expression, Cygb protein by immunohistochemistry, and tail growth length.

**Statistical analyses**

The normally distributed data are presented as the mean ± SE. One-way ANOVA was used to analyse the value of the Cygb, PGC-1α mRNA expression, and the length of the tail to the control group; the correlation...
between the values of Cygb and PGC-1α mRNA with the length of the tail using Spearman test. Differences were considered to be statistically significant at the 0.05 level of confidence.

**Results**

The growth length of the house gecko tail was measured from the proximal to distal portion of the tail on each observation day. The results of the tail regeneration are shown in Fig. 1 showed that growth of 33 house gecko (*Hemidactylus platyurus*) tail from day 1 to day 30. The results show different patterns for each of group of observation. The tail growth was relatively slow from day 1 until day 13, and then, it increased sharply from day 13 until day 21. However, from day 21 to day 30 the growth again became slower. In general, the growth curve of the house gecko tail regeneration is significantly different from day 1 to day 30 (p < 0.05) according to the Kruskal-Wallis test.

**Histological Observation**

Day 1 until day 10 is the wound-healing period and the remodelling phase. The cells in this period were active during proliferation, differentiation, and migration. In this phase, the epithelial layer was formed, closing the wound area. The fibroblasts spread in the dermis, and the nerve ganglion cells were formed. The formation of the endothelial cells of the blood vessels in the endodermis occurred on day 5, and the endothelial cells were enlarged on day 8. The epidermis and basal lamina cells grew and became thicker. On day 10, granules with stem cells appeared in the dermis, and new blood vessels appeared in some tissues (Fig. 2 A-E).

The regenerating tissue entered the granulation phase on day 13 until day 17. The blood vessel grew faster to prepare the tissue for the regeneration of the house gecko tail. The granules with stem cells began to spread more rapidly than before in the dermis. The epidermis and dermis thickened (Fig. 2 F-G).

In the maturation phase, the adipose tissue and muscle tissue in the regenerating tail tissue grew faster. The epidermis and dermis became more compact. The bulge formation along the epithelial layer occurred on day 30 after autotomy (Fig. 2 H-I).

**Immunohistochemistry**

In immunohistochemistry assays, the Cygb proteins spread to several cells from day 1 until day 30 after autotomy began. The Cygb protein was in the nucleus of the epithelial cells, neuron cells, adipose cells, fibroblast-like cells, muscle cells, and red blood cells (Fig. 3). The spread of the Cygb protein occurred during tissue regeneration of the house gecko tail.
Expression of Cygb and PGC-1α mRNA

The expression of Cygb mRNA during the tail tissue regeneration of the house gecko was relatively higher than that of the control (C) from day 1 to day 30 during the tissue regeneration process. The expression reached the peak on day 8 and decreased slightly until day 30, but the expression was still higher than that of the control (Fig. 4). The result of the ANOVA was obtained with \( p < 0.05 \), which showed that the expression of mRNA Cygb differed significantly on every growth day (additional file-Table 1).

The expression of PGC-1α mRNA on day 1 was relatively low. The expression increased sharply on day 3 and day 5, but on day 8, it decreased progressively until day 30. Although the expression of the PGC-1α decreased, its expression was higher than that of the control (Kruskal-Wallis test, \( p \)-value < 0.05), which showed that the mRNA PGC-1α expression was varied each day of the observation (Fig. 5). The variation in the data of each group was identified using the Man-Whitney test (additional file-Table 2).

A comparison of the curves of the mRNA Cygb and mRNA PGC-1α expression (Fig. 6) showed different patterns. The mRNA Cygb expression was higher than the mRNA PGC-1α expression, but the expression of the PGC-1α increased before that of Cygb. Whereas the PGC-1α expression increased sharply on day 3 and day 5, the Cygb expression had not yet increased significantly. When the expression of the Cygb rose significantly and reached the peak on day 8, the expression of the PGC-1α had decreased. Although the expression of PGC-1α had decreased by day 8, its expression was relatively higher than that of the control during the study, as well as being greater than the expression of the mRNA Cygb, which also was still relatively higher than that of the control until day 30 (Fig. 6).

The correlation between mRNA expression and the length of the tail

The correlation between the mRNA Cygb expression and tail length growth was significant at \( p < 0.05 \), with a negative value (\( r = -0.388 \)). The correlation with the PGC-1α mRNA (\( r = -0.465 \)) was significant at \( p < 0.05 \).

Discussion

This research is very important as an initial research into the molecular biology of the tissue regeneration process. Studies on Cygb and PGC-1α expression in tissue regeneration process are very limited. The house gecko (\textit{Hemidactylus platyurus}) from the Geckonidae family was used as a model animal because of its ability for autotomy and its ability in the tissue regeneration process. Furthermore, the taxonomy of the house gecko is closest to mammals of all animals that have a high ability for tissue regeneration. [18] [19] [20]

The tissue regeneration process in the house gecko is relatively fast and needs only 30 days to completely regenerate the tail tissue. The growth time of gecko tail is almost similar as the growth of lizard tail. [21] The tail is an organ that is composed completely of tissue, including the epithelium,
dermis, muscles, bones, nerves, connective tissue, blood, and adipose tissue. Accordingly, the house gecko tails are suitable as a simple model organ for the study of the tissue regeneration process.

The growth length of the house gecko tail showed three different phases. The growth in the first 13 days was relatively slow, the growth on day 13 until day 21 increased significantly, and on day 21 to day 30 the growth slowed again. These three different phases showed the differences of cell and tissue activity. This is also similarly observed in the growth of lizard tail, with first phase indicates wound healing, followed by granulation in the second phase, and maturation in the third phase.

The first 13 days of wound healing phase is the period of tissue regeneration process, indicated by the high activity of cells. The characteristics of this phase include cell migration, proliferation, and differentiation. The initial phase of wound healing involved the inflammatory process and wound remodelling. According to Mescher, the wound-healing phase occurs at the beginning of regeneration and is characterized by the process of cell proliferation, migration and differentiation of macrophages, fibroblasts, and progenitor cells.

The high activity of cells in this period showed that cells need oxygen and high energy. In the planaria regeneration process, the cells do proliferate and differentiate, requiring more energy. For regeneration, cells require a good supply of oxygen. Likewise, in regeneration of the house gecko tail, an increase in the requirements for high energy causes an increase in oxygen demand for the metabolic processes to produce the energy. Actually, beginning with day 1, no blood vessel was present to distribute oxygen. The supply oxygen was poor, but the activity of the cells was still high. The high expression of Cygb in wound healing phase of the observations shows an effort by the tissue to meet the need for oxygen. Although the Cygb expression increased slowly to reach its peak, its expression remained higher than the control until day 30. When the Cygb expression reached its peak, the activity of the fibroblasts and nerve cells was high, as shown in the histological analysis. This result suggests that the increase in the Cygb expression indicated its role in supplying oxygen to the respiratory chain within the mitochondria. The high expression of Cygb in the tissue regeneration process until day 30 likely maintained the oxygen in the tissue. In a study conducted by Avivi on the brains of Spalax mole rats, mRNA and Cygb protein were present when the brain was in a normoxic state, and their presence increased when the brains experienced hypoxic conditions. According to Schmidt, the presence of a protein of Cygb in hypoxia is needed because it can bind the oxygen from red blood cells and carry it into the mitochondria. Cygb protein is one of the extra erythrocyte haemoglobins other than the myoglobin (Mb) in muscle tissues and neuroglobin (Ngb) in neuron cells.

The result of immunohistochemistry showed that Cygb proteins spread in various cells. With immunohistochemistry staining, Cygb proteins were found in epithelial cells, red blood cells, muscle cells, and neurons. Cygb has a role in oxygen storage according to the research of Schmidt, who found Cygb in tissue fibrosis when the oxygen supply was reduced. Another function of Cygb is as an oxygen sensor. Cygb can regulate the activity of proteins that are able to respond to the change of oxygen levels in the tissue. Cygb is also thought to be involved in the synthesis of extracellular matrix proteins, such as
collagen, as evident from the presence of Cygb in a number of fibroblasts, chondroblasts, and osteoblasts. Cygb contributes to lipid cell signalling, thus increasing the expression of antioxidants and acts as a protector from oxidative stress such as that provided by the hydrogen peroxidase enzyme. [14] In a study using Spalax rats it was found that Cygb was found to spread in neurons and fibroblasts. [24] Singh found Cygb in fibroblasts that would form connective tissue. They also proved the role of Cygb in skeletal muscle regeneration because it was found in the nucleus lamina basal cells during the regeneration process of skeletal muscles and because the basal cells of lamina, which lack Cygb, experienced disruption in the process of cell proliferation and differentiation. [25]

The correlation of Cygb mRNA expression and the tail length of the house gecko was significant and negative. The negative correlation indicated that the expression of these genes stimulated the growth of the tail indirectly, and that growth occurs only after the expression of Cygb mRNA. This finding suggests that, after the Cygb diffused oxygen to mitochondria, the cells were activated to proliferate, migrate, and differentiate, with the subsequent tail growth being significant. Because a high need for oxygen exists, and the oxygen from the environment does not increase, the tissue remains in a relatively hypoxic state. According to Gauron, an imbalance of oxygen demand with oxygen supply causes the tissue to experience a hypoxic state. [2] Cygb is able to store oxygen and will release it under hypoxic conditions. [11] [12] Therefore, Cygb is likely involved in adaptive responses to injuries. The Cygb protein is a member of the hexacoordinate globin family, which has a strong oxygen-binding ability. [11] [12] The affinity of Cygb to oxygen is so great that it is thought that Cygb has a role as a factor in oxygen diffusion to the mitochondria. Cygb will carry oxygen into the mitochondria for the oxidative phosphorylation reaction, as well as the function of myoglobin in muscle cells. This function is the same as that of myoglobin in the muscle cells that play a role in maintaining the oxidative phosphorylation process. [10] [12] [13]

It has been observed that high energy level would be required to support the high activity of cells in the tissues. [7] The expression of PGC-1α on the first day of observation was relatively low. The expression of PGC-1α increased progressively and reached its peak earlier than Cygb. This indicates an increase in mitochondrial biogenesis, which shows a high need for energy. Jornayvaz proved for the first time that PGC-1α protein plays a role in stimulating mitochondrial biogenesis so that it can increase energy in tissues. [17] A study by Osuma showed an increase in glycolysis reactions for tissue regeneration in planaria. [7] In our opinion, the amount of energy needed for the regeneration processes is insufficient if taken only from the glycolysis process, and energy is also required from an aerobic metabolic process that produces also produces 36 ATP. Therefore, this regeneration process requires many cellular organelles in the form of mitochondria. This need was indicated by the increased expression of PGC-1α. The expression of PGC-1α was increased to maintain and regulate the differentiation process of the various cells. The expression of the PGC-1α gene decreased at second phase of tissue regeneration, but its expression was maintained above that of the control until the last day of observation. Therefore, the cell has sufficient energy during the tissue regeneration process. The expression of the PGC-1α gene was maintained to keep the high energy supply in the tissue until day 30. Gene expression on each observation day was significantly different, proving that the expression of the PGC-1α gene was active.
The synergy of the Cygb and PGC-1α expression shows an interesting pattern in tissue regeneration process. The peak of PGC-1α expression occurred earlier, and the increase of Cygb expression occurred after the day peak of PGC-1α. This interesting expression pattern shows that the tissue tried to meet the needs of the number of mitochondria in the cell before the Cygb expression reached its peak, which then supported oxygen diffusion into the mitochondria. The peak expression of the PGC-1α and Cygb genes occurred in the first phase of wound healing, which showed that this phase requires high oxygen and energy. Cell migration, cell proliferation, and cell differentiation occurred in preparation for the regeneration phase after wound healing phase and stimulated the expression of Cygb and PGC-1α to meet oxygen and energy needs before the tissue regeneration phase began. Nakatani et al. ported that the Cygb presence in hypoxic tissue that Cygb stores oxygen in this tissue. [26] Cygb also acts as an oxygen sensor that regulates the protein activity in response to oxygen level changes in tissue. Cygb is also thought to be involved in extracellular matrix protein synthesis, such as collagen, as Cygb was found in fibroblasts, chondroblasts, and osteoblast. In addition, Cygb contributes to lipid cell signalling, thus increasing the expression of antioxidants and acts as a protector from oxidative stress such as that caused by the hydrogen peroxidase enzyme. [27]

In the second phase, some new tissue appeared, making the tissue more compact and complex. According to Alibardi, cell granulation that contained stem cells formed in this phase and had a role in the formation of new blood vessels, new connective tissue, new muscle tissue, and the other tissues. [28] The stem cells aggregated in the tail tissue of the house gecko, appearing in the dermis in this phase, and has a role in forming the muscle tissue, connective tissue, blood vessels, and adipose tissue. Cygb expression is still high in this phase to maintain the oxygen in the tissue.

It was observed that the third phase acted as the actual tissue regeneration period. In this period, the various new tissues were formed, and the spinal cord tissue became more elongated. Therefore, this phase resulted the significant growth of tail tissue of the house gecko. The morphogenesis of the new tail tissue began, forming the thorns in the skin tail, membranes of skin, and the other tail accessories. The tail length did not show significant growth in this period.

It was found in this study that dynamics of Cygb and PGC-1α gene expression play an important role in the tissue regeneration process of house gecko tail. It was concluded that tissue regulation in house gecko tail will occur when the right tissue carries out mitochondrial biogenesis and when the appropriate tissue uses oxygen for cell respiration. However, the results of this study cannot be directly interpolated to humans due to the animal model used in this study is based on species with distant taxonomic levels to human, mainly chosen because of its high tissue regeneration capability. It is therefore recommended that this study be further pursued using mammalian experiments, attempting at stimulation of gene expression (including the Cygb and PGC-1α), so that it might be implicated in human.

Conclusions
The high expression of Cygb showed the high oxygen demand for cell metabolism during tissue regeneration. The expression of PGC-1α earlier than Cygb expression showed a role for PGC-1α in the formation of mitochondria, which were required to support cell respiration and metabolic processes. The pattern of Cygb and PGC-1α expression curves were observed to be dynamic, indicating that it correlated with the tissue regeneration function in house gecko tail.

**Abbreviations**

**ATP**: Adenosine Triphosphate; **Cygb**: Cytoglobin; **DNA**: Deoxyribonucleic acid; **HE**: Haematoxylin-eosin; **HRP**: Horseradish Peroxidase; **mRNA**: messenger Ribonucleic Acid; **mtTFA**: mitochondria Transcription Factor A; **Nrf-1**: nucleus regulates nuclear respiratory factor; **O\(_2\)**: Oxygen; **PGC-1α**: peroxisome proliferator-activated receptor gamma coactivator-1α; **qPCR**: quantifying Polymerase Chain Reaction.

**Declarations**

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**Availability of data and material**

All data generated or analysed during this study are included in this published article [and its supplementary information files]

**Authors’ contributions**
MSA and SWJ contributed in the formulation of idea and experimental design used in this research. TNO contributed in conducting the research, analysis of data and writing of the first draft of the manuscript. EAA contributed to providing permission and ensuring appropriate use of animal model in the research. All authors contributed in the performing of the experiments and acquisition of data. All authors read, reviewed the manuscript, involved in its critical revision before submission, and approved the final manuscript.

**Ethics approval and consent to participate**

House gecko were collected under permits issued by Zoology Laboratory Indonesian Institute of Sciences (LIPI) Cibinong, cq. Herpetologist, Dr rer. nat. Evy Ayu Arida), and all animal experimental procedures were approved by Health Research Ethics Committee Faculty of Medicine Universitas Indonesia-Cipto Mangunkusumo Hospital no. 672/UN2.F1/ETIK/VII /2. The committee give the permission for using house gecko (*Hemidactylus platyurus*) as an animal model in this research (Chairman Prof Dr. dr Rianto Setiabudy, SpFK and Vice Chairman Prof. dr. RitaSita Sitorus, SpM(K), PhD.)

**Consent for publication**

Not Applicable

**Competing interests**

The authors declare that they have no competing interest

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**Additional files**

File 1:

**Additional Fig. 1** The growth of house gecko (*Hemidactylus platyurus*) tail on day-3 (A) and on day-13 (B) after autotomy

File 2:

**Table 1.** Post hoc test of varied data of Cygb mRNA between each growth-day group

(p < 0.05)
Table 2. Man-Whitney test (p value) for each group between groups for PGC-1α

Table 3. The results for primer DNA of Cygb, PGC-1α, and 18S genes designed by using multiple alignment

Author’s information

TNO graduated from Doctoral Biomedical Program, Faculty of Medicine University of Indonesia and the lecturer of Biotechnology Department, Esa Unggul University, Indonesia

VJT a researcher and lecturer in Department of Pathology, Faculty of Animal Health, Agriculture Institute of Bogor, Indonesia

AAJ a researcher and lecturer in Department of Histology, Faculty of Medicine, Universitas Indonesia

EAA an herpetologist researcher in Zoology Laboratory of Indonesian Institute of Sciences (LIPI), Cibinong -Bogor, Indonesia

MSA and SWA are the Professor at Department of Biochemistry & Molecular Biology, and researchers at Center of Hypoxia and Oxidative Stress Studies (CHOSS) Faculty of Medicine Universitas Indonesia

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Figures
Figure 1

The growth of the house gecko tail (cm) from day 1 to day 30. * indicates significantly different growth between two periods of growth—between day 13 and day 17 and between day 17 and day 21 (p < 0.05) with the Kruskal-Wallis test.

Figure 2

Histological analysis of regeneration of lizard tail tissue after autotomy On day 1, an epithelial layer was formed, closing the wound area (single arrow), and fibroblasts (double arrow) spread in the dermis; (B). The nerve ganglion cells formed on day 3 (single arrow); (C). On day 5, the endothelial cells of the blood vessels formed in the endodermis (single arrow); (D). On day 8, the endothelial cells were enlarged (single arrow), in the epidermis (double arrow), shown here with the basal lamina cells (short arrow); (E). On day 10, the stem cells are aggregated; (F). On day 17, blood vessels can be observed in the regenerating tissue (single arrow); (G). Day 21, adipose tissue (double arrow) and muscle tissue (single arrow) are apparent in the regenerating tail tissue; (H). Day 25, epidermis is more compact; (I). A bulge forms in the epidermis cells on day 30. The magnification was 40 x 10.
Figure 3

Analysis of immunohistochemistry for the Cygb protein in tissue regeneration of the house gecko tail (A). Positive control using duodenal mice; (B) Negative controls; (C) Cygb protein found in the red blood cell on day 1 (D) Cygb spread in nerve cells on day 3; (E) Cygb protein in muscle cells on day 5 (F) Cygb protein spread along epithelial cell on day 8. (The magnification was 40 x 10)

![Cygb Expression Graph](image)

Figure 4

Expression of Cygb mRNA from day 1 until day 30. n =30, (mean ± SEM), the distribution of the data is normal (p >0.05), and the data varied between growth days according to ANOVA (p <0.05).
Figure 5

mRNA PGC-1α expression relative to the control (K) from day 1 until day 30. n =30, the distribution data was not normal (p< 0.05), and the data varied between growth days using the Kruskal-Wallis test (p< 0.05) between data from day 1 and day 3.
Figure 6

The comparison curve between mRNA Cygb and mRNA PGC-1α expression from day 1 to day 30 in tail tissue regeneration of the house gecko (Hemidactylus platyurus). The Cygb expression was high during the tissue regeneration process, and the PGC-1α expression was higher on day 3 to day 5 and had decreased by day 8, but the expression was still higher than that of the control during the tissue regeneration process.
Figure 7

The correlation test between mRNA expression of Cygb and PGC-1α with the growth of tail length. (A) The correlation of the mRNA Cygb and length of tail is -0.388 (B) The correlation of the mRNA PGC-1 and length of tail is -0.465. These correlations were significant at p < 0.05.

Figure 8

A tail-autotomized house gecko (Hemidactylus platyurus) on day 1 post-autotomy.
Supplementary Files

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