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The changes of age-related molecules in the trophocytes and fat cells of queen honeybees (*Apis mellifera*)

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Abstract – Queen honeybees (*Apis mellifera*) have much longer lifespans than worker bees. Although the expression of age-related molecules in the trophocytes and fat cells of young and old workers have been determined, the expression of age-related molecules in queens is unknown. In this study, we examined the expression of age-related molecules in the trophocytes and fat cells of young and old queens. Molecular analyses detected no differences in telomerase activity or telomere lengths between trophocytes and fat cells from young and old queens, indicating that these cells do not divide in adulthood. Further assays showed that old queens has higher non-homogeneous cellular morphology, senescence-associated β -galactosidase (SA- β -Gal) activity, accumulation of lipofuscin granules, lipid peroxidation, and protein oxidation compared to young queens. These results demonstrate that age-related molecules can be used to evaluate aging in the trophocytes and fat cells of queens and to lay the foundation for further study of the mechanisms that delay cellular aging in the trophocytes and fat cells of queens.

aging / trophocyte / fat cell / queen / honeybee

1. INTRODUCTION

Cellular senescence is a complicated process of progressive deterioration. Aging leads to a gradual diminishing of biological functions and increased incidences of age-associated diseases. Understanding the biology of aging will not only clarify the mechanisms involved in life-span extension, but can also lead to the improved health of organisms.

Age-related molecules have been used as indices of aging in many organisms (Tian et al. 1998; Kishi et al. 2003; Genade et al. 2005; Zheng et al. 2005; Hsu et al. 2008; Hsu and Chiu 2009). These age-related molecules include SA- β -Gal (Dimri et al. 1995), lipofuscin granules

(Nakano et al. 1995), lipid peroxidation (Draper and Hadley 1990; Inal et al. 2001; Almeida et al. 1998), protein oxidation (Oliver et al. 1987; Welis-Knecht et al. 1993), telomerase activity (Davis et al. 2005; Argyle et al. 2003), and telomere length (Davis et al. 2005; Hastings et al. 2004; Argyle et al. 2003). Numerous studies have shown that the expression of age-related molecules in cells and tissues increases or decreases with advancing age. Accordingly, the fluctuation of age-related molecules has been used to evaluate the degree of aging in organisms (Mecocci et al. 1999; Hsu et al. 2008; Hsu and Chiu 2009; Hsieh and Hsu 2011).

Honeybees (*Apis mellifera*) are an excellent model system for studying aging because they live in large colonies, they are easily manipulated, and their genome has been sequenced. Therefore, honeybees have been evaluated in

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many studies on aging (Remolina et al. 2007; Corona et al. 2007; Amdam and Page 2005; Amdam et al. 2004; Tofilski 2000; Robertson and Gordon 2006; Jemielity and Keller 2007; Rueppell et al. 2007a). A variety of honeybee specimens, including spermathecae, muscle, ventriculi, hemolymph plasma, semen, brain, trophocytes, and fat cells, have been used to study oxidative stress (Weirich et al. 2002; Seehuus et al. 2006a, b; Williams et al. 2008; Collins et al. 2004) and aging (Hsieh and Hsu 2011).

Especially, workers and queens develop from the same genome, but workers have a short lifespan (15–38 days in the summer and 150–200 days in the winter) while queens have a long lifespan (1–2 years, with a maximum of up to 8 years) (Page and Peng 2001; Omholt and Amdam 2004; Rueppell et al. 2007b; Remolina and Hughes 2008). This provides a unique resource for studying the causes of longevity. Comparing workers and queens, the following differences have been established:

- (1) queens eat royal jelly, whereas workers eat glandular secretions, honey, and pollen (Winston 1987).
- (2) queens live in the hive except during mating flights and colony fission. They generally lay 1,500–2,000 eggs per day throughout their lives (Camazine 1991). In contrast, workers spend only the first 2 weeks of life in the hive. Workers perform tasks such as comb-building, cell-cleaning, and brood-nursing before transitioning to the foraging stage. In the foraging stage, workers fly up to 21 km per day to collect nectar and pollen for the hive (Neukirch 1982).

We recently assayed the expression of age-related molecules in the trophocytes and fat cells of young and old workers (Hsieh and Hsu 2011). Trophocytes, which are large and irregularly shaped, and fat cells, which are small and spherical, attach to one another to form a single layer of cells around each segment of the honeybee abdomen. Trophocytes and fat cells are immersed in body fluid, and have the

potential to be used to test anti-aging drugs when drugs are microinjected into the body fluid (Hsieh and Hsu 2011). Therefore, trophocytes and fat cells are good target cells for cellular senescence studies. The assay of age-related molecules in the trophocytes and fat cells of workers showed that old workers exhibited more non-homogeneous cellular morphology, SA- β -Gal, lipofuscin granules, lipid peroxidation, and protein oxidation than newly emerged workers (Hsieh and Hsu 2011). In this study, we examined the expression of age-related molecules in the trophocytes and fat cells of young and old queens.

2. MATERIALS AND METHODS

2.1. Queen honeybees

Queen honeybees (*Apis mellifera*) were purchased from a single commercial breeder (Hsinchu, Taiwan) from different hives, and collected on the same dates for the same experiments in May. The age of young queens was approximately 2 months old. The age of old queens was about 16 months old.

2.2. Light microscopy (LM)

Trophocytes and fat cells were freshly isolated from a young and an old queen, fixed in 4% formaldehyde at room temperature, mounted onto glass slides, and viewed under LM (Olympus BX-61, Tokyo, Japan). This experiment was repeated three times.

2.3. Transmission electron microscopy (TEM)

Trophocytes from a young and an old queen were fixed in 2.5% glutaraldehyde in a 0.1 M phosphate buffer containing 0.35 M sucrose at pH 7.4 for 30 min at 25°C, and postfixed in 1% osmium tetroxide in a 0.1 M phosphate buffer with 0.35 M sucrose at pH 7.4 for 2 h. Trophocytes were dehydrated via an ethanol series and embedded in Spurr's resin. Thin sections (60–90 nm thick) were cut with a diamond knife, stained with uranyl

acetate and lead citrate, and then examined using a TEM (JEOL JEM-2000EXII, Tokyo, Japan), operating at an accelerated voltage of 100 kV (Hsu and Li 1993; Hsu 2004). This experiment was repeated three times.

2.4. Telomerase activity assay

Telomerase activity was measured using the telomere repeat amplification protocol (TRAP) by using the TRAPeze XL Telomerase Detection Kit (Chemicon, Temecula, CA, USA; S7707). Briefly, 0.2 g of trophocytes and fat cells was isolated from three young and old queens, treated with 1.5 ml ice-cold lysis buffer (0.5% 3-([3-cholamidopropyl]-dime-thylammonio)-1-propane sulfonate (CHAPS), 10 mM Tris-HCl, pH 7.5, 1 mM MgCl₂, 1 mM EGTA, 10% glycerol, 5 mM β -mercaptoethanol, and 1 mM PMSF), then incubated on ice for 30 min. After centrifugation at 15,000 g for 30 min at 4°C, DNA concentration in the supernatant was measured by UV absorbance before it was stored at -80°C. Telomerase extension products (2 μ g/ μ l) were amplified by a three-step PCR (94°C for 30 s, 59°C for 30 s, 72°C for 60 s) for 38 PCR cycles with TS primer (5'-AATCCGTCGAGCAGAGTT-3') and CX primers (5'-CCCGGGCCTAACCTAACCTAA-3' for bees) for negative control, young queens, and old queens. Positive control (293 T cells) was mixed with TS primer and CX primers (5'-GCGCGGCTAACCC TAACCCTAACCTACCC-3' for mammal). These products were resolved in 10% polyacrylamide (Kim and Wu 1997). Telomerase was quantified by normalizing the band intensities of the characteristic every 6-bp telomerase-specific ladder band by using Image J software (NIH, Bethesda, MA, USA). This experiment was repeated three times.

2.5. Telomere length assay

Telomere length analysis was performed by Southern blot hybridization using the Telo TAGGG Determination Kit (Roche, Mannheim, Germany; 12209136001). Briefly, high-molecular-weight genomic DNA was isolated from trophocytes and fat cells from three young and old queens by using the EasyPure Genomic DNA Mini Kit (Bioman, Taipei, Taiwan; EP-2500), digested with proteinase

K and RNase in lysis buffer (10 mM Tris-HCl, 100 mM NaCl, 100 mM EDTA, 0.5% SDS and 0.1 mg/ml RNase), extracted with isopropanol-ethanol, and verified by standard agarose gel electrophoresis. Next, 0.5 μ g/ μ l of genomic DNA was digested with Hind III at 37°C for 3 h and subjected to 0.8% agarose gel electrophoresis for 4 h at 75 V. Digested DNA was then transferred to Genescreen Plus Hybridization Transfer Membrane (PerkinElmer, CA, USA; NEF986001PK). A dig-labeled oligonucleotide probe (5'-(GGATT)₅-dig-3') was hybridized to the membrane, and detected using enhanced chemiluminescence (TeloTAGG Telomere Length Assay) (Okazaki et al. 1993). Telomere length was analyzed according to the manufacturer's protocol (Roche, Mannheim, Germany; 12209136001) with ImageQuant image analysis software (version 5.2, Molecular Dynamics, CA, USA). This experiment was repeated three times.

2.6. SA- β -Gal expression assay

SA- β -Gal expression was carried out as described previously (Dimri et al. 1995). Briefly, trophocytes and fat cells were isolated from a young and an old queen, fixed in 4% paraformaldehyde at room temperature, immersed in SA- β -Gal staining solution at 37°C, washed with PBS, mounted onto glass slides, and viewed under LM (Olympus BX-61, Tokyo, Japan). SA- β -Gal area was determined using QWin image processing and analysis software (version 2.5, Leica, Wetzlar, Germany). This experiment was repeated three times.

2.7. Lipofuscin granules assay

The level of lipofuscin was determined as previously described (Brunk and Terman 2002). Briefly, trophocytes and fat cells were isolated from a young and an old queen, mounted onto glass slides, and viewed under a confocal laser scanning microscope (Leica TCS SP2; Leica, Wetzlar, Germany) with blue (450–490 nm) excitation light and a 520-nm emission filter at room temperature. The area of lipofuscin expression was determined using QWin image processing and analysis software (version 2.5, Leica, Wetzlar, Germany). This experiment was repeated three times.

2.8. Lipid peroxidation assay

Sample preparation was carried out as previously described (Hsieh and Hsu 2011). Briefly, trophocytes and fat cells from three young and old queens were freshly homogenized in 1.3 ml of phosphate buffer containing protease inhibitors (leupeptin, pepstatin and phenylmethylsulfonyl fluoride) and centrifuged at 5000g for 10 min at 4°C. The resulting supernatant was immediately used for the assays described below. Protein concentration was determined using the protein assay reagent (#500-0006, Bio-Rad, Hercules, CA, USA). Lipid peroxidation assay was carried out as previously described (Hsieh and Hsu 2011). The fresh supernatant described above was mixed with trichloroacetic acid (TCA) and thiobarbituric acid (TBA). The absorbance of resulting mixture was then measured using a Thermo spectrophotometer at a wavelength of 532 nm at room temperature. Malondialdehyde (MDA) concentration was calculated by multiplying the absorbance by the MDA-TBA complex absorbance coefficient ($1.56 \times 10^5 \text{ cm}^{-1} \text{ M}^{-1}$), and expressed as nanomoles per gram of protein. This experiment was repeated three times.

2.9. Protein oxidation assay

Protein oxidation assay was carried out as previously described (Hsieh and Hsu 2011). Briefly, the fresh supernatant described above was mixed with 2,4-dinitrophenylhydrazones (DNPH) in 2 M HCl or 2 M HCl alone (control), incubated for 1 h at room temperature, precipitated with TCA, and centrifuged

at 7,800 g for 3 min. The resulting pellet was washed with ethanol/ethyl acetate and dissolved in 6 M guanidine (pH 2.3). Protein oxidation, expressed as nanomoles of carbonyl groups per mg of protein, was calculated using the differential absorbance of experimental (DNPH in HCl) and control (HCl) samples at a wavelength of 370 nm, and the extinction coefficient of aliphatic hydrazones ($22.0 \text{ mM}^{-1} \text{ cm}^{-1}$). This experiment was repeated three times.

2.10. Statistical analysis

Differences in mean values between the two age groups were examined using two-sample *t*-test. Differences in telomerase activity were determined by one-way ANOVA and Tukey's HSD for pairwise comparisons. A *p*-value of less than 0.05 was considered statistically significant. *N* represents the number of independent experiments.

3. RESULTS

3.1. Cellular morphology of trophocytes and fat cells in young and old queens

The morphology of trophocytes and fat cells under LM showed a greater accumulation of brown material in old queens than in young queens (Figure 1a, b). Trophocytes were further examined under TEM, old queen trophocytes showed loose organelle morphology, dark organelle inclusion, a crowded distribution of

Figure 1. The LM images showing the morphology of the trophocytes and fat cells of a young queen (a) and an old queen (b). Black arrows point to trophocytes. White arrows point to trachea. Arrowheads point to fat cells. Scale bar, 50 μm .

organelles, and oil accumulation compared to young queens (Figure 2a, b).

3.2. Telomerase activity and telomere length do not change with age in trophocytes and fat cells

To explore whether the trophocytes and fat cells of queens can divide, we assayed telomerase activity and telomere length in the trophocytes and fat cells of young and old queens. All queens showed a telomerase amplification pattern similar to the negative control, indicating a lack of telomerase activity in these cells (Figure 3a). Statistical analyses further confirmed these results ($P < 0.01$) (Figure 3c). Telomere lengths were the same in young and old queens, suggesting that telomere length does not change with age. Telomere lengths ranged from 8.89 ± 0.01 kb to 53.27 ± 0.20 kb in young queens and 8.86 ± 0.03 kb to 53.16 ± 0.51 kb in old queens (Figure 3b). The average telomere length was 17.43 ± 0.90 kb in young queens and 16.89 ± 1.02 kb in old queens (Figure 3d). These results indicate that trophocytes and fat cells in queens do not divide during adulthood.

3.3. SA- β -Gal and lipofuscin granule accumulation increase with age in trophocytes and fat cells

To evaluate the expression of age-related molecules in trophocytes and fat cells, we analyzed the expression levels of SA- β -Gal and lipofuscin in young and old queens. SA-

β -Gal activity was not detected in the trophocytes and fat cells of young queens; however, SA- β -Gal activity was clearly observed in cells of old queens (Figure 4a). Statistical analyses revealed that the area within trophocytes and fat cells that stained positive for SA- β -Gal activity increased with age ($P < 0.05$), indicating that SA- β -Gal expression in these cells is associated with aging (Figure 4b). Lipofuscin granules showed little to no accumulation in the trophocytes and fat cells of young queens, whereas lipofuscin granules accumulated to high levels in the cells of old queens (Figure 4c). Statistical analyses showed that the area within trophocytes and fat cells occupied by lipofuscin increased with age ($P < 0.05$), indicating that lipofuscin accumulation is associated with aging (Figure 4d).

3.4. Lipid peroxidation, protein oxidation, and ROS levels increase with age in trophocytes and fat cells

To further examine molecular damage in trophocytes and fat cells with age, we assayed lipid peroxidation and protein oxidation in young and old queens. Lipid peroxidation was assessed by determining the levels of MDA, a metabolite of lipid peroxidation. The mean MDA concentrations in trophocytes and fat cells were 0.13 ± 0.01 nmol/mg protein in young queens and 0.27 ± 0.01 nmol/mg protein in old queens ($P < 0.05$), suggesting that lipid peroxidation is associated with aging (Figure 5a). Protein oxidation was assessed by determining

Figure 2. The TEM image showing the morphology of trophocytes in young queens (a) and old queens (b). O, oil body. Scale bar, 1 μ m. Arrow in (b) shows mitochondria.

Figure 3. Telomerase activity and telomere length in the trophocytes and fat cells of young and old queens. **a** Telomerase activity. *P*: positive control (293 T cells); *N*: negative control; *Y*: young queens; *O*: old queens; *M*: marker. **b** Telomere lengths. *M*: marker; *Y*: young queens; *O*: old queens; *asterisks*: bands from the manufacturer's marker. **c** Quantification of telomerase activity in **a**. Bars represent mean $\pm$ SEM ($N=3$ for each group). Asterisks indicate statistical significance as determined by one-way ANOVA (** $P < 0.01$). **d** Quantification of telomere length in **b**. Bars represent mean $\pm$ SEM ($N=3$ for each group).

the amino acid carbonyl contents using DNPH. The mean carbonyl-group levels in trophocytes and fat cells were 2.19 ± 0.20 nmol/mg protein in young queens and 7.04 ± 0.28 nmol/mg protein in old queens ($P < 0.01$), indicating that protein oxidation is associated with aging (Figure 5b).

4. DISCUSSION

In this study, we compared the cellular morphology and expression levels of age-related molecules in the trophocytes and fat

cells of young and old queen honeybees. Telomerase activity and telomere lengths showed no obvious differences with age, indicating that the trophocytes and fat cells do not divide in adulthood. Assays that measured age-related molecules showed that the cells of old queens had increased SA- β -Gal expression, lipofuscin granules accumulation, lipid peroxidation, and protein oxidation compared to young queens. These results indicate that age-related molecules can be used to evaluate aging in the trophocytes and fat cells of queens.

Figure 4. SA- β -Gal activity and lipofuscin granule accumulation in the trophocytes and fat cells of young and old queens. **a** Cellular SA- β -Gal activity was monitored by an enzymatic assay that employed X-gal as the substrate. Higher SA- β -Gal expression is associated with a more intense blue stain. *Arrows* point to trophocytes. *Arrowheads* point to fat cells. Scale bar, 25 μ m. **b** Quantification of area that stained positive for SA- β -Gal activity. *Bars* represent mean $\pm$ SEM ($N=3$ for each group). *Asterisks* indicate statistical significance as determined by the two-sample t -test (* $P<0.05$). **c** Bright-green-colored dots indicate lipofuscin granules. Micrographs were captured using fluorescence (lower panel) and bright field microscopy (upper panel). *Arrows* point to trophocytes. *Arrowheads* point to fat cells. Scale bar, 50 μ m. **d** Quantification of area that contained lipofuscin granules. *Bars* represent mean $\pm$ SEM ($N=3$ for each group). *Asterisks* indicate statistical significance as determined by the two-sample t -test (** $P<0.01$).

4.1. The cellular morphology of trophocytes and fat cells

We previously assayed the cellular morphology of trophocytes in young and old workers (Hsieh and Hsu 2011). The cellular morphology showed remarkable differences between young and old workers. These differences are due to the accumulation of damaged or dysfunctional

biological molecules and organelles (Hsieh and Hsu 2011). In this study, the cellular morphology of trophocytes and fat cells in young and old queens also showed obvious differences. These differences in cellular morphology and inclusion between young and old queens may result from age-related accumulation of damaged or dysfunctional biological molecules and organelles.

Figure 5. Lipid peroxidation (a) and protein oxidation (b) in the trophocytes and fat cells of young and old queens. Bars represent mean $\pm$ SEM ($N=3$ for each group). Asterisks indicate statistical significance as determined by the two-sample t -test (* $P<0.05$, ** $P<0.01$).

4.2. Telomerase activity and telomere length

Previous studies have shown that telomerase activity decreases with increased age in sheep fibroblasts (Davis et al. 2005) and in human germline and embryonic tissues (Wright et al. 1996). Furthermore, the absence of telomerase activity results in telomeric shortening in dogs (Nasir et al. 2001), donkeys (*Equus asinus*), and horses (*Equus equus*) (Argyle et al. 2003). However, telomerase activity exhibits no apparent relationship with age in the muscles of zebrafish (*Danio rerio*) (Kishi et al. 2003) or *N. rachovii* (Hsu et al. 2008). Among insects, including silkworms (*Bombyx mori*) (Sasaki and Fujiwara 2000), flour beetles (*Tribolium castaneum*) (Osanai et al. 2006), and worker bees (Hsieh and Hsu 2011), telomerase activity does not appear to be age-dependent. Similarly, this study found no difference in telomerase activity with advancing age in the trophocytes and fat cells of queens.

Insect telomeres consist of a simple repetitive DNA sequence, (TTAGG) $_n$, which differs from that of mammals, (TTAGGG) $_n$ (Okazaki et al. 1993; Sahara et al. 1999; Lorite et al. 2002; Osanai et al. 2006; Moyzis et al. 1988). Telomere shortening results in cellular senescence in rat cardiac tissue (Hastings et al. 2004), dog tissues (Nasir et al. 2001), sheep fibroblasts

(Davis et al. 2005), donkey and horse cells (Argyle et al. 2003), human cells (Lindsey et al. 1991), and muscles from *N. rachovii* (Hsu et al. 2008). Telomere lengths did not shorten with age in the trophocytes and fat cells of worker bees (Hsieh and Hsu 2011). Similarly, in this study, telomere lengths did not shorten with age in the trophocytes and fat cells of queens. Female ants (*Lasius niger*) also found that telomere length did not shorten (Jemielity et al. 2007).

The results of telomerase activity and telomere lengths indicate that trophocytes and fat cells in queens do not divide during adulthood. This finding corresponds to previous studies showing that trophocytes and fat cells in workers do not divide in adulthood (Hsieh and Hsu 2011; Ward et al. 2008). Therefore, the trophocytes and fat cells of honeybees are suitable for studying aging.

4.3. SA- β -Gal and lipofuscin granules

SA- β -Gal expression increases with age. It has been used as a marker to assess cellular senescence in dermal fibroblasts and epidermal keratinocytes (Dimri et al. 1995), endothelial and smooth muscle cells from human and rabbit blood vessels (van der Loo et al. 1998; Kurz et al. 2000), the skin and dermis of zebrafish (*Danio rerio*) (Kishi et al. 2003), the skin and

dermis of the annual fish *Nothobranchius furzeri* (Genade et al. 2005), the gills of the annual fish *Nothobranchius rachovii* (Hsu et al. 2008), and the trophocytes and fat cells of worker bees (Hsieh and Hsu 2011). In this study, the expression levels of SA- β -Gal in trophocytes and fat cells increased with the age of the queens, suggesting that cellular senescence increases with age. This result is consistent with previous studies (Dimri et al. 1995; van der Loo et al. 1998; Kurz et al. 2000; Kishi et al. 2003; Genade et al. 2005; Hsu et al. 2008) and is similar to the findings in worker bees (Hsieh and Hsu 2011).

Lipofuscin accumulates with age, and has been used as a marker in the evaluation of cellular senescence in the skin, dermis, and muscle of zebrafish (*Danio rerio*) (Kishi et al. 2003), brain and heart of rats (Nakano et al. 1995), neonatal cardiac myocytes of rats (Brunk and Terman 2002), liver and caudal peduncle of *N. furzeri* (Genade et al. 2005), gills of *N. rachovii* (Hsu et al. 2008), and the trophocytes and fat cells of worker bees (Hsieh and Hsu 2011). In this study, we found that the accumulation of lipofuscin in trophocytes and fat cells increased with the age of queens, indicating that cellular senescence increases with age. This result is consistent with previous studies (Kishi et al. 2003; Nakano et al. 1995; Brunk and Terman 2002; Genade et al. 2005; Hsu et al. 2008), and is similar to the findings in worker bees (Hsieh and Hsu 2011).

4.4. Lipid peroxidation and protein oxidation

Lipid peroxidation increases with age, and has been used as a marker in the assessment of cellular senescence in the adrenal glands of rats (Almeida et al. 1998), muscles of *N. rachovii* (Hsu et al. 2008), and the trophocytes and fat cells of worker bees (Hsieh and Hsu 2011). In this study, we found that lipid peroxidation increases with the age of queens, suggesting that cellular senescence increases with age. This result is consistent with previous studies (Almeida et al. 1998; Hsu et al. 2008) and

similar to the finding in worker bees (Hsieh and Hsu 2011).

Protein oxidation increases with age, and has been used as a marker in the examination of cellular senescence in the muscle of *Drosophila melanogaster* (Sohal et al. 1993; Das et al. 2001), brain and liver of rats (Tian et al. 1998), lens cells from humans (Welis-Knecht et al. 1993), muscles of *N. rachovii* (Hsu et al. 2008), and trophocytes and fat cells of worker bees (Hsieh and Hsu 2011). In this study, we found that protein oxidation increases with the age of queens, revealing that cellular senescence increases with age. This finding is consistent with previous studies (Sohal et al. 1993; Das et al. 2001; Tian et al. 1998; Welis-Knecht et al. 1993; Hsu et al. 2008), and is similar to finding in worker bees (Hsieh and Hsu 2011). Protein carbonylation can result from ROS, oxidative cleavage of proteins, and lipid-derived aldehydes (Draper et al. 1986). It is more commonly caused by lipid-derived aldehydes than amino acid side chain oxidation (Yuan et al. 2007).

Increased SA- β -Gal expression, lipofuscin granule accumulation, lipid peroxidation, and protein oxidation may reflect an age-related decline in the efficiency of degradation and repair processes (Terman and Brunk 1998a, b; Ozawa 1997; Richter 1995) and a gradual accumulation of tissue damage (Sitte et al. 2000a, b).

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Modifications des molécules liées à l'âge dans les trophocytes et les cellules adipeuses des reines d'abeilles (*Apis mellifera*)

Viellissement / trophocyte / cellule adipeuse / reine / abeille

Altersabhängige molekulare Veränderungen im Fettkörper von Königinnen der Honigbiene (*Apis mellifera*)

Alterung / Trophozyten / Fettzelle / Königin / Honigbiene

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