

Age- and sex-associated changes in prosaposin and its receptors in the lacrimal glands of rats

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Summary. Prosaposin, a saposin precursor, is a potent neurotrophic factor found in several tissues and various biological fluids. Saposin-deficient patients have different ophthalmic disorders, indicating a relationship between ocular health and prosaposin. However, there is little information about prosaposin on the ocular surface. Because ocular functions are diverse and depend on age and sex, we examined whether prosaposin and its receptors, G protein-coupled receptor 37 (GPR37) and GPR37L1, are expressed in the major ocular glands, the extra orbital lacrimal gland (ELG), and harderian gland (HG) of rats and whether sex and aging affect their expression. Immunohistochemical analyses revealed that prosaposin and its receptors were expressed in the ELGs and HGs of rats, although their expression varied based on the type of gland, age, and sex. Prosaposin, GPR37, and GPR37L1 were expressed in the basolateral membranes and cytoplasm of acinar cells of the ELGs, and their immunoreactivities were higher in female rats of menopausal age than age-matched male rats. However, such age- and sex-related differences in the immunoreactivities of prosaposin, GPR37, and GPR37L1 were not observed in the HGs. Triple immunofluorescence labelling revealed that prosaposin, GPR37, and GPR37L1 were co-localised in the acinar and ductal cells in the ELGs, although the degrees of colocalization varied according to the age and sex of the

rats. Together, the present results showed that prosaposin and its receptors were expressed in the major ocular glands of rats, and their immunoreactivities to the ELGs differed considerably with age and sex.

Key words: Prosaposin, Lacrimal glands, Aging, Sex, Rat

Introduction

The stimulation of lacrimal gland secretion occurs through a neural reflex arc originating from the ocular surface (Botelho, 1964). Nerves surrounding the basolateral side of the acinar cells in lacrimal glands initiate the secretion (Rocha et al., 2008). Dysregulation of lacrimal secretion causes various ocular surface diseases. For example, dry eye disease involves hypolacrimation due to aging, or autoimmune or sex-related diseases (Fox et al., 1984; Gayton, 2009). The prevalence of various ocular diseases differs between sexes, with postmenopausal females having a higher prevalence than males (Schaumberg et al., 2003; Fairweather et al., 2008; Gayton, 2009), indicating that lacrimal functions are altered with age and sex.

Previous studies have shown that numerous biologically active growth factors including neurotrophins, which are secreted by the lacrimal gland and distributed via tears over the ocular surface, have biological functions in the lacrimal system (Kasayama et al., 1990; Tervo et al., 1990; Wilson et al., 1991; Li et al., 1996; Klenkler et al., 2007). It has been suggested that neurotrophins are involved directly or indirectly in

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DOI: 10.14670/HH-18-137

the maintenance and stimulation of lacrimal glands in an autocrine and/or paracrine manner (Tepavcevic et al., 2003; Chen et al., 2005). Neurotrophic factors affect the proliferation, migration, differentiation, and survival of cells on the ocular surface (Kruse and Tseng, 1993; Gener-Galbis et al., 1993). Some of these factors are increased in lacrimal glands as well as in tears during ocular surface injuries (Tervo et al., 1997; Muzi et al., 2010). Furthermore, increased levels of neurotrophins have been found in the inflamed conjunctiva of patients with vernal keratoconjunctivitis (Lambiase et al., 1995). Although it has been suggested that neurotrophins participate in the regulation of physiological and pathological processes of the ocular surface, very little is known about sex- and age-related changes in neurotrophins in lacrimal glands.

Prosaposin, a saposin precursor, is a potent neurotrophic factor (Bradova et al., 1993; O'Brien et al., 1994) that is found in several tissues and various biological fluids such as the cerebrospinal fluid, breast milk, seminal fluid, pancreatic juice, and bile (Hineno et al., 1991; Kondoh et al., 1991; Hiraiwa et al., 1993). Prosaposin receptors, including G protein-coupled receptor 37 (GPR37) and GPR37L1, are also expressed in various neuronal and nonneuronal tissues (Meyer et al., 2013; Shimokawa et al., 2013; Nabeka et al., 2014; Li et al., 2017; Islam et al., 2018). Patients deficient in prosaposin and/or saposins show clinical syndromes such as Krabbe disease, metachromatic leukodystrophy-like disease, or Gaucher-like lysosomal storage disease (Beutler and Grabowski, 2001; Motta et al., 2016). Indeed, prosaposin levels are increased to protect cells from toxicity (Meyer et al., 2013; Nabeka et al., 2014; 2015; Li et al., 2017) or apoptotic agents (Koochekpour et al., 2007; 2012; Wu et al., 2012), providing a survival advantage to the cells. Furthermore, dysfunction or loss of saposins can result in many ophthalmic disorders such as macular grayness and irregularly pigmented retinas (Van Den Berghe et al., 2004), indicating a relationship between ocular diseases and prosaposin. Although prosaposin is a clinically significant and potent neurotrophin, little is known about its localisation and distribution in ocular glands.

In this study, we examined whether prosaposin and its receptors, GPR37 and GPR37L1, are expressed in the major ocular glands of rats. Because the functions of ocular glands vary based on age and sex, this study also examined whether sex and age affect the expression of prosaposin and its receptors in lacrimal tissues. The results of this study provide comprehensive analyses of prosaposin and its receptors in major ocular glands in untreated young to postmenopausal female rats, as well as in age-matched male rats.

Materials and methods

Animals

Female and male Wistar rats were used in this study.

The rats were housed at a constant temperature (22°C) under a 12:12 h light-dark cycle and given food and water *ad libitum*. All experimental procedures were consistent with the ethical regulations and guidelines for animal experimentation at Ehime University School of Medicine, Japan (05A261).

Sample collection and tissue preparation

Rats become sexually mature at 2 months (Sengupta, 2013) and female rats enter menopause at 18 months (Durbin et al., 1966). Therefore, we collected tissues from female rats at four different reproductive ages (young adult/pubertal age [2 months], mature adult [6 months], menopause [18 months], and post-menopause [27 months]), as well as from similarly aged male rats. The rats were anesthetised with an intraperitoneal injection of chloral hydrate (10 mg/kg), and six rats from each sex at each age were transcardially perfused with a fixative solution containing 4% paraformaldehyde in 0.1 M phosphate-buffered saline (PBS). After fixation, the major ocular glands, extra orbital lacrimal gland (ELG) and Harderian gland (HG) were removed (Fig. 1a). The tissues were immersed in 4% paraformaldehyde solution overnight at 4°C. Then the samples were dehydrated and embedded in paraffin.

Histopathological analyses

Serial coronal sections (7 µm) of the ELGs and HGs from both sexes at each age were stained with haematoxylin, dehydrated in ascending concentrations of ethanol, cleared in xylene, and mounted in Entellan™ new (Merck KGaA, Darmstadt, Germany). The tissues were observed using the Nikon Eclipse E800 light microscope (Nikon, Tokyo, Japan), and images were acquired using a digital camera attached to the microscope (Nikon Digital Sight DS-L2, Nikon).

Cell counts

Serous cells in the ELGs were counted using a light microscope. The cells in the ELGs of male and female rats at different ages were counted in visible sections at a 20× magnification (representing an area of 0.14 mm² of tissue). In each cross-section, three fields were randomly selected and the cells were counted. The number of cells was calculated and averaged across six cross-sections of each rat in each sex at each age; there were six rats in each sex at each age point.

Immunohistochemical staining of prosaposin and its GPR37 and GPR37L1 receptors

The routine avidin-biotin complex method was used to detect prosaposin and its GPR37 and GPR37L1 receptors. Briefly, the sections of paraffin-embedded ELGs and HGs were dewaxed and rehydrated. The sections were boiled for 20 min in citrate buffer (10 mM

citric acid, 0.05% Tween 20, pH 6.0) at 95°-100°C using a steamer to retrieve the antigen (Kanai et al., 1998). After boiling, the slides were allowed to cool for 20 min. Then the sections were rinsed in PBS-Tween 20 (PBST) three times for 5 min each. After rinsing, the sections were treated with 5% bovine serum albumin, 1% normal goat serum, 0.1% fish gelatin, and 0.1% NaN₃ in PBS for 1 h at room temperature, followed by incubation with one of the following primary antibodies overnight at 4°C: rabbit polyclonal anti-prosaposin (2 µg/mL; Nakaku, Nagoya, Japan), rabbit polyclonal anti-GPR37 (5 µg/mL; Abnova, Taipei, Taiwan), or rabbit polyclonal anti-GPR37L1 (5 µg/mL; LifeSpan BioSciences, Seattle, WA, USA). These antibodies were affinity purified with oligopeptides, and species reactivity was confirmed by immunoblotting and immunostaining (Shimokawa et al., 2013; Li et al., 2017; Islam et al., 2018). Following incubation with primary antibodies, the sections were washed three times for 10 min each in PBST and reacted with biotinylated swine anti-rabbit IgG secondary antibody (0.5 µg/mL, Dako, Glostrup, Denmark) for 2 h at room temperature. After rinsing three times for 10 min each in PBST, the avidin-biotin-peroxidase complex (1:300; Dako) was incubated for 1 h at room temperature. Then the sections were immersed in 3,3'-diaminobenzidine (Sigma-Aldrich, St. Louis, MO, USA) with 0.0033% H₂O₂ for approximately 10 min. After rinsing with distilled water, the sections were dehydrated in ascending concentrations of ethanol, cleared in xylene, and mounted in Entellan™ new (Merck). Finally, the sections were observed using the Nikon Eclipse E800 light microscope, and the images were acquired using a digital camera attached to the microscope. The specificity of antibody staining was also tested in ELGs and HGs using a negative staining procedure with normal rabbit IgG instead of primary antibodies; the ELG and HG sections were processed as described above. Minimal nonspecific staining was detected (data not shown).

Triple immunofluorescence staining of prosaposin and its GPR37 and GPR37L1 receptors

Triple immunofluorescence staining was used for the co-localisation of prosaposin, GPR37, and GPR37L1 in the ELGs. The deparaffinised sections were blocked as described above. After blocking, the sections were incubated overnight at 4°C in a solution containing rabbit polyclonal prosaposin (2 µg/mL; conjugated to Alexa Fluor 594), rabbit polyclonal GPR37 (5 µg/mL; conjugated to Alexa Fluor 488), and rabbit polyclonal GPR37L1 (5 µg/mL; conjugated to Alexa Fluor 405). Prosaposin, GPR37, and GPR37L1 antibodies were generated by Eurofins Genomics (Tokyo, Japan). Briefly, the specific antibodies were produced by immunising rabbits with synthetic oligopeptides based on the amino acid sequences specific to prosaposin (M19936), GPR37 (NP_476549.1), or GPR37L1 (NP_665727.2). The

specificities of the antibody staining were tested in our previous studies (Li et al., 2017; Islam et al., 2018). Following incubation with the antibodies, the sections were washed three times for 10 min each in PBST, mounted in Vectashield (Vector Laboratories; Burlingame, CA, USA), and examined using high resolution confocal images obtained using a Nikon A1 confocal microscope equipped with a 100× objective lens (Nikon).

Colocalization analysis

Colocalization analyses were conducted using the Fiji Coloc 2 plugin (https://imagej.net/Coloc_2). The colocalizations between pairs of two antibodies in single image planes of triple stainings were analyzed using Pearson's correlation coefficient. Six random images per rat at each age for each sex were analyzed; there were six rats in each sex at each age.

Statistical analyses

All computed parameters were quantified and compared between male and female rats. Statistics were performed using the mean values for each rat, and all data are expressed as the mean ± standard error. The data were analysed with two-way analysis of variance and further analysed with t-tests at each age when a significant interaction was observed. A value of $p < 0.05$ was considered statistically significant.

Results

Histopathological analyses

The gross necropsy of the rat lacrimal system showed that the ELG was located subcutaneously a few millimetres to the temporal side of the eye and anterior to the ear, while the HG was located in the deep part of the orbit (Fig. 1a). Haematoxylin staining of the ELG revealed the presence of serous cells with narrow lumens at different ages (Fig. 1c-j). Histological analyses demonstrated age-related acinar atrophies in the ELGs of rats. The presence of inflammatory cell infiltration around the acini and ducts was observed at 18 months of age, which was further increased at 27 months of age. Quantitative analyses were performed to determine the number of serous cells in the ELG of male and female rats at different ages (Fig. 1b). Although the numbers of acinar cells in the ELG decreased at older ages, they did not differ between male and female rats at any age examined. However, the HG is a tubulo-alveolar gland and consists of tubules with wide lumina lined by a single layer of epithelial cells (Fig. 2k-r), so age-dependent changes in the intracellular space and diameter of the tubules in the HGs were observed. No prominent sex-dependent changes in the size and shape of cells were observed in the HGs at any age.

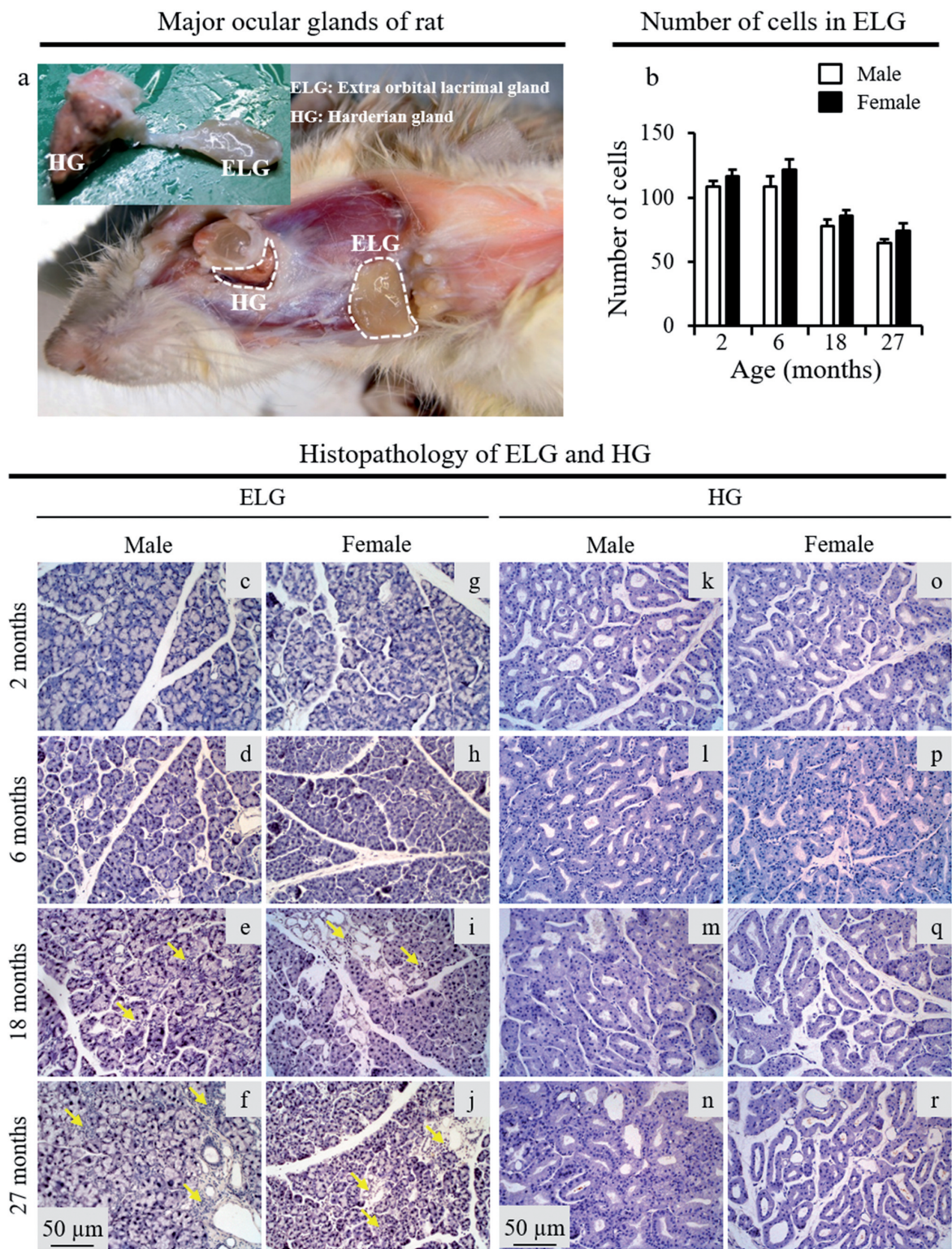


Fig. 1. Assessment of the histopathology of the extra orbital lacrimal gland (ELG) and Harderian gland (HG) in male and female rats of different ages. **a.** Gross necropsy of the rat lacrimal system showed that ELGs were located subcutaneously a few millimetres to the temporal side of the eye and anterior to the ear, while HGs were located in the deep part of the orbit. **b.** Histogram showing the number of serous cells in the ELGs of male and female rats at different ages. **c-r.** Cross-sectional images of the ELGs and HRs stained with haematoxylin, showing age-related changes in acinar cells. Representative images obtained from paraffin-embedded ELGs of male (**c-f**) and female (**g-j**) rats, and HRs of male (**k-n**) and female (**o-r**) rats at different ages. Data in (**b**) are expressed as the mean $\pm$ standard errors of six rats at each age for each sex. Arrows indicate lymphocytic infiltration around acini and ducts in ELGs.

Immunoreactivities of prosaposin, GPR37, and GPR37L1 in the ELGs of male and female rats

The expression profiles of prosaposin in the ELGs of male and female rats at different ages are shown in Figure 2. Prosaposin immunoreactivity was evident in acinar cells of the ELGs at all ages, although expression at the cellular level was different between male and female rats at some ages (Fig. 2a-p). Weak staining was observed in the acinar cells of male rats at older ages (Fig. 2a-h), while relatively strong prosaposin immunoreactivity was observed in female rats (Fig. 3i-

p). Prosaposin immunoreactivity was observed in the basolateral membranes of serous cells in both male and female rats; however, it was most frequently observed in the cytoplasm of acinar cells in female rats, particularly those of menopausal age. GPR37 and GPR37L1 immunoreactivities in the ELG of male and female rats are shown in Figures 3 and 4, respectively. GPR37 immunoreactivity was detected in the cytoplasm and nucleus of ELGs of rats; however, similar to prosaposin, its expression at the cellular level was different between males and females, particularly at older ages (Fig. 3). In male rats, GPR37 immunoreactivity was observed in the

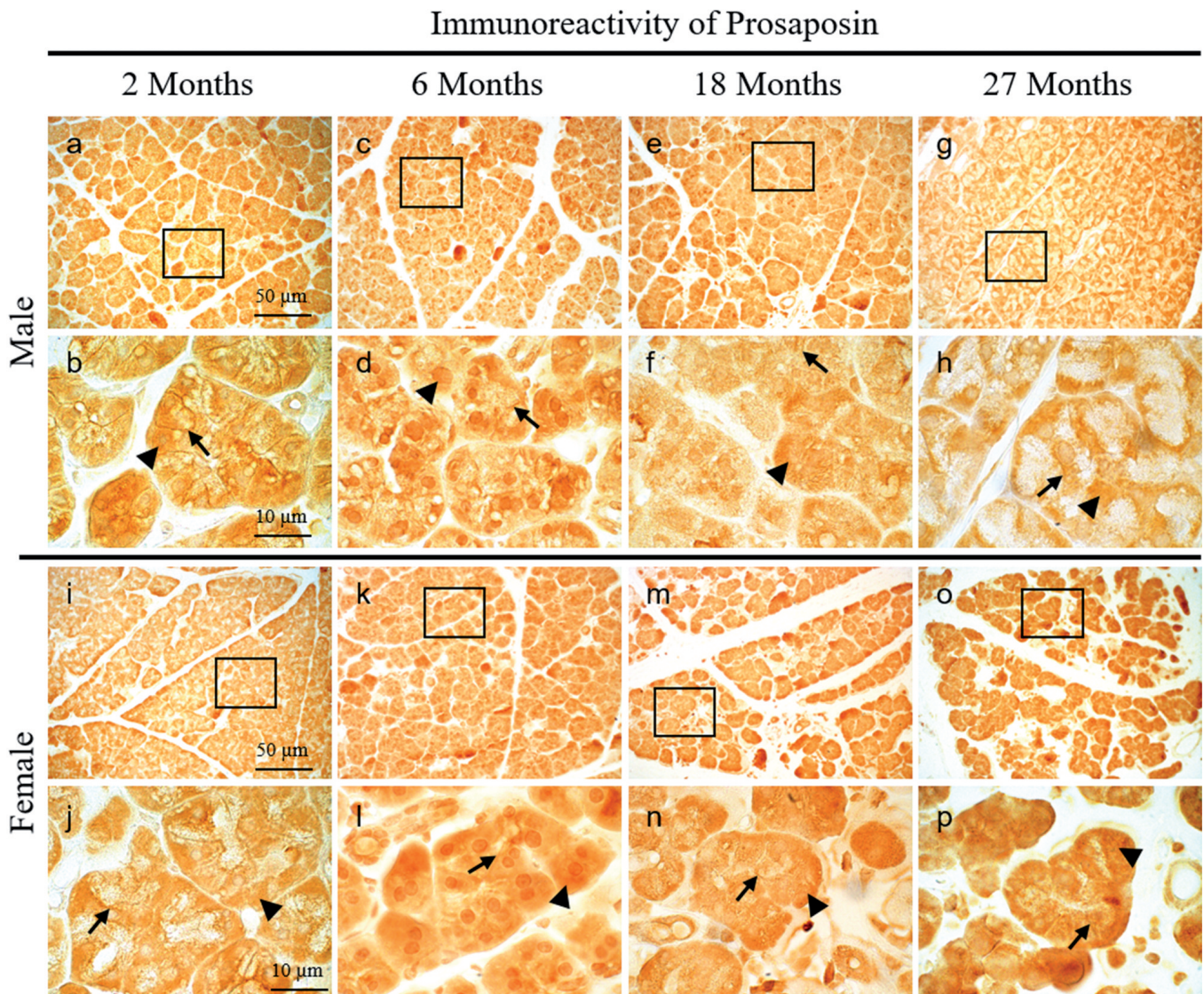


Fig. 2. Immunohistochemical analyses of the expression and localisation of prosaposin (prosaposin) in the extra orbital lacrimal gland (ELG) of rats. **a-p.** The ELGs of 2-, 6-, 18-, and 27-month-old male and female rats were processed for immunohistochemistry with an anti-prosaposin antibody. Representative images obtained from the paraffin-embedded ELGs of male (**a-h**) and female (**i-p**) rats of different ages. Arrows indicate the basolateral membranes of cells. Arrowheads indicate the cytoplasm of cells.

cytoplasm of a few acinar cells, and the expression decreased with age, particularly at 18 and 27 months of age (Fig. 3a-h). GPR37 immunoreactivity in female rats was abundant in both the basal layer and cytoplasm of acinar cells, and the expression increased with age (Fig. 3i-p). GPR37L1 immunoreactivity was also detected in the ELGs of rats (Fig. 4). However, the immunoreactivity of GPR37L1 was mainly observed in the apical and basolateral membranes of acinar cells, distributed among acini in a “mosaic” pattern in some acinar cells of the ELGs in male and female rats. GPR37L1 expression in the acinar cells of both male

(Fig. 4a-h) and female (Fig. 4i-p) rats decreased at older ages, particularly in male rats.

Prosaposin, GPR37, and GPR37L1 immunoreactivities in the HGs of male and female rats

Figure 5 shows the immunoreactivities of prosaposin (a-h), GPR37 (i-p), and GPR37L1 (q-x) in the HGs of young and older male and female rats. Immunoreactivities of prosaposin and GPR37L1 were observed in the cytoplasm of acini in the alveolus at young and older ages. No prominent age- or sex-

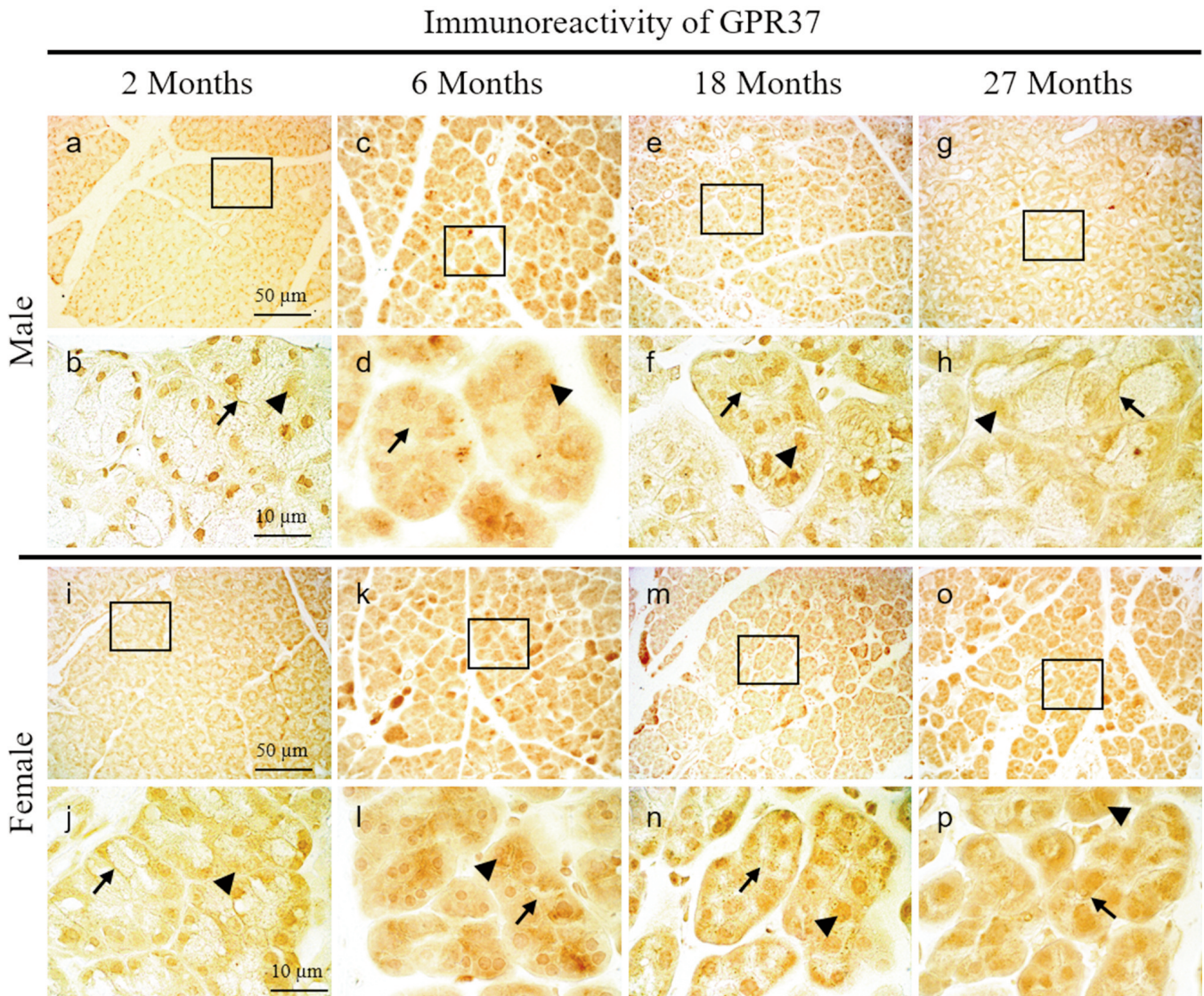


Fig. 3. Immunohistochemical analyses of the expression and localisation of G protein-coupled receptor 37 (GPR37) in the extra orbital lacrimal gland (ELG) of rats. **a-p.** The ELGs of 2-, 6-, 18-, and 27-month-old male and female rats were removed and processed for immunohistochemistry with anti-GPR37 antibody. Representative images obtained from the paraffin-embedded ELG of male (**a-h**) and female (**i-p**) rats at different ages. Arrows indicate the basolateral membranes of cells. Arrowheads indicate the cytoplasm of cells.

dependent changes in the expression of prosaposin and its receptors were observed in the cells of HGs in this study.

Co-localisation of prosaposin, GPR37, and GPR37L1 in the ELGs of male and female rats

Figure 6 shows high-resolution confocal images of triple immunofluorescence for prosaposin, GPR37, and GPR37L1 in the ELG staining of young and older male and female rats. The triple labelling results showed that prosaposin and its receptors were co-localised primarily in the basolateral membranes of acinar cells and

cytoplasm of some ELG cells, although immunoreactivities varied between male and female rats at older ages (Fig. 6a-p). Age-related accumulation and co-localisation of prosaposin and its receptors, dominantly GPR37, were also observed in acinar cells of older rats, which is very prominent in females. Figure 7 shows high-resolution confocal images of triple immunofluorescence of prosaposin, GPR37, and GPR37L1 in striated ducts of ELGs of young and older age male and female rats. The immunoreactivities showed that prosaposin and its receptors were co-localised in ductal cells of the ELG. Moderate immunoreactivities of prosaposin, GPR37, and

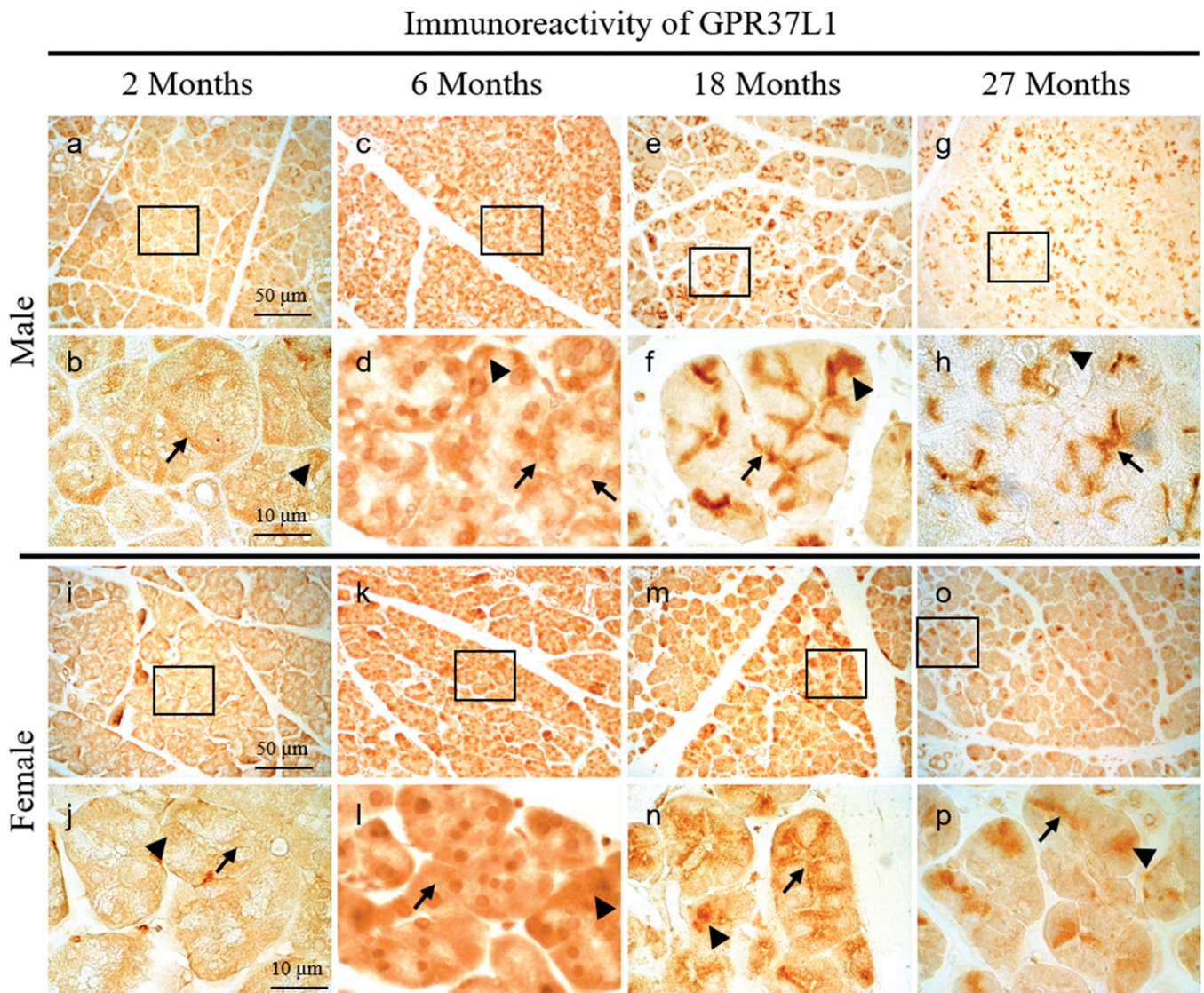


Fig. 4. Immunohistochemical analyses of the expression and localisation of G protein-coupled receptor 37L1 (GPR37L1) in the extra orbital lacrimal gland (ELG) of rats. **a-p.** The ELGs of 2-, 6-, 18-, and 27-month-old male and female rats were removed and processed for immunohistochemistry with anti-GPR37L1 antibody. Representative images obtained from the paraffin-embedded ELGs of male (**a-h**) and female (**i-p**) rats at different ages. Arrows indicate the basolateral membranes of cells. Arrowheads indicate the cytoplasm of cells.

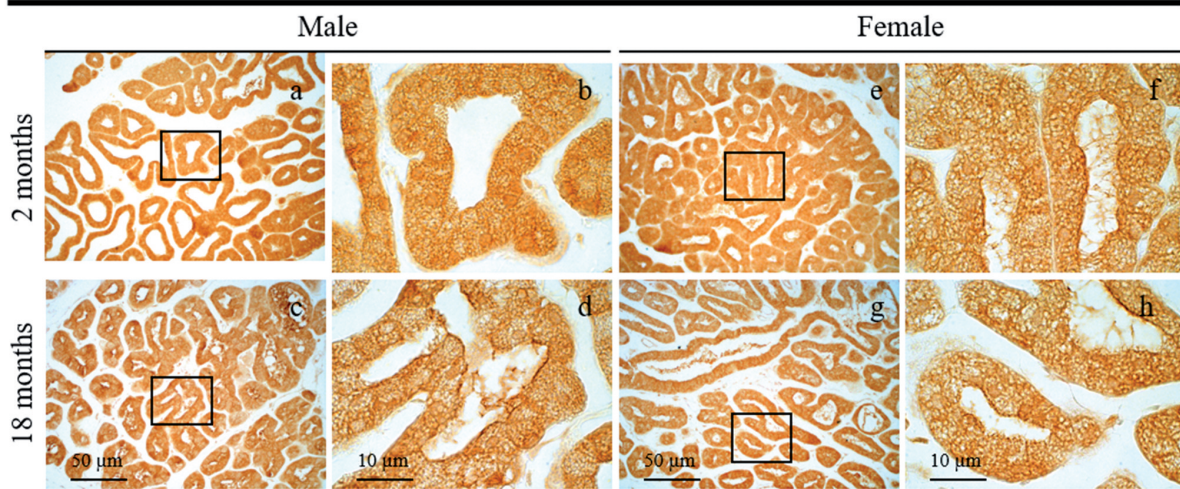
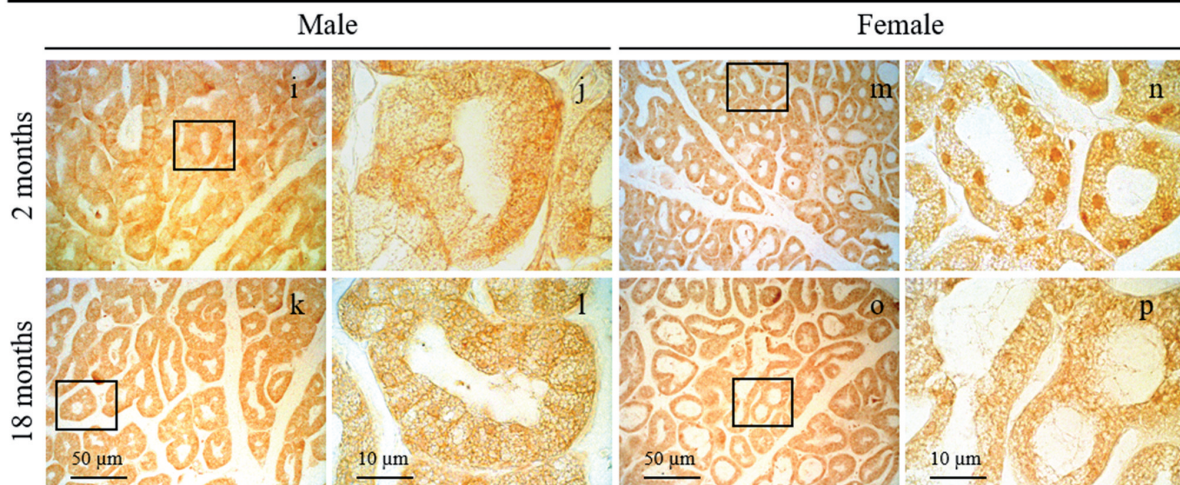
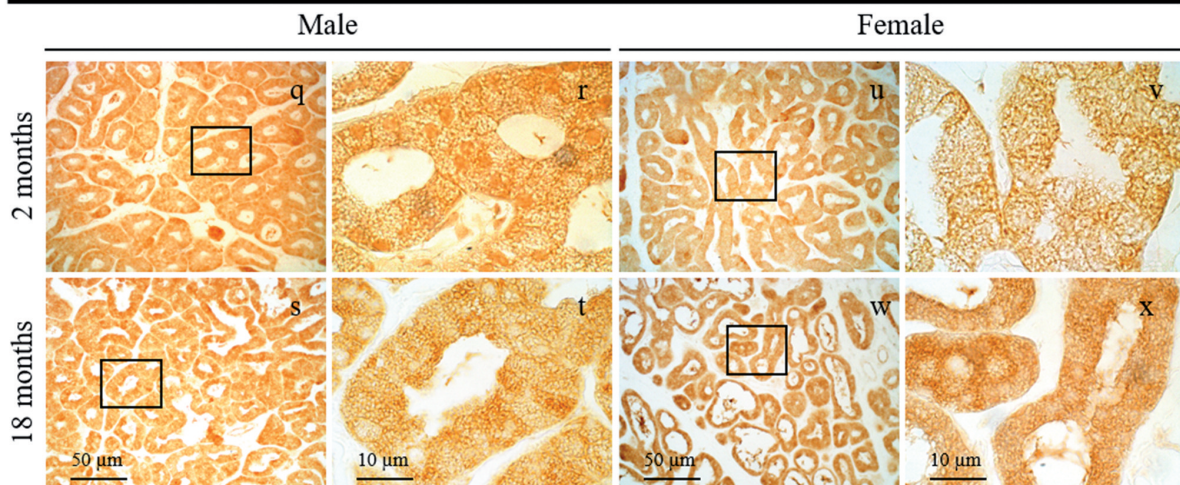
Immunoreactivity of Prosaposin in HG**Immunoreactivity of GPR37 in HG****Immunoreactivity of GPR37L1 in HG**

Fig. 5. Immunohistochemical analyses of the expression and localisation of prosaposin (prosaposin), G protein-coupled receptor 37 (GPR37), and G protein-coupled receptor 37L1 (GPR37L1) in the Harderian gland (HG) of rats. The HGs of 2- and 18-month-old male and female rats were removed and processed for immunohistochemistry. Immunostaining was performed with antibodies directed against prosaposin (a-h), GPR37 (i-p), or GPR37L1 (q-x). Representative images obtained from the paraffin-embedded HGs of young adult (2-month-old) and older (18-month-old) male and female rats.

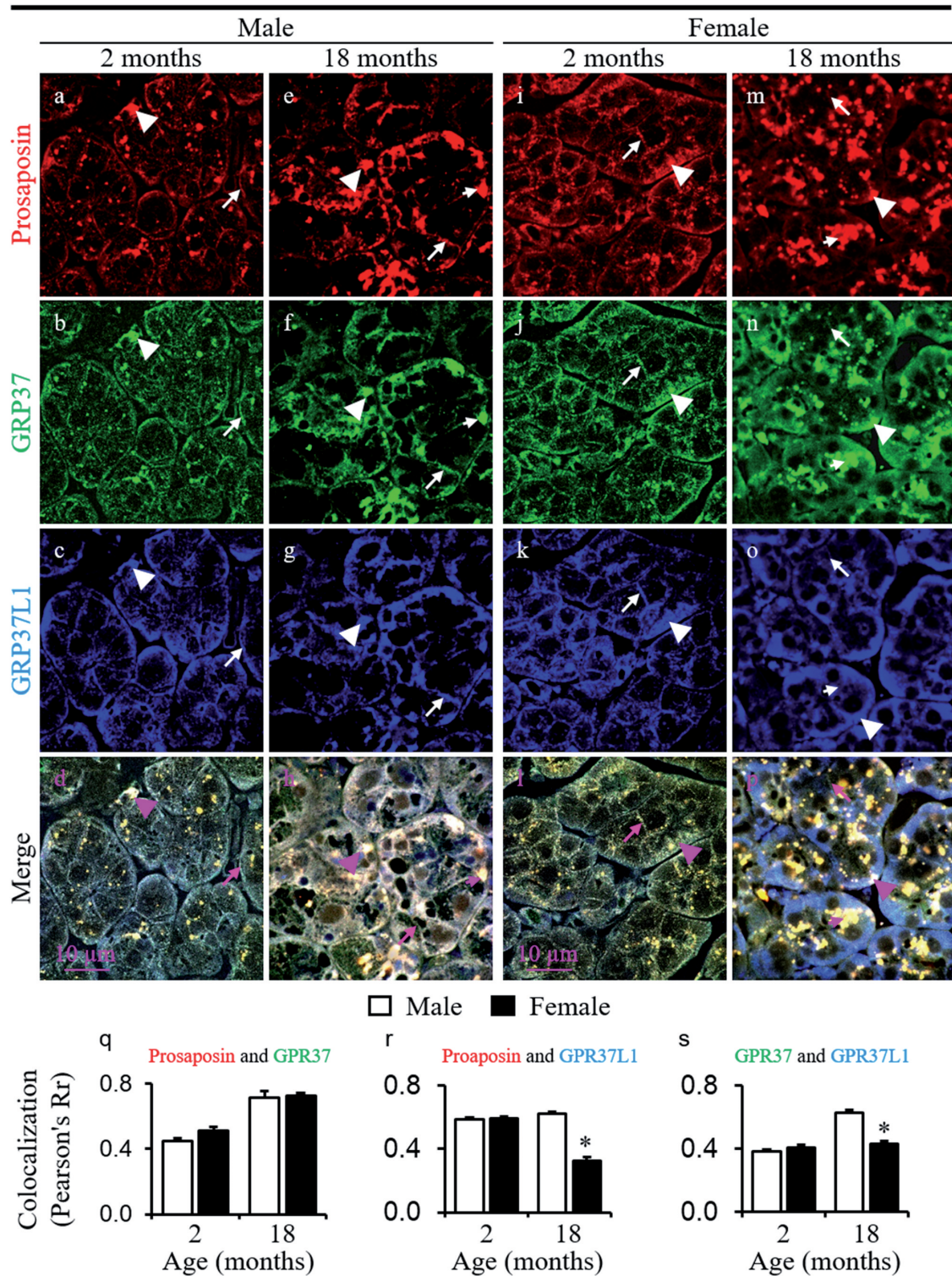


Fig. 6. Triple-labelled immunofluorescence staining performed with antibodies directed against prosaposin (prosaposin), G protein-coupled receptor 37 (GPR37), and G protein-coupled receptor 37L1 (GPR37L1). **a-p.** Representative confocal images show the localisation patterns of prosaposin, GPR37, and GPR37L1 in the extra orbital lacrimal glands (ELGs) of 2- and 18-month-old male (**a-h**) and female (**i-p**) rats. Each panel represents the immunoreactivities of prosaposin (red), GPR37 (green), and GPR37L1 (blue) with sites of co-localisation (white or yellow). **q-s.** Colocalization analysis using the Fiji Coloc 2 plugin (Pearson's correlation coefficient, Rr) was conducted to examine pixel intensity correlations over space in single confocal images. Prosaposin, GPR37 and GPR37L1 triple staining analyzed in the acini of ELGs showed varying degrees of colocalization. Data in (**q-s**) are expressed as the mean $\pm$ standard errors. Six random images per rat at each age for each sex were analyzed; there were six rats in each sex at each age. Large arrows indicate basolateral membranes of cells. Small arrows indicate aging-related accumulation of prosaposin and its receptors in acinar cells. Arrowheads indicate the cytoplasm of cells. *Significantly different from female rats at each age (P < 0.01).

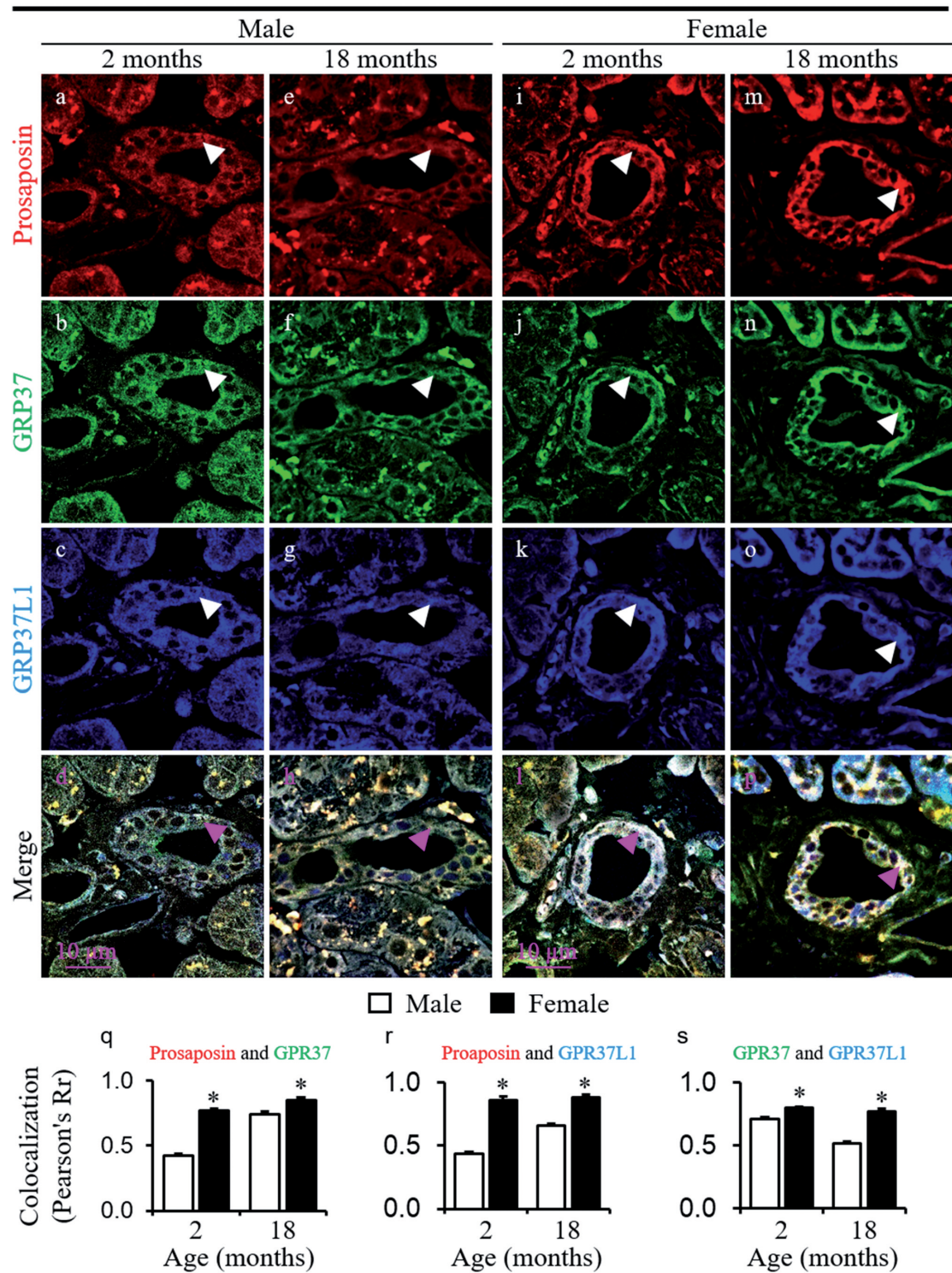


Fig. 7. Triple-labelled immunofluorescence staining with antibodies directed against prosaposin (prosaposin), G protein-coupled receptor 37 (GPR37), and G protein-coupled receptor 37L1 (GPR37L1). **a-p.** Representative confocal images show the co-localisation patterns of prosaposin, GPR37, and GPR37L1 in the striated ducts of extra orbital lacrimal glands (ELGs) of 6- and 18-month-old male (**a-h**) and female (**i-p**) rats. Immunoreactivities of prosaposin, GPR37, and GPR37L1 were co-localised in the secretory granules of ductal cells (arrowheads) in the ELGs of male and female rats. Each panel represents the immunoreactivities of prosaposin (red), GPR37 (green), and GPR37L1 (blue) with sites of co-localisation (white or yellow). **q-s.** Colocalization analysis using the Fiji Coloc 2 plugin (Pearson's correlation coefficient, Rr) was conducted to examine pixel intensity correlations over space in single confocal images. Prosaposin, GPR37 and GPR37L1 triple staining analyzed in the ducts of ELGs showed varying degrees of colocalization. Data in (**q-s**) are expressed as the mean $\pm$ standard errors. Six random images per rat at each age for each sex were analyzed; there were six rats in each sex at each age. Arrowheads indicate the cytoplasm of cells. *Significantly different from female rats at each age ($P < 0.01$).

GPR37L1 were observed in the ductal cells of ELGs of male rats (Fig. 7a-h) and female rats (Fig. 7i-p). Unlike acinar cells in the ELG, the immunoreactivities of prosaposin and its receptors in ductal cells did not vary between male and female rats.

Colocalization analysis using Pearson's correlation coefficient showed varying degrees of colocalization between prosaposin and its receptors, GPR37 and GPR37L1 in the acinar cells (Fig. 6q-s) and ducts (Fig. 7q-s) of ELGs. At a young age, a relatively lower degree of colocalization between prosaposin and GPR37 was observed in acinar cells of ELGs, whereas it increased at older age (Fig. 6). Although the degree of colocalization between prosaposin and its receptors did not differ in sexes at young age (Fig. 6q-s), the colocalization between prosaposin and GPR37L1, and GPR37 and GPR37L1 decreased in female rats at older age compared to age-matched male rats (Fig. 6r,s). On the other hand, the degrees of colocalization between prosaposin and its receptors in ducts of ELGs were more prominent in female rats than age-matched male rats at both young and older ages (Fig. 7q-s).

Discussion

Lacrimal glands produce various growth factor families including neurotrophins, which play a role in lacrimal gland physiology and maintenance of the ocular surface (Ohashi et al., 1989; Wilson et al., 1991; van Setten et al., 1992; Li et al., 1996; Yoshino et al., 1996; Ghinelli et al., 2003). Growth factors increase in lacrimal glands as well as in tears during ocular surface injuries (Tervo et al., 1997; Muzi et al., 2010), suggesting that they respond to ocular surface functions during pathological conditions. In this study, we provided evidence that immunoreactivities of the potent neurotrophin, prosaposin, and its GPR37 and GPR37L1 receptors were detectable in the acinar cells of major ocular glands, the ELGs and HGs of rats. Therefore, prosaposin originating from the major ocular glands has been thought to regulate lacrimal functions. Furthermore, the prosaposin immunoreactivity in lacrimal glands varied according to the age and sex of the rats, being more abundant in female glands at menopausal age. Similar age- and sex-dependent changes in prosaposin and its receptors have also been found in salivary glands (Islam et al., 2018), which are structurally and functionally similar to lacrimal glands, indicating the possibility of prosaposin regulation of oral and ocular health, particularly in cases where sex and age are affected unequally, such as xerostomia and dry eye disease.

In addition to acinar cells, widespread immunoreactivities of prosaposin and its receptors were observed in the ductal system of lacrimal glands. Prosaposin immunoreactivity in the ductal system showed a very similar distribution pattern to other growth factors (i.e., exclusive localization in the

epithelial cells of ducts) (Obata et al., 1993; Li et al., 1996; Ghinelli et al., 2003), suggest the possibility of a systemic action of prosaposin via endocrine secretion of ductal cells. Although little is known about prosaposin in tears, the existence of prosaposin in various biological fluids such as cerebrospinal fluid, milk, seminal fluid, pancreatic juice, and bile (Hineno et al., 1991; Kondoh et al., 1991) suggests that it may be present in tears and play roles on the ocular surface. This possibility is also supported from the observations that other neurotrophins are secreted from the lacrimal glands found in tears and are distributed over the ocular surface, where they affect cellular proliferation, migration, differentiation, and survival (Tervo et al., 1990; Li et al., 1996; Klenkler et al., 2007). Although the present study examined the expression profiles rather than the functions of prosaposin, based on the localisation and variations in major ocular glands, prosaposin originating from lacrimal glands might regulate functions of lacrimal tissues as well as distant ocular surface tissues during normal and pathological conditions in an autocrine and/or paracrine manner.

Because the neurotrophin family of growth factors and their receptors are present in lacrimal glands (Nguyen et al., 1997; Ghinelli et al., 2003) and lacrimal glands are highly responsive to sex hormones (Azzarolo et al., 1997), there may be cross-talk among neurotrophins, lacrimal glands, and gonadal organs. Neurotrophins play roles in prostatic cancer (Walch and Marchetti, 1999) and breast cancer (Hondermarck, 2012). Prosaposin, a potent neurotrophin, is expressed with its receptor in gonadal organs and plays important roles in normal and pathological conditions (Sun et al., 1994; La Sala et al., 2015). Consistent with its possible function, prosaposin is overexpressed in the cells of prostate and breast cancers and is thought to enhance androgen and estrogen receptors to play roles in the development and progression of cancers (Koochekpour et al., 2012; Wu et al., 2012). Although the actual role of prosaposin in lacrimal glands remains unknown, because changes in the expression of prosaposin and its receptors in various tissues indicate the tissue's pathological condition, we cannot exclude the possibility that ocular surface tissues, particularly in menopausal female rats when prosaposin and its receptors are strongly expressed, might be in advanced pathological conditions. In addition, because prosaposin plays important roles in gonadal organs, we cannot exclude the possibility of cross-talk between lacrimal prosaposins and gonadal secretion, particularly at older ages when sex hormone production levels have declined.

In conclusion, we found that prosaposin and its receptors were expressed in the major ocular glands, ELGs and HGs, of rats. The identification of prosaposin and its receptors is the first step toward further studies that elucidate the specific roles of this potent neurotrophic factor in the regulation of ocular surface functions in normal and pathological conditions.

Acknowledgements. This work was supported in part by grants to M.S.I.K. from the Japan Society for the Promotion of Science (No. 15K20005 and 18K07500) and to S.M (No. 18K08945).

Additional Information. The authors declare no competing financial interests.

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Accepted June 19, 2019