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Anatomy

Restricted localization of ultimobranchial body remnants and parafollicular cells in the one-humped camel (*Camelus dromedarius*)

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ABSTRACT. Parafollicular cells (C-cells) exist within the thyroid glands and display different distributions within the glands among mammalian species. In the one-humped camel (*Camelus dromedarius*), localization of the C-cells remains under debate. We herein investigated appearance of C-cells and the remnants of the ultimobranchial body, origin of C-cells, in the thyroid glands of one-humped camels. Macroscopically, a white mass was present at one-third the length from the cranial end of the thyroid glands where the cranial thyroid artery entered. In addition, large fossae were frequently found adjacent to the white mass. Histologically, the mass was mainly composed of connective tissues, thyroid follicles, and two types of cell clusters: one was composed of cells with clear cytoplasm and the other was composed of non-keratinized epidermoid cells. The mass and the fossae contained p63-positive cells, indicating that they consisted of ultimobranchial body remnants. Calcitonin was expressed in cells with clear cytoplasm, which were localized just beneath the fossae and in the cell clusters of the white mass. C-cells also resided in both subfollicular and interfollicular spaces adjacent to the white mass, but gradually decreased toward the periphery. C-cells tended to display round shapes in the ultimobranchial body remnants and subfollicular spaces, and spindle shapes in interfollicular spaces. In conclusion, we demonstrated that the ultimobranchial body remnants were limited to the region around the entrance of cranial thyroid artery and vein, and C-cells were mainly concentrated within and around the ultimobranchial body remnants.

KEW WORDS: camel, C-cell, thyroid gland, ultimobranchial body remnant

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In mammals, the thyroid glands contain two types of hormone-producing cells: follicular epithelial cells producing thyroglobulins, and parafollicular cells (C-cells) producing calcitonin [7, 8]. The follicular epithelial cells develop from the thyroid primordium, which buds from the foregut endoderm and descends ventral to the foregut tube [7]. C-cells originate from the ultimobranchial body, which buds from the fourth pharyngeal pouch in mammals [8]. The ultimobranchial body migrates caudally, fuses to the thyroid primordium, spreads throughout the thyroid lobe, and eventually differentiates into C-cells [8]. The ultimobranchial body often remains in the thyroid glands as solid cell nests, follicles, and cysts [12, 19, 20]. In mammals, the fourth pharyngeal pouches also give rise to parathyroid glands IV (superior or internal parathyroid glands) except in rodents such as mice, rats, and hamsters, which lack these glands [8]. The parathyroid glands IV are embedded within the thyroid glands, but are separated by connective tissues [14].

Generally, mammalian C-cells exist at high densities in the central region of the thyroid lobes and around the parathyroid glands IV, but are not found in the isthmus of the thyroid glands [14, 17]. However, the distribution of C-cells shows species-specific patterns, indicating that the dispersion pattern of the ultimobranchial body differs among mammalian species [14, 17]. In one-humped camels (*Camelus dromedarius*), the localization of C-cells in the thyroid glands remains controversial. In several studies,

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researchers did not find C-cells in the thyroid glands of one-humped camels at all [1, 5, 6, 10], but Ahmadpanahi and Yousefi reported that the thyroid glands contained C-cells in about 5% of the cell population, and Al-Ramadan also found the calcitonin-positive C-cells within the ultimobranchial body remnants [2, 3]. However, the precise distribution of C-cells in the thyroid glands of one-humped camels has not been clarified yet. In the present study, we histomorphologically investigated the localization of C-cells and the remnants of ultimobranchial body in the thyroid glands of one-humped camels.

MATERIALS AND METHODS

In this study, we used apparently healthy one-humped camels slaughtered for human consumption at slaughterhouse in Zagazig, Sharkia, Egypt. After the one-humped camels were slaughtered, a total of twelve thyroid glands from four males and two females between 3 and 5 years of age were collected, and fixed with 10% neutral buffered formalin. The use of the thyroid glands for this study was agreed by the slaughterhouse.

The fixed thyroid glands were post-fixed with glutaraldehyde overnight at 4°C, followed by 1% osmium tetroxide in 0.1 M phosphate buffer for 1 hr, and treated with 0.5 and 1% tannic acid. The specimens were dehydrated using graded alcohol and dried by HCP-2 critical point dryer (Hitachi, Tokyo, Japan). The dried specimens were sputter-coated with an E-1030 ion sputter coater (Hitach), and examined on an S-4100 SEM (Hitachi) with an accelerating voltage of 10 kV.

The formalin-fixed, paraffin-embedded thyroid glands were cut into 3-μm-thick slices and stained with hematoxylin and eosin (HE), periodic acid-Schiff (PAS), and Masson's trichrome (MT). Immunohistochemistry was performed to detect calcitonin and p63, which are markers for C-cells and ultimobranchial body remnants, respectively [15, 19]. Deparaffinized sections were heated with Histo VTone® (Nacalai Tesque, Kyoto, Japan) for 30 min at 90°C, treated with 0.3% hydrogen peroxidase/methanol solution for 30 min to eliminate endogenous peroxidase, blocked with blocking reagent (Nichirei, Tokyo, Japan), and incubated overnight at 4°C with rabbit anti-human calcitonin polyclonal antibody (prediluted, DAKO, Glostrup, Denmark) or mouse anti-human p63 monoclonal antibody (clone 4A4, prediluted, Nichirei). The calcitonin antibody reacts to one-humped camel calcitonin [3]. The p63 antibody is raised against human p63 aa 1-205. Although it has not been reported that the p63 antibody reacts to one-humped camel p63, aa 1-205 of p63 in one-humped camel has 98% homology to that in human (https://www.ncbi.nlm.nih.gov/). Next, the sections were treated with appropriate secondary antibodies (Nichirei) for 30 min, followed by treatment with streptavidin-peroxidase (Nichirei) for 30 min at approximately 25°C. The immunopositive reactions were developed using a 3,3′-diaminobenzidine tetrahydrochloride-H_2O_2 solution. The sections were then counterstained with hematoxylin.

RESULTS

From the lateral view, the thyroid lobes of one-humped camels were oval in shape, and the two lobes were connected by an isthmus at their caudal end (Fig. 1A). The internal parathyroid glands were embedded around the cranial pole as reported previously [4], and the external parathyroid glands and thymic tissues were localized along the thyroid lobes (Fig. 1A). Cross sections of the thyroid lobes at about 5-mm intervals revealed that white mass was present at about one-third the length from the cranial end of the thyroid gland where the cranial thyroid artery/vein entered, and we termed this area the "cranial thyroid hilum" in this study (Fig. 1B). In addition, macroscopically visible large fossae were found between the white mass and the cranial thyroid hilum in six out of twelve thyroid glands, extending ventrodorsally (Fig. 1C) as well as craniocaudally (Fig. 1D). The fossae branched toward the central region of the thyroid glands, i.e., toward the white mass (Fig. 1E).

Histologically, a branch of the cranial laryngeal nerve was found in the cranial thyroid hilum, and the small parathyroid glands were found in the same region in three out of twelve thyroid glands (Fig. 2A). The fossae branched toward the central region of the thyroid glands, consistent with the morphological observation (Figs. 1E and 2B). The fossae were lined with simple squamous, simple cuboidal, stratified cuboidal or pseudostratified columnar epithelium with a few ciliated cells, and cells with clear cytoplasm were present just beneath the lining cells (Fig. 2C and 2D). Unlike thyroid follicles, the fossae were filled with neither aniline blue positive nor PAS positive intrafollicular colloids (Fig. 2B and 2D). The same histological structures as the fossae were found in 11 out of 12 thyroid glands although the size was variable. The white mass was mainly composed of connective tissues and thyroid follicles (Fig. 2B). We also found two additional types of cell clusters within the white mass: one was composed of cells with clear cytoplasm, and another was composed of non-keratinized epidermoid cells (Fig. 2E and 2F). The ultimobranchial body marker, p63, was expressed in the basal cells of the fossae, two of the cell cluster types, and several follicles in the white mass (Fig. 2G–J). These results indicated that the white mass consisted of remnants of the ultimobranchial body.

In one-humped camels, calcitonin-positive C-cells were found at high density within the ultimobranchial body remnants as reported previously [3]. Calcitonin was expressed in cells with clear cytoplasm just beneath the lining of the fossae and in the cell clusters (Fig. 2K and 2L). In addition, C-cells were also found in both subfollicular space and interfollicular space outside of the ultimobranchial body remnants. The C-cells were frequently found around the ultimobranchial body remnants, mostly as solitary cells (Fig. 2M). C-cells gradually decreased towards the periphery, tending to be arranged focally rather than uniformly (Fig. 2N), and were not found in the periphery of the thyroid glands (Fig. 2O). C-cells were of various shapes in the one-humped camels. In the ultimobranchial body remnants and the subfollicular space, C-cells were mainly round to oval in shape (Fig. 2K, 2L, and 2P). C-cells in the interfollicular space tended to show oval to spindle shapes (Fig. 2P).

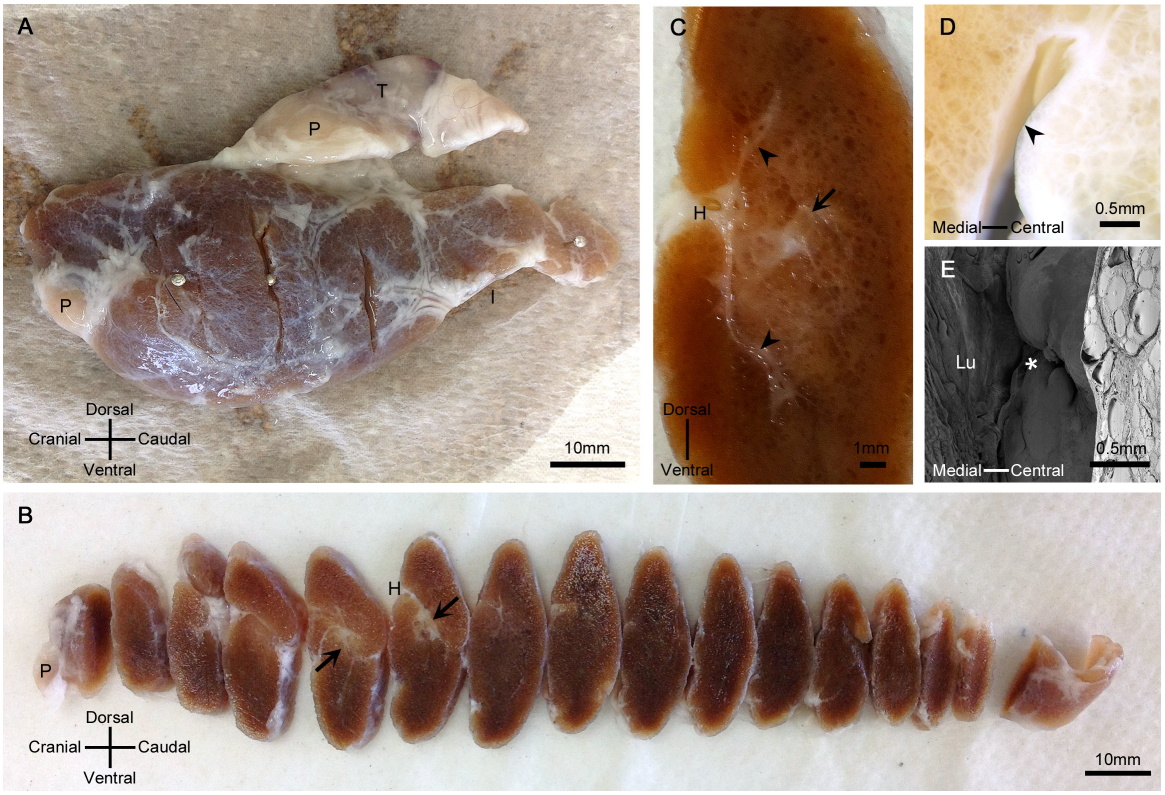

Fig. 1. Morphological features of the thyroid glands in the one-humped camel. (A–C) Lateral view (A) and cross-section (B) of the left thyroid gland, and cross-section at one-third the length from the cranial end of the thyroid gland (C), fixed with 10% neutral buffered formalin. (D and E) cross-section of the fossae under a stereomicroscope (D) and under a scanning electron microscope (E). H, cranial thyroid hilum; I, isthmus; Lu, lumen of the fossae; P, parathyroid gland; T, thymic tissue. Arrows indicate white mass, arrowheads indicate fossae, and asterisk indicates the fossae extending toward the central region of the thyroid glands.

DISCUSSION

This study demonstrated histomorphological localization of the ultimobranchial body remnants and C-cells in the thyroid glands of one-humped camels. Although Al-Ramadan found the ultimobranchial body remnants in the thyroid glands of one-humped camels [3], we are the first to demonstrate that they are localized around the cranial thyroid hilum. The ultimobranchial body remnants are normally found in the mammalian thyroid glands in various shapes, such as solid cell nests, follicles, and cystic structures [12, 19, 20]. Solid cell nests are composed of two cell types: p63-positive main cells and calcitonin-positive C-cells, which are the majority and minority populations, respectively [19]. Mixed follicles, composed of main cells and differentiated follicular cells, are also recognized as ultimobranchial body remnants [19]. In one-humped camels, as in other mammalian species, solid cell nests and thyroid follicles containing p63-positive main cells were found in the thyroid glands. Unlike typical solid cell nests composed of non-keratinized epidermoid cells [9], cell clusters composed of an abundance of C-cells and few main cells were also present in one-humped camels. Similar structures are found in dogs and are known as “C-cell complexes”, which are recognized as persistent ultimobranchial bodies that have incompletely fused with the thyroid glands [14]. We consider that the clusters of C-cells in one-humped camels are analogous to C-cell complexes rather than to solid cell nests. On the other hand, the ultimobranchial body also frequently remained in the form of large fossae. The mammalian species frequently possessing large fossa originating from the ultimobranchial body have not been reported. Together, the restricted localization of the ultimobranchial body remnants and the large fossae might represent unique characteristics of one-humped camels.

Although mammalian C-cells are mainly concentrated in the central region of the thyroid lobes and around the parathyroid glands IV, the distribution patterns of the C-cells differ among mammalian species. In dogs, most of the C-cells accumulate as C-cell complexes adjacent to the parathyroid glands IV [11–13]. In monkeys, C-cells appear as single cells or as a small group of cells, and are limited to the narrow region around the parathyroid glands IV in the middle of the thyroid lobe [14]. In sheep, solitary C-cells are concentrated in the deep central region, and gradually decrease towards the periphery [17]. In mice lacking parathyroid glands IV, C-cells are densely concentrated in the middle one third of the thyroid lobes [12]. In one-humped camels, C-cells were restricted to both the ultimobranchial body remnants, mainly as C-cell complexes, and the subfollicular or interfollicular spaces around the ultimobranchial body remnants as solitary cells. Thus, our results indicate that the restricted distribution of C-cells in one-humped camels differs from that in other mammalian species. The shapes of the C-cells also show

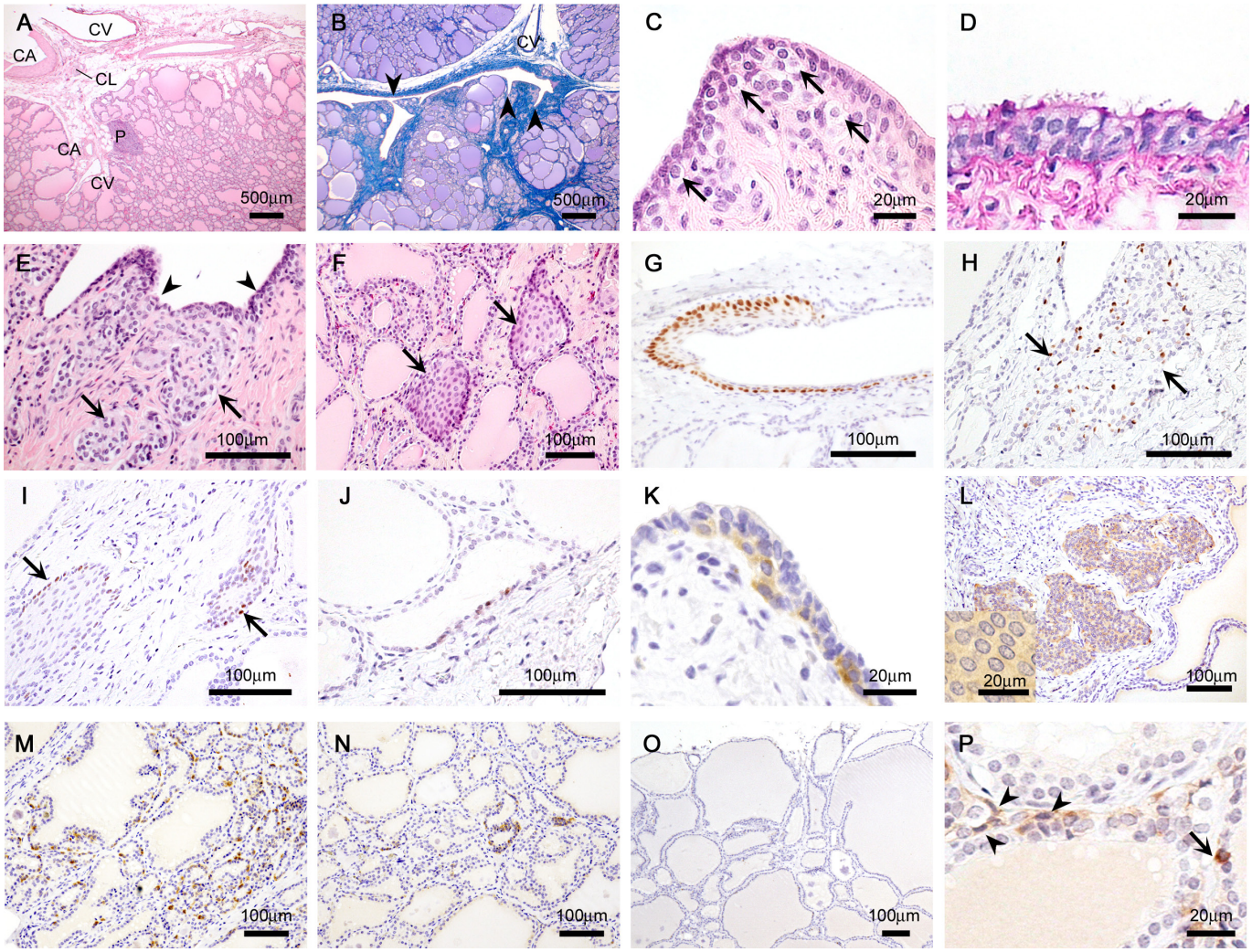

Fig. 2. Histological features of the thyroid glands in the one-humped camel. (A and B) Thyroid glands around the cranial thyroid hilum stained with HE (A) and MT (B). CA, cranial thyroid artery; CL, cranial laryngeal nerve; CV, cranial thyroid vein; P, parathyroid gland. Arrowheads indicate fossae. (C and D) Epithelial structures of the fossae stained with HE (C) and PAS (D). Arrows indicate cells with clear cytoplasm. (E and F) Cell clusters composed of cells with clear cytoplasm (E) and non-keratinized epidermoid cells (F) stained with HE. Arrows indicate cell clusters and arrowheads indicate the fossae. (G-J) Immunohistochemistry for p63 in the epithelium of the fossae (G), in the cell clusters composed of cells with clear cytoplasm (H), in the non-keratinized epidermoid (I), and in the follicles localized within the mass (J). Arrows indicate cell clusters. (K and L) Immunohistochemistry for calcitonin beneath the epithelium of the fossae (K) and cell clusters composed of cells with clear cytoplasm (L). Inset indicates higher magnification of the cell clusters. (M–O) Immunohistochemistry for calcitonin at central region around the mass (M), caudal region of the thyroid glands (N), and periphery of the thyroid glands (O). (P) Higher magnification of subfollicular and interfollicular spaces. Arrow indicates round-shaped C-cells in subfollicular space, and arrowheads indicate elongated C-cells in interfollicular space.

species-specific differences in mammals. C-cells are mainly round to oval in mice, rats, cats, dogs, and monkeys [13], while in pigs, cows and sheep, C-cells show more elongated shapes [13, 18]. In one-humped camels, C-cells tended to display round shapes in the ultimobranchial body remnants and subfollicular spaces, and spindle shapes in interfollicular spaces. Previous research may have overlooked C-cells in the thyroid glands of one-humped camels owing to the non-uniform distribution and shapes shown in this study [1, 5, 6, 10].

The migration route of the ultimobranchial body from the fourth pharyngeal pouch to the thyroid glands remains unclear. The ultimobranchial body descends to the thyroid gland along with the blood vessels in mice [7], and the pyriform sinus fistula originating from the ultimobranchial body runs with the cranial thyroid artery in cotton rats (*Sigmodon hispidus*) [16]. In cats and dogs, ultimobranchial body remnants are often localized adjacent to the parathyroid glands IV [11, 12]. These previous studies suggest a close relationship between blood vessels and the migration route of the ultimobranchial body and parathyroid glands IV. In one-humped camels, both the ultimobranchial body remnants and the small parathyroid glands were located around the cranial thyroid hilum. Within the thyroid glands, fossae of the ultimobranchial body remnants extended ventrodorsally and craniocaudally, and branched toward the central region of the thyroid glands where C-cells densely accumulated. C-cells also accumulate around

the ultimobranchial body remnants in other mammalian species [9, 16, 19], indicating close relationship between the fossae and C-cell distribution. Putting these findings together, we hypothesize that the ultimobranchial body enter along the cranial thyroid hilum, and disperse in the same direction as the fossae in the thyroid glands of one-humped camels.

In conclusion, we demonstrated that the ultimobranchial body remnants were limited to a region around the cranial thyroid hilum, and that C-cells were mainly concentrated within and around the ultimobranchial body remnants in one-humped camels. Their restricted localization, a unique condition found only in one-humped camels, might be helpful for clarifying the direction of dispersion of the ultimobranchial body within the thyroid glands.

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