Immunohistochemical localization of autophagosomal membrane-associated protein LC3 in granular cell tumor and schwannoma

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Abstract Granular cell tumor (GCT) is a neoplasm derived from Schwann cell or (in cases arising in the neurohypophysis) pituicyte and is characterized by abundant cytoplasm filled with numerous eosinophilic granules, which have been considered autophagolysosomes on the basis of their ultrastructure. To confirm that the formation of these granules is related to an autophagy phenomenon, 12 cases of GCT (including two cases of GCT of the neurohypophysis) were studied immunohistochemically using an antibody against LC3 (microtubule-associated protein 1 light chain 3, a specific marker of autophagy). All cases of GCT showed granular immunoreactivity for LC3 in the cytoplasm of tumor cells, indicating that the formation of intracytoplasmic granules in GCT is closely related to an autophagy phenomenon. For elucidation of the relationship between GCT and schwannoma, 20 cases of schwannoma were similarly studied using the anti-LC3 antibody. In eight of 20 cases, a small number of tumor cells showed granular immunoreactivity for LC3, suggesting an increased autophagic activity in some schwannomas and further reinforcing the close relationship between GCT and schwannoma.

Keywords Granular cell tumor · Schwannoma · Autophagy · Immunohistochemistry · LC3

Introduction

Granular cell tumor (GCT) is a neoplasm with distinctive histopathological and ultrastructural characteristics. It commonly arises in the skin and subcutaneous tissue, various visceral organs, especially the breast and upper aerodigestive tracts, and in the neurohypophysis [1]. Regarding the histogenesis of GCT, cases of GCT arising in the skin, subcutaneous tissue, and visceral organs are widely believed to originate from Schwann cells [2–7]. The histogenesis of GCT in the neurohypophysis remains controversial, but derivation from pituicyte has been proposed [8, 9]. Regardless of their localization or origin, the histopathology of GCT is quite uniform. It consists of a proliferation of large polyhedral cells with abundant cytoplasm which is loaded with numerous eosinophilic granules. Although these granules have been considered autophagosomes or autophagolysosomes based on their ultrastructural features [2, 3, 10], their exact nature and the process of their formation remain to be elucidated. It is also unknown why a large number of these granules are formed specifically in GCT.

In recent years, various genes and proteins related to autophagy (autophagocytosis) have been identified, and it has become possible to investigate the autophagy phenomenon by immunohistochemical methods, employing antibodies against these autophagy-related proteins [11, 12]. We investigated cases of GCT immunohistochemically employing an antibody that was raised against microtubule-associated protein 1 light chain 3 (LC3) [11]. This protein is considered to be specifically localized to the membranes of autophagosomes, and immunohistochemical studies employing this antibody have demonstrated its usefulness in the elucidation of the autophagy phenomenon [11, 12].
To clarify further the histogenetic relationship between GCT and schwannoma, we also examined cases of schwannoma for LC3 immunoreactivity.

Materials and methods

The surgical pathology files at Osaka Red Cross Hospital during the recent 6 years were searched for cases of GCT. Ten consecutive cases of GCT were retrieved for the study, and in all of these cases, the tumors exhibited a typical histopathological appearance of GCT. The clinicopathological features of individual cases are presented in Table 1. These consisted of four cases from the skin or subcutaneous tissue, five cases from the digestive tract, and one case from the trachea. Two GCTs of the neurohypophysis which were found incidentally at autopsies were also examined similarly. Twenty cases of schwannoma, which are listed in Table 2, were also retrieved from the surgical pathology files. These comprised 10 cases in which the tumors arose in the skin or subcutaneous tissue and another 10 cases in which the tumors arose in the cranial nerves or spinal nerve roots. Only tumors demonstrating histopathological features that were typical of conventional schwannoma were used for the study, and questionable or problematic cases were excluded. All tumors examined in this study had been fixed in 10% neutral buffered formalin and embedded in paraffin.

Immunohistochemical study was performed on paraffin sections using the Envision Plus detection system (Dako, Glostrup, Denmark), and polyclonal or monoclonal primary antibodies against the following substances were employed: LC3 (polyclonal; Medical & Biological Laboratories, Nagoya, Japan; 1:1,000), CD68 (clone KP1, Dako; 1:100), and S-100 protein (clone ER-PR8, Dako; 1:500). Two GCTs of the neurohypophysis were also examined for the expression of glial fibrillary acidic protein (GFAP, polyclonal; Dako; 1:100). Heat-induced epitope retrieval using a hot bath was performed before applying immunostains for LC3, CD68, and S-100 protein.

Results

A summary of the results of immunohistochemical study is presented in Tables 1 and 2. In all cases of GCT, tumors consisted of an alveolar, trabecular, or diffuse proliferation of large polyhedral cells that had abundant cytoplasm loaded with numerous eosinophilic granules of variable sizes (Fig. 1a). These granules were strongly or weakly positive for periodic acid-Schiff reaction. The cytoplasm of tumor cells exhibited intense, coarsely or finely granular immunoreactivity for LC3 in all cases (Fig. 1b). The cytoplasm was similarly immunoreactive for CD68 and S-100 protein. Two GCTs of the neurohypophysis (Fig. 2a) also showed granular immunoreactivity of the cytoplasm for LC3 (Fig. 2b). Both tumors were also immunoreactive for CD68 and S-100 protein, but only one of the two tumors exhibited focal immunoreactivity for GFAP.

In 8 of 20 schwannomas examined, a small number of large polyhedral cells loaded with eosinophilic granules ("granular cells") were scattered within the tumors (Fig. 3a). These cells were easily overlooked or often difficult to distinguish from reactive macrophages on hematoxylin–eosin sections, but they showed intense granular immunoreactivity and stood out clearly on sections immunostained for LC3 (Fig. 3b). These LC3-immunoreactive cells within schwannoma were also immunoreactive for CD68 and S-100 protein on serial sections. However, immunohistochemistry for these two markers was not useful for the detection of "granular cells," because almost all tumor cells were immunoreactive for S-100 protein, and CD68 was intensely immunoreactive in reactive macrophages within the tumors. Most schwannomas contained both Antoni A and B areas in variable proportions within a given tumor, and the distribution of "granular cells" tended to be more prevalent in Antoni A area than in Antoni B area.

Discussion

Abundant intracytoplasmic granules that accumulate in tumor cells of GCT have been considered autophagosomes or autophagolysosomes on the basis of their ultrastructural features [2, 3, 10]. However, as pointed out by some investigators [13], the electron microscopic identification of intracytoplasmic inclusions as autophagy-related structures

Table 1  Clinicopathological findings and results of LC3 immunohistochemistry in granular cell tumors

| Case | Age/gender | Site/diameter (mm) | LC3 |
|------|------------|-------------------|-----|
| 1    | 54/F       | abdomen/40        | +   |
| 2    | 76/F       | face/18           | +   |
| 3    | 60/M       | inguinal region/20| +   |
| 4    | 35/F       | chest wall/10     | +   |
| 5    | 56/F       | cecum/7           | +   |
| 6    | 35/F       | stomach/10        | +   |
| 7    | 66/M       | esophagus/5       | +   |
| 8    | 41/M       | esophagus/5       | +   |
| 9    | 42/M       | esophagus/5       | +   |
| 10   | 13/F       | trachea/15        | +   |
| 11   | 74/M       | neurohypophysis/1  | +   |
| 12   | 59/F       | neurohypophysis/4  | +   |
is occasionally not straightforward, and some structures unrelated to autophagy can be misinterpreted as autophagosomes. It is also unknown why autophagolysosomes are accumulated in a large amount specifically in tumor cells of GCT. Some intrinsic metabolic abnormalities within tumor cells of GCT probably result in focal cytoplasmic degradation and an accumulation of numerous autophagolysosomes.

Monoclonal antibodies against CD68 molecules, such as KP1 and PGM-1, have been employed as a marker for lysosome or phagolysosome [14–16], and several investigators demonstrated the immunoreactivity of GCT for these antibodies [14–16], which was also confirmed in our present study. We further applied an anti-LC3 antibody for the investigation of GCT and demonstrated that all GCTs examined showed immunoreactivity for LC3. LC3 is a protein located on the inner and outer membranes of autophagosomes [11, 12], and the findings of our study provide additional evidence for the autophagosomal origin of intracytoplasmic granules in tumor cells of GCT. Although the specificity of LC3 as a marker for autophagosome at the light microscopic level leaves some room for reservation because LC3 has been shown to be incorporated into protein aggregates independent of autophagy [12], the immunohistochemical reactivity of GCT for LC3 can be reasonably interpreted as representing an autophagy phenomenon in the light of their well-known ultrastructural features [2, 3, 10].

Concerning the histogenesis of GCT, the Schwann cell origin has been widely accepted in recent years [2–7]. The immunoreactivity of GCT for S-100 protein has been well known [5–7], and a recent study concerning many cases of peripheral nerve sheath tumors arising in the gastrointestinal tract demonstrated the immunoreactivity for CD56, }

| Case | Age/gender | Site/diameter (mm) | Antoni A & B | Granular cells | LC3 |
|------|------------|--------------------|--------------|----------------|-----|
| 1    | 51/F       | shoulder/25        | A=B          | +              | +   |
| 2    | 48/F       | retroperitoneum/38 | A>B          | –              | –   |
| 3    | 92/F       | orbit/25           | A>B          | +              | +   |
| 4    | 46/F       | hand/13            | A>B          | –              | –   |
| 5    | 29/F       | forearm/17         | A>B          | –              | –   |
| 6    | 58/M       | forearm/29         | A<B          | –              | –   |
| 7    | 36/M       | finger/9           | A<B          | –              | –   |
| 8    | 61/M       | knee/13            | A>B          | –              | –   |
| 9    | 60/F       | thigh/40           | A>B          | –              | –   |
| 10   | 52/F       | upper arm/15       | A>B          | +              | +   |
| 11   | 64/M       | cauda equina/4     | A            | +              | +   |
| 12   | 42/M       | thoracic root/18   | A<B          | –              | –   |
| 13   | 62/F       | lumbar root/18     | A>B          | +              | +   |
| 14   | 23/F       | acoustic nerve/15  | A>B          | –              | –   |
| 15   | 76/M       | acoustic nerve/12  | A            | +              | +   |
| 16   | 75/F       | acoustic nerve/20  | A<B          | –              | –   |
| 17   | 71/F       | cauda equina/10    | A            | +              | +   |
| 18   | 33/F       | acoustic nerve/19  | A            | +              | +   |
| 19   | 75/M       | lumbar root/23     | A>B          | –              | –   |
| 20   | 58/F       | acoustic nerve/27  | A            | –              | –   |

**Table 2** Clinicopathological findings and results of LC3 immunohistochemistry in schwannomas

**Fig. 1** a A subcutaneous GCT. Large polyhedral cells having abundant cytoplasm loaded with numerous eosinophilic granules proliferated diffusely (hematoxylin–eosin stain). b The intracytoplasmic granules in GCT showed an immunoreactivity for LC3 (immunoperoxidase method)
calretinin, and α-inhibin (as additional markers of Schwann cell differentiation) in GCT [7]. Immunohistochemical studies employing antibodies against various proteinaceous components of the myelin sheath have shown that intracytoplasmic granules in GCT contain degradation products of the myelin sheath, thus emphasizing further the close relationship between Schwann cell and GCT [6]. However, the relationship between GCT and ordinary schwannoma remains unclear. In an ultrastructural study of schwannoma, Sian and Ryan reported the occurrence of many cells containing intracytoplasmic osmiophilic granules in the Antoni B area and pointed out the similarity of these granules to those found in GCT [17]. A few cases of schwannoma have been reported in which scattered or aggregated “granular cells” were found [4, 18]. In the present study, we examined schwannoma for the presence of LC3-immunoreactive granules in the cytoplasm. In 8 of 20 schwannoma cases, a small number of tumor cells showed cytoplasmic swelling, and the swollen cytoplasm contained numerous granules that were immunoreactive for LC3. This finding probably corresponds to the immunoreactivity for CD68 of autophagosomes in Schwann cells in cases of degeneration of nerve fibers and also in schwannoma and neurofibroma [15, 16]. The immunohistochemistry for LC3 is superior to that for CD68 in detection of “granular cells” within schwannoma, because, while not only “granular cells” but also reactive macrophages showed immunoreactivity for CD68, only the former was highlighted by immunohistochemistry for LC3. The presence of “granular cells” immunoreactive for LC3 indicates increased autophagic activity in some cases of schwannoma and further reinforces the close relationship between GCT and schwannoma.

Some neoplasms other than schwannoma, for example smooth muscle tumors [19], astrocytoma [20], glioblastoma [21], oligodendrogliaoma [22], and anaplastic medulloblastoma [23], are known to rarely exhibit a “granular cell change” of the cytoplasm. Mentzel et al. studied the “granular cell change” in smooth muscle tumors but found that the change was not related topographically to areas showing degenerative features such as necrosis, hemorrhage, hyalinization, or myxoid change [19]. Although it remains to be clarified whether these tumors also show immunoreactivities for proteins specific to the autophagy-related processes, this finding has something in common with findings we obtained in schwannoma, that is, the occurrence of “granular cells” was not always associated with degenerative changes represented by the Antoni B area in schwannoma. The role of autophagy is diverse and includes the adaptation to nutrient starvation by producing amino acids and the constitutive removal or turnover of cytosolic proteins and redundant or damaged organelles [11, 12, 24]. Autophagy phenomenon therefore does not necessarily indicate degenerative changes of the cells, but it is a dynamic adaptive response to sublethal stress [24].

In conclusion, we demonstrated immunoreactivity for LC3, an autophagy-specific marker, in tumor cells in all...
cases of GCT. Eight of 20 cases of schwannoma contained LC3-immunoreactive “granular cells.” These results indicate that intracytoplasmic granules in tumor cells of GCT are actually autophagosomes or autophagolysosomes and the activity of autophagy is increased in some cases of schwannoma, supporting a close histogenetic relationship between GCT and schwannoma.

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Conflict of interest statement We declare that we have no conflict of interest.

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