Pathology - Research and Practice 215 (2019) 1–4

Contents lists available at ScienceDirect

Pathology - Research and Practice

journal homepage: www.elsevier.com/locate/prp

Review

Structural biology of intraepithelial neuroendocrine cells in the larynx: Literature review☆

Asterios Triantafyllou[a,b,*], Kenneth O. Devaney[c], Jennifer L. Hunt[d], Alessandra Rinaldo[e], Alfio Ferlito[f]

[a] Department of Pathology, Liverpool Clinical Laboratories, Royal Liverpool University Hospital, Liverpool, UK
[b] School of Dentistry, University of Liverpool, Liverpool, UK
[c] Department of Pathology, Allegiance Health, Jackson, MI, USA
[d] Department of Pathology, University of Arkansas for Medical Sciences, Little Rock, AR, USA
[e] University of Udine, School of Medicine, Udine, Italy
[f] Coordinator of the International Head and Neck Scientific Group, Padua, Italy

ARTICLE INFO

Keywords:
Electron microscopy
Histology
Immunohistochemistry
Larynx
Neuroendocrine cells

ABSTRACT

Current knowledge of laryngeal neuroendocrine cells in man and other vertebrates is reviewed. Particular attention is paid to differences in the distribution of neuroendocrine cells between squamous and respiratory laryngeal mucosa, foetal versus post-natal spatial arrangements, relation to the laryngeal cavity and nerve fibres, and immunoreactivities of these cells. Methodological deficiencies and gaps in knowledge are outlined. Comparisons with neuroendocrine cells in lung and gut are drawn, caution with regard to existing histogenetic models of laryngeal neuroendocrine neoplasia is advised and lines of future research are suggested.

1. Introduction

Common experience would predict that various mechanical and/or chemical factors targeting the laryngeal mucosa would elicit reflexes ranging from retching to choking [3]. These reflexes may, in turn, evoke allergic reactions or secretion of mucus [33], depending on the presence of free nerve endings, tactile or chemo-receptors and intraepithelial neuroendocrine cells [3]. These neuroendocrine cells may be acting as endocrine or paracrine agents [33], akin to the intestinal Kulchitsky cells and other components of the diffuse neuroendocrine system. Although laryngeal nerve endings and localization of neuropeptides have been investigated [1,13,28] and much attention has been paid to laryngeal neuroendocrine neoplasms [9,14,26], our understanding of intraepithelial neuroendocrine cells in the normal or non-neoplastic laryngeal mucosa is still incomplete. There is only a passing reference to these cells in specialised texts [7]. An understanding of the various cells and tissues of head and neck is expected from pathologists specializing in this area – hence, aspects of the structural biology of laryngeal neuroendocrine cells are critically reviewed here. The literature search was based on the use of PubMed and the MeSH terms 'neuroendocrine cells', 'APUD cells' (since 'APUD' preceded 'neuroendocrine') and 'larynx'. Laryngeal paraganglia and paraganglionic tissue in laryngeal nerves [5,16] are outside the scope of this review.

2. Methodological considerations

Current approaches to the investigation of intraepithelial laryngeal neuroendocrine cells are largely based on morphology. Ideally, such an approach would aim at detecting and mapping out the in-situ arrangements and numbers of these cells with the combined use of argentaffin and argyrophil special stains, other techniques of conventional histochemistry, electron microscopy, immunohistochemistry employing a wide range of antibodies, and morphometry. This array of investigations would allow assessing the sensitivity and specificity of the respective technologies as well as comparing the distribution and density of the neuroendocrine cells between various topographical areas of the larynx. Unfortunately, the published investigations available to date (see 'Neuroendocrine cells in the laryngeal mucosa of vertebrates other than man' and 'Neuroendocrine cells in the laryngeal mucosa of man' below) do not fully satisfy such requirements, inasmuch as most are useful compromises.

Controversies apart, despite the availability of various technologies and reagents, many recent investigations of intraepithelial laryngeal neuroendocrine cells have chiefly employed the widely available and

☆ This article was written by members of the International Head and Neck Scientific Group (www.IHNSG.com).

* Corresponding author at: Department of Pathology, NHS Liverpool Clinical Laboratories, Duncan Building, Daulby Street, Liverpool, L69 3GA, UK.
E-mail address: A.Triantafyllou@liverpool.ac.uk (A. Triantafyllou).

https://doi.org/10.1016/j.prp.2018.10.027
Received 19 August 2018; Received in revised form 21 October 2018; Accepted 22 October 2018
0344-0338/ Crown Copyright © 2018 Published by Elsevier GmbH. All rights reserved.

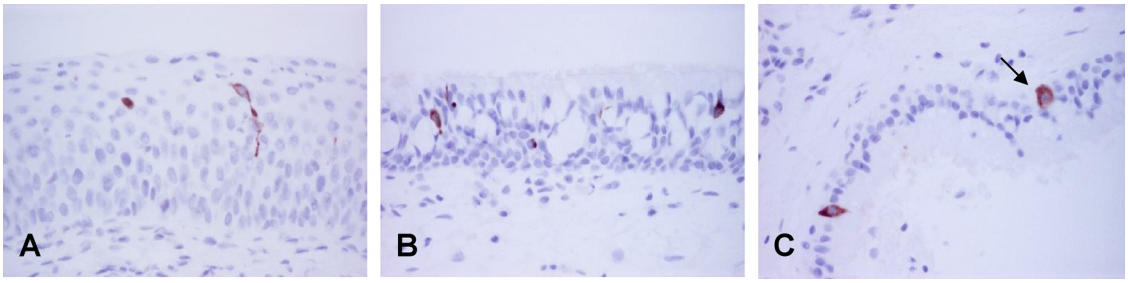

Fig. 1. Varying numbers of neuroendocrine cells staining for synaptophysin in squamous (A) and respiratory (B) laryngeal epithelia, and lining of an underlying cyst (C). While most appear slender and columnar with the unstained nuclei situated at different levels, the cell in the upper right part of (C) (arrow) is basally situated and resembles conventional Kulchitsky cells. The cavity of the larynx is towards the upper part of (A) and (B); the lumen of the cyst is towards the lower right part of (C) (objective magnification ×20).

often not overly technically demanding immunohistochemistry. This usually influences the study design in turn. Further limiting these investigations is the fact that desirable information on the topographical distribution and density of neuroendocrine cells is often frustratingly lacking; moreover, human studies are often restricted to pathological material.

3. Neuroendocrine cells in the laryngeal mucosa of vertebrates other than man

Early investigations focussed on rodents and applied histochemistry and/or electron microscopy to specially preserved tissues [8,17]. These investigations are dating back to an era when the notion of APUD (amine precursor uptake and decarboxylation) cells [22,23,25], which eventually led to the current perception of a diffuse neuroendocrine system, enjoyed popularity. It was at that time that biogenic amines were histochemically localized in cells of the laryngeal epithelium of rat [8]. Despite this 'endocrine-like' feature [8,23], the nature of those intraepithelial cells was not established. Their electron microscopy correlation also appears open to question. Fig. 3 in Ewen et al. [8] illustrates an 'endocrine-like' cell situated among conventional columnar ciliated and mucous cells. This 'endocrine-like' cell lacks small basally-situated dense-cored vesicles, and instead shows large apical electron-dense secretory granules. Accordingly, it would be better interpreted as a serous exocrine cell. This interpretation is supported by a later electron microscopic investigation that established the presence of serous cells in the respiratory epithelium of the rat larynx [17]; this investigation also reported rare singly occurring Kulchitsky-type cells with characteristic dense-cored cytoplasmic vesicles, therein. Fig. 9 in Lewis and Prentice [17] illustrates the differences between the adluminal serous and basal Kulchitsky-type cells, but that observation was not pursued further.

A more recent and informative investigation in the larynx of cat [33] applied both electron microscopy and immunohistochemistry. That investigation reported an increased occurrence of bipolar, neuroendocrine cells in the subglottic epithelium, when compared with the glottis and supraglottis. The neuroendocrine cells showed cytoplasmic, preferentially subnuclear, dense-cored vesicles and a microvillous apex reaching the mucosal surface [33]. The latter feature is supported by an immunohistochemical investigation in the frog (see Fig. 21 in Bodegas et al. [4]).

It appears that while intraepithelial neuroendocrine cells in the lungs can occasionally be clustered to form 'neuroepithelial bodies' [12], those in the larynx of rodents and amphibia occur as single, individual units [4,11,17]. McDowell et al., however, observed neuroepithelial bodies in the larynx of hamster foetuses, though these structures became inconspicuous in the postnatal setting [20]. These authors also drew attention to differential expressions of immunoreactive substances in the intraepithelial laryngeal neuroendocrine cells, which were related to age, topography or cellular arrangement [20].

It is of interest that experimental manipulations including antigenic stimulation and exposure to hypercapnic hypoxia effect increased numbers of APUD and 'endocrine' cells in the laryngeal epithelium of guinea pigs [18] and rats [32], respectively. Those APUD cells were elongated and argyrophilic [18]; the morphologically similar 'endocrine' cells were positive for calcitonin gene-related peptide and protein gene product 9.5 [32].

4. Neuroendocrine cells in the laryngeal mucosa of man

A 1984 investigation used special staining to identify dendritic argyrophilic cells in respiratory epithelia of the larynx in 2 out of 13 autopsies; these cells were different from melanocytes, interpreted as APUD cells and occurred at different levels within the epithelia [27]. The presence of such cells was not echoed by Woodruff et al. [31]. However, while investigating neuroendocrine carcinomas of the larynx, those authors observed cells with weak calcitonin immunoreactivity in glands of 9 out of 21 'normal' laryngeal specimens; photomicrographs of these cells were not provided [31].

A more recent immunohistochemical investigation of 20 human laryngectomies [6], achieved localisation of neuroendocrine cells in the non-neoplastic mucosal epithelium. These cells were positive for chromogranin and synaptophysin, though did not express CD56 or calcitonin; they appeared in a 1:20 neuroendocrine:epithelial cell ratio and were preferentially associated with the laryngeal respiratory epithelium (ventricle, subglottis) rather than with the squamous epithelium (glottis, supraglottis). This localisation broadly corresponds with the topographical distribution of similar cells in the rat and cat [17,33]. The human laryngeal neuroendocrine cells appeared to be basally located, individual units of a bipolar or stellate silhouette, which did not reach the mucosal surface [6]. Such features correspond with unpublished pathological experience (Fig. 1A, B), but contrast with the aforementioned observations in cat and frog in which laryngeal neuroendocrine cells do reach the mucosal surface [4,33]. The issue had not been pursued by step-sectioning. In addition, the slender elongated neuroendocrine-like cells that were immunoreactive for protein gene product 9.5, described by Hauser-Kronberger et al. in the normal larynx of man, traversed the entire thickness of the mucosal epithelium and were associated with similarly immunoreactive nerve fibres [13].

Established immunoreactivities of laryngeal neuroendocrine cells in man and other vertebrates are summarized in Table 1. Methodological differences in the respective investigations, however, preclude detailed comparative and/or evolutionary considerations.

5. Neuroendocrine cells in the laryngeal glands?

An early investigation reported argyrophilic cells in the salivary-type glands of larynx in the guinea-pig [15]. The photomicrographs indicate that these cells are in 'myoepithelial' and ductal arrangements. An argyrophil staining technique in which reaction product depends on

Table 1
Immunoreactivities of neuroendocrine cells in the normal or non-neoplastic laryngeal mucosa.

Authors	Species	Immunoreactivities
Gomi et al. [11]	Salamander	serotonin
Bodegas et al. [4]	Frog	bombesin, chromogranin, endothelin-1, substance P
McDowell et al. [20]	Hamster	calcitonin, calcitonin gene-related peptide, protein gene product 9.5, serotonin
Yu et al. [33]	Cat	calcitonin gene-related peptide, neuron-specific enolase, protein gene product 9.5, serotonin, substance P
Yamamoto et al. [32]	Rat	calcitonin gene-related peptide, protein gene product 9.5
Chung et al. [6]	Man	chromogranin, synaptophysin
Hauser-Kronberger et al. [13]		protein gene product 9.5

the addition of an extraneous reducer, would also demonstrate argentaffin tissue components having an innate reducing capacity [24]. At the same time, lipofuscin is common in salivary glandular parenchyma [10,29] - and lipofuscin also possesses argentaffin qualities [24]. Finally, it is unlikely that intraepithelial, neuroendocrine cells are present in normal salivary glands [29]. These arguments suggest that the argyrophilic cells in laryngeal glands [15] correspond to lipofuscin-laden parenchymal cells rather than to neuroendocrine cells. Whether the stained elements in Fig. 1C correspond to newly-formed or pre-existing neuroendocrine cells incorporated in the cyst lining, is speculative. The observations by Woodruff et al. [31] have been discussed above.

6. Discussion

Observations indicate that the intraepithelial laryngeal neuroendocrine cells can reach the mucosal surface [4,33] and thus are of the open type [7]. This in turn suggests that they are capable of responding to stimuli from the laryngeal cavity. Their association with nerve fibres also suggests that they may share some kinship with bipolar neurones like the olfactory cells. This suggestion fits with the predilection of the neuroendocrine cells for respiratory-type laryngeal mucosa [17,33], and with their microvillous apices in cat [33]. On these grounds, the laryngeal neuroendocrine cells appear to differ from the intestinal Kulchitsky cells, which are often conical with a base facing the stroma and an apex not reaching the lumen [19] (closed type [7]).

The neuroendocrine cells in mucosa and, possibly, glandular epithelia of the human larynx are obvious candidates for the cell of origin of neuroendocrine neoplasms in that organ [14,27,31]. An innate proliferative capacity of those cells would fit with this notion. Indeed, such a capacity can be reconciled with the finding of increased numbers of such cells in rodents following antigenic or oxygenation challenging [18,32] (see 'Neuroendocrine cells in the laryngeal mucosa of vertebrates other than man'). Caution should be, however, exercised in this interpretation. The neuroendocrine neoplasms are usually located in the supraglottic larynx [26] while normal neuroendocrine cells are common in the subglottic sites [6,33]. In addition, the results of the experimental manipulations explored above [18,32] may reflect acquisition of neuroendocrine phenotypes by reserve or undifferentiated, basal cells of laryngeal epithelia, in response to an altered microenvironment, and not the proliferation of pre-existing differentiated neuroendocrine cells. On these grounds, it seems difficult and even futile to attempt to establish the histogenesis of laryngeal neuroendocrine neoplasms. Perhaps, these reflect clonal proliferations of an unidentified cell expressing neuroendocrine histochemical, immunohistochemical and ultrastructural phenotypes generated via as yet unidentified innate genetic/molecular events, possibly in combination with environmental factors. The latter may influence the development of the more common, supraglottic and thus more superficial, laryngeal neuroendocrine neoplasms. Epidemiological investigations examining laryngeal neuroendocrine neoplasms in a setting of asthma or pulmonary insufficiency may be a means of exploring this hypothesis. It is noted that a series of 54 moderately-differentiated neuroendocrine laryngeal carcinomas reported a 68% history of long-term smoking [30].

The present review has indicated possible deficiencies and gaps in knowledge, and future work may address these. Physiological investigations exploring the effect of mechanical, chemical or neural stimulation can be envisaged. Current evidence favours an endodermal rather than neurocristic origin of the gut and thyroid neuroendocrine and APUD cells [2,21] and it may well be that the situation is similar in the larynx. Molecular approaches to the unravelling of factors influencing development and differentiation of laryngeal neuroendocrine cells and their role in histogenetic models of laryngeal neoplasia, are desirable.

Competing interests

None declared.

References

[1] K. Albegger, C.E. Hauser-Kronberger, A. Saria, A.H. Graf, G. Bernatzky, G.W. Hacker, Regulatory peptides and general neuroendocrine markers in human nasal mucosa, soft palate and larynx, Acta Otolaryngol. 111 (1991) 373–378.
[2] A. Andrew, B. Kramer, B.B. Rawdon, The origin of gut and pancreatic neuroendocrine (APUD) cells–the last word? J. Pathol. 186 (1998) 117–118.
[3] R.M. Bradley, Sensory receptors of the larynx, Am. J. Med. 108 (Suppl. 4a) (2000) 47S–50S.
[4] M.E. Bodegas, L.M. Montuenga, P. Sesma, Neuroendocrine diffuse system of the respiratory tract of Rana temporaria: an immunocytochemical study, Gen. Comp. Endocrinol. 100 (1995) 145–161.
[5] B. Carlsöö, A. Dahlqvist, S. Domeij, S. Hellström, H.H. Dedo, K. Izdebski, Carotid-body-like tissue within the recurrent laryngeal nerve: an endoneural chemosensitive micro-organ? Am. J. Otolaryngol. 4 (1983) 334–341.
[6] J.H. Chung, S.S. Lee, Y.S. Shim, S.Y. Kim, S.Y. Nam, D.H. Kim, J.K. Cho, A study of moderately differentiated neuroendocrine carcinomas of the larynx and an examination of non-neoplastic larynx tissue for neuroendocrine cells, Laryngoscope 114 (2004) 1264–1270.
[7] R.A. DeLellis, Y. Dayal, The neuroendocrine system, in: S.E. Mills (Ed.), Histology for Pathologists, fourth ed., Wolters Kluwer/Lippincott Williams & Wilkins, Philadelphia, 2012pp. 1255–1276.
[8] S.W. Ewen, G. Bussolati, A.G. Pearse, Uptake of L-dopa and L-5-hydroxytryptophan by endocrine-like cells in the rat larynx, Histochem. J. 4 (1972) 103–110.
[9] A. Ferlito, A. Coca-Pelaz, J.P. Rodrigo, A. Triantafyllou, K.O. Devaney, J.L. Hunt, B. Perez-Ordoñez, P.J. Slootweg, D. Bell, J.A. Bishop, A. Rinaldo, New tumor phenotypes reported in the larynx in the last decades: a critique, Am. J. Otolaryngol. 36 (2015) 494–497.
[10] J.R. Garrett, The ultrastructure of intracellular fat in the parenchyma of human submandibular salivary glands, Arch. Oral Biol. 8 (1963) 729–734.
[11] T. Gomi, Y. Kikuchi, D. Adriaensen, J.P. Timmermans, M.H. De Groodt-Lasseel, A. Kimura, H. Naruse, Y. Ishikawa, K. Kishi, D.W. Scheuermann, Immunocytochemical survey of the neuroepithelial endocrine system in the respiratory tract of the Tokyo salamander, Hynobius nebulosus tokyoensis TAgo, Histochemistry 102 (1994) 425–431.
[12] J.R. Gosney, Endocrine pathology of the lung, in: P.P. Anthony, R.N.M. MacSween (Eds.), Recent Advances in Histopathology, vol. 16, Churchill Livingstone, Edinburgh, 1994pp. 147–165.
[13] C. Hauser-Kronberger, G.W. Hacker, P. Franz, K. Albegger, O. Dietze, CGRP and substance P in intraepithelial neuronal structures of the human upper respiratory system, Regul. Pept. 72 (1997) 79–85.
[14] J.L. Hunt, A. Ferlito, H. Hellquist, A. Rinaldo, A. Skálová, P.J. Slootweg, S.M. Willems, A. Cardesa, Differential diagnosis in neuroendocrine neoplasms of the larynx, Adv. Anat. Pathol. 24 (2017) 161–168.
[15] S. Kirkeby, P. Romert, Argyrophilic cells in the larynx of the guinea-pig demonstrated by the method of Grimelius, J. Anat. 123 (Pt 1) (1977) 87–92.
[16] W. Lawson, F.G. Zak, The glomus bodies ("paraganglia") of the human larynx, Laryngoscope 84 (1974) 98–111.
[17] D.J. Lewis, D.E. Prentice, The ultrastructure of rat laryngeal epithelia, J. Anat. 130 (1980) 617–632.

[18] A.M. Marchevsky, S. Keller, J.R. Fogel, J. Kleinerman, Quantitative studies of argyrophilic APUD cells in airways. III. The effects of sensitization and anaphylactic shock, Am. Rev. Respir. Dis. 129 (1984) 477–480.
[19] P. Masson, Carcinoids (argentaffin-cell tumors) and nerve hyperplasia of the appendicular mucosa, Am. J. Pathol. 4 (1928) 181-212.19.
[20] E.M. McDowell, S.P. Sorokin, R.F.Jr Hoyt, Ontogeny of endocrine cells in the respiratory system of Syrian golden hamsters. I. Larynx and trachea, Cell Tissue Res. 275 (1994) 143–156.
[21] M. Nilsson, D. Williams, On the origin of cells and derivation of thyroid cancer: C cell story revisited, Eur. Thyroid J. 5 (2016) 79–93.
[22] A.G. Pearse, The diffuse neuroendocrine system: peptides, amines, placodes and the APUD theory, Prog. Brain Res. 68 (1986) 25–31.
[23] A.G. Pearse, The cytochemistry and ultrastructure of polypeptide hormone-producing cells of the APUD series and the embryologic, physiologic and pathologic implications of the concept, J. Histochem. Cytochem. 17 (1969) 303–313.
[24] A.G.E. Pearse, Histochemistry, 2nd ed., Theoretical and Applied vol. 2, Churchill Livingstone, Edinburgh, 1972.
[25] A.G. Pearse, The APUD cell concept and its implications in pathology, Pathol. Annu. 9 (1974) 27–41.
[26] B. Perez-Ordonez, J.A. Bishop, D.R. Gnepp, J.L. Hunt, L.D.R. Thompson, Neuroendocrine tumours, in: A.K. El-Naggar, J.K.C. Chan, G.R. Grandis, T. Takata, P.J. Slootweg (Eds.), WHO Classification of Head and Neck Tumours, 4th ed., IARC, Lyon, 2017pp. 95–98.
[27] C. Pesce, F. Tobia-Gallelli, C. Toncini, APUD cells of the larynx, Acta Otolaryngol. 98 (1984) 158–162.
[28] N. Takahashi, N. Nakamuta, Y. Yamamoto, Morphology of P2X3-immunoreactive nerve endings in the rat laryngeal mucosa, Histochem. Cell Biol. 145 (2016) 131–146.
[29] A. Triantafyllou, J.L. Hunt, K.O. Devaney, A. Ferlito, A perspective of comparative salivary and breast pathology. Part I: microstructural aspects, adaptations and cellular events, Eur. Arch. Otorhinolaryngol. 271 (2014) 647–663.
[30] B.M. Wenig, V.J. Hyams, D.K. Heffner, Moderately differentiated neuroendocrine carcinoma of the larynx. A clinicopathologic study of 54 cases, Cancer 62 (1988) 2658–2676.
[31] J.M. Woodruff, A.G. Huvos, R.A. Erlandson, J.P. Shah, F.P. Gerold, Neuroendocrine carcinomas of the larynx. A study of two types, one of which mimics thyroid medullary carcinoma, Am. J. Surg. Pathol. 9 (1985) 771–790.
[32] Y. Yamamoto, T. Kusakabe, Y. Hayashida, T. Yoshida, H. Matsuda, Y. Atoji, Y. Suzuki, Laryngeal endocrine cells: topographic distribution and adaptation to chronic hypercapnic hypoxia, Histochem. Cell Biol. 114 (2000) 277–282.
[33] Y.C. Yu, J. Miyazaki, T. Shin, Neuroendocrine cells in the cat laryngeal epithelium, Eur. Arch. Otorhinolaryngol. 253 (1996) 287–293.