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The origin of neuroendocrine tumors and the neural crest saga

Juan Rosai[1,2]

[1]*International Center for Oncologic Pathology Consultations, Centro Diagnostico Italiano, Milan, Italy and* [2]*Genzyme Genetics, New York, NY, USA*

In this essay, the role of the neural crest in the development of the vertebrate embryo is briefly described. The techniques used to document the neural crest origin of various cell types and the tumors arising from them are discussed, with emphasis on Le Douarin's quail-chick chimera model. The current dogma on the origin of the cells of the diffuse endocrine system is presented, and some personal conjectures based on the microscopic appearances of various types of normal, vestigial and neoplastic human tissues are offered to the reader as 'food for thought.'
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The biology of the diffuse neuroendocrine system, its controversial embryologic derivation, and the variegated number of tumors that can give rise to constitute one of the most fascinating chapters of pathology. The saga had rather modest beginnings, consisting in the description by normal anatomists of scattered cells throughout the intestinal mucosa, which were identifiable through their reactivity for chromium and silver salts, and which seemed to be morphologically and functionally different from the numerically more conspicuous cells present in the same mucosa. One of the better-known studies of these cells was carried out by Nicholas (Nikolai) Kultschitsky (1856–1925), Professor of Histology at the University of Kharkov (Russia), in his paper 'Zur Frage über den Ban des Darmkanals.' In this contribution, written in 1897, he pointed out the different polarity that these cells exhibited when compared with the mucus-secreting and absorbing cells of the same mucosa, suggesting that their secretory product was not emptied into the intestinal lumen but rather toward the basilar pole of the mucosa and possibly into the vessels. These cells, which were recognized independently by several other investigators, were variously called enterochromaffin cells, argentaffin cells, clear cells, enteroendocrine cells, and Kultschitsky cells, the latter term honoring the individual who had studied them in greatest detail. As an aside, it could be mentioned here that Kultschitsky was a very distinguished and influential scientist, to the point of being given by the Czar the title of Imperial Minister of Education for all of Russia. The advent of the Bolshevik revolution led to an end to all of these activities. He was incarcerated and later worked in a soap factory for years to save his life. He and his family managed to escape to England on a British battleship together with the surviving members of the Russian Royal family and eventually secured a position with Bayliss and Starling at the University College (London), where he continued his research, tragically cut short by a bizarre accident, a fall into an elevator shaft in the University building on the day of his 69th birthday.[1]

The next important chapter of this account was written by Siegried Oberdorfer[2] while at the University of Munich, who coined in 1907 the term *Karzinoid Tumoren* (*carcinoid or carcinoma-like tumor*) for a morphologically distinct type of intestinal neoplasm composed of tight nests of small uniform cells, which had already been identified but not pursued further by T Langhans, O Lubarsch, and WB Ramson.[3] As another interesting historical aside, Helena ('Leni'), the daughter of Dr Oberdorfer, worked at Harvard University, married Dr Castrillon, a Colombian anesthesiologist, and moved to Colombia (South America), to become the first female full-time professor at the University of Antioquia and the first pediatrician of the country.[4]

Correspondence: Professor J Rosai, MD, Department of Pathology, International Center for Oncologic Pathology Consultations, Centro Diagnostico Italiano, Via Saint Bon, 20, 20147 Milano, Italy.
E-mail: rosai@cdi.it
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Although the possible endocrine nature of Kultschitsky's cells and the relationship of these cells to carcinoid tumors had been suggested by other authors, it was the genius of Pierre Masson, at the time a young Assistant at the Pasteur Institute in Paris, that linked these isolated observations into a more encompassing theme, that of the endocrine tumors of the gastrointestinal tract. His original paper, written with A Gosset in 1914,[5] refers specifically to the appendix, but it soon became obvious that the observations made in it also applied to other portions of the gastrointestinal tract. The fact that Gosset and Masson entitled their paper 'Endocrine tumors of the appendix' leaves little doubt as to their views of the nature of Oberndorfer's carcinoid tumors.

While those developments were taking place in the field of pathology, normal anatomists were probing further on the subject of neuroendocrine cells of the gastrointestinal mucosa and finding out that such cells were present in greater or lesser amount throughout the entire digestive tract. The most comprehensive evaluation of this system was made by F Feyrter, Professor of Pathology at the University of Gottingen, who put the concept of the 'diffuse' endocrine system on solid grounds.[6] With the advent of enzyme histochemistry, electron microscopy, and immunohistochemistry, the existence of a complex system of endocrine cells dispersed throughout the digestive tract became established. Just as importantly, it became increasingly evident that such a system was not exclusive of this organ system but that it also manifested itself—although it a much more restricted manner—in practically all mucosal-lined organs of the body. Another significant discovery was the realization that the cells of the diffuse neuroendocrine system could be divided into two distinct categories: those that discharged their hormonal content into the blood to exert their function throughout the entire body ('true' endocrine cells), and those that limited their action to a restricted anatomic field delimited by the dendrite-like prolongations present in those cells ('paracrine' cells). For the purposes of this discussion, no distinction is made in this article among these two types of cells. Actually, the terms endocrine, (neuro)endocrine, enteroendocrine, and diffuse endocrine system are used interchangeably throughout the text.

In the 1960s, a consensus had been reached by which the cell of the diffuse neuroendocrine system could be accurately defined on the basis of their properties: representation in all types of glandular mucosae, basal-type polarity, and the presence of intracytoplasmic secretory granules showing varying types of affinity for silver stains (argentaffin and argyrophil cells), which appeared as dense core granules at the electron microscopic level. The next step was to sort out the numerous members of this family into specific types on the basis of the above-listed criteria. At the same time, pathologists were beginning to subdivide the tumors composed of those cells (still generically called carcinoid tumors) into subtypes on the basis of their histologic appearance, type of secretory product, and behavioral features. One of the most original efforts in that direction was that of ED Williams, who proposed in 1963, while a Research Assistant at the Bernhard Baron Institute of Pathology of London Hospital, a classification of carcinoid tumors based on their derivation from different embryonic segments of the gut, that is, foregut, midgut, and hindgut.[7] Although this scheme was eventually superseded by more specific distinctions largely based on immunohistochemical markers, it was of great importance in suggesting a possible relationship between tumor morphology and topography, the latter seen as a function of embryology.

The embryologic approach to the understanding of neuroendocrine cells and the tumors thereof acquired great importance through the work of AGE Pearse and his associate Julia Polak.[8,9] These investigators postulated the existence of a common biochemical pathway in these cells, consisting in the uptake and decarboxylation of amine precursors and expressed by the catchy acronymical designation APUD. More closely related to the subject of this essay, they postulated a common embryologic origin of these cells from the neural crest, a transient neural structure unique to vertebrates located on both sides of the embryonic neural plate, at the junction with the normal ectoderm. The neural crest, first described by Wilhelm His in 1868 and named as such by Arthur Milnes in 1879, is composed of a pluripotent cell population, which in the course of the normal embryonic development migrates throughout the body to give rise to many divergent derivatives, including neurons (ganglion cells) and glia of the sensory, sympathetic and enteric systems, melanocytes, the chromaffin cells of the adrenal medulla and extraadrenal paraganglia, thyroid C cells, and the bones, cartilage, and connective tissues of the face.[10] Pearse and Polak[11] based their theory of the neural crest origin of the endocrine cells of the digestive tract largely on observations derived from the application of formaldehyde-induced fluorescence (the 'APUD-FIF' technique), a highly sensitive method, which, however, is of very difficult interpretation as far as the identity of the positive cells is concerned. More sophisticated techniques developed by other investigators provided results that casted serious doubts on the neural crest origin of these cells. One such experiment was carried out by Pictet *et al*[12] using pancreatic anlage of rats as a model. The authors removed the entire ectoderm of embryonic rats prior to the formation of the neural crest, cultured the mesoderm for 11 days, and found that in every case in which a pancreas had developed, insulin was detected and/or B cells were observed. The authors reasonably concluded that these findings eliminated the possibility of a neural crest origin for these cells.

A very ingenious model applied to the study of this issue was devised by the French embryologist Nicolle LeDouarin at the Embryology Institute of the CNRS and the College of France.[13] It consisted in creating chick-quail chimeras by replacing segments of chick neural crest with isotopic and isochronous segments of quail neural crest, to then follow the migration of the latter in the chimeric animals, taking advantage of the fact that quail cells are easily identified in Feulgen-stained (or even hematoxilin-eosin stained) preparations because of the presence within their nucleus of a large globular clump of heterochromatin resembling a nucleolus. Through this clever stratagem, LeDouarin and her coworkers were able to confirm that ganglion cells of the submucous and myenteric plexus of the gastrointestinal tract, cells of paraganglia, melanocytes, and thyroid C cells were indeed of neural crest origin, in that they all exhibited the quail signature. Instead, the cells of the gastrointestinal diffuse endocrine system lacked this marker, indicating a lack of participation of quail neural crest in their genesis and indirectly favoring a local origin from endodermally derived cells.[14] Largely as a result of the observations made possible by this original model, the belief in a neural crest origin for the APUD cells was discarded and replaced by the Cheng and Leblond's scheme, in which the gut endocrine cells are shown to have the same endodermal origin as the other cell components of the intestinal mucosa, that is, absorptive cells, mucus-secreting (goblet) cells, and Paneth cells.[15]

An observation in support of this alternative scheme is the existence of the so-called amphicrine cells, that is, cells that combine features of endocrine and exocrine cells (manifested, for instance, by the combined cytoplasmic presence of mucin and neurosecretory-type granules), a finding that would be very difficult to rationalize within the neural crest theory framework. An analogous observation at the neoplastic level is the presence of malignant tumors combining endocrine and exocrine features, such as so-called goblet cell carcinoid tumor of the appendix, adenocarcinoma-small cell neuroendocrine carcinoma of the bowel and other sites, and mixed follicular-medullary and papillary-medullary carcinoma of the thyroid gland.[16]

Thus, it would seem as if the riddle of the origin of the diffuse endocrine cells and their tumors has been settled once and for all.[17,18] Yet, there are some lingering facts that do not quite fit this appealing scheme, facts that suggest that the whole story is yet to be told. The reader should be warned that the considerations I am making below did not emerge from the results of an ingenious experimental model like the one devised by Le Douarin or even from a systematic review of microscopic preparations of the various settings in which normal or neoplastic neuroendocrine cells are present. Rather, they represent the condensation of life-long reflections (some might say divagations) based on the many writings on the subject (particularly the early works of master histologists), on random microscopic observations made on routine and consult material, and on discussions held over the years with people who were as fascinated as myself by the subject, guided by the belief that nothing in cell biology is casual, confident that static histology can still teach us something about function, and aware of the fact that pathologic anatomy can throw light on the corresponding normal anatomy ('pathology illuminating biology,' in the felicitous expression of Pierre Masson). Let's take a look at these observations:

1. *The neural-like phenotype of (neuro)endocrine cells*. Whatever the ultimate origin of neuroendocrine will prove to be, there is no question that their phenotype has distinctly neural features, including the expression of allegedly specific neural markers.[19] They are not called 'neuroendocrine' for nothing. Of course, we know that a common phenotype does not necessarily mean a common histogenesis. Yet, the neural-like properties of (neuro)endocrine cells are so blatant as to render one incredulous to the notion that the nervous system does not have a role of some kind in their development, specific location, and ultimate function.[20] Significantly, these neural-like features are not uniformly distributed throughout the diffuse endocrine system. There is instead a 'gradient' of neural as opposed to epithelial features in the system, which relates to topography and which is generally ignored. Thus, the neuroendocrine cells located in the larynx, lung, thymus, and thyroid (C cells) are the most 'neural-like' cells of the system, a feature that becomes obvious in the corresponding tumors. It is in neoplasms of this subset that one observes at the electron microscopic level a profusion of neurotubules and dendrite-like prolongations containing synaptic vesicles; it is at these sites that spindle cell (Schwann cell-like) variants of these tumors are found; and it is in these locations that rare examples of pigmented (melanotic) variants have been described, melanin being a quintessential neural product. Conversely, the (neuro)endocrine cells of the digestive tract and their tumors lack almost always these features (or exhibit them is a very abortive manner) and show instead epithelial-like qualities. Nowhere is this fact more obvious than in the pancreas, where the (neuro)endocrine cells detach from their mucosal companions to be on their own through the formation of the miniendocrine glands known as Langerhans' islets. It would seem as if the more specialized the cell is concerning its endocrine role, the more epithelial and the less neural it becomes. One would assume that this increasing specialization along epithelial lines in detriment of the neural features is the result of a genetic reprogramming leading to progressive expression of epithelial-type genes

coupled with progressive decrease of the expression of neural-type genes.[21–23] It is tempting to believe that this genetic reprogramming is related to evolution and that it represents a response to the changing physiologic needs of the organism. We realize that this is too much of a leap to ask the readers to make, and would be satisfied if they were simply to accept the notion that (neuro)endocrine cells have truly neural-like properties, and that these properties are much more developed in some sites (larynx, lung, thymus, thyroid) than in others (digestive tract).

2. *The interplay between peripheral nerve endings and (neuro)endocrine cells of the overlying epithelium*. Whether the cells of the diffuse (neuro)endocrine system derive or not from the neural crest, an interplay seems to exist between these cells and neural crest-derived nerve endings, which is not immediately apparent under normal conditions but which becomes evident in at least three conditions, all of them associated with vestigial structures: the microscopic carcinoid tumors found in the tip of obliterated appendices, the so-called gangliocytic paraganglioma of the ampulla of Vater, and the banal benign melanocytic nevus of the skin. The vestigial nature of the appendix vermiformis in too well known to be commented upon. The vestigial nature of the structure upon which the gangliocytic paraganglioma develops becomes evident when one realizes that this is the site of the ventral pancreatic anlage, an atavistic structure rich in pancreatic polypeptide (PP) cells which in humans is superseded by (and incorporated into) the evolutionarily more recent dorsal pancreatic anlage, destined to form most of the adult pancreas.[24] The atavistic nature of cutaneous melanocytic nevi has been commented upon by several early authors, some of whom have made an imaginative comparison with the tactile corpuscles of reptiles and have pointed out that melanocytic nevi are practically non-existent in other mammals such as dogs or cats, which from the point of view of skin adnexal (and specifically hair follicles) structures are much more evolved than humans.

In all three situations, we have a deeply seated neuroma-like component made up of S-100–protein-positive Schwann-like spindle cells,[25] and a (neuro)endocrine component, which in the appendiceal carcinoid tumor is represented by the carcinoid cells, in the gangliocytic paraganglioma by the PP-positive pancreatic (neuro)endocrine cells, and in the compound/intradermal nevus by the nevus cells. In all three instances, the topographic relationship of the (neuro)endocrine cells is just as close if not closer to the Schwann cell-like spindle cells than to the accompanying epithelial component, almost as if these (neuro)endocrine cells were emerging from the terminal sprouts of the Schwann-like spindle cells. In our opinion, this intimate relationship, which is at the base of the proposal made in this article, has been consistently underevaluated. Thus, appendiceal carcinoid tumors are generally thought to arise from Kultschitzky's cells of the appendiceal mucosa (which is by definition absent in the obliterated tip of this organ) rather than from the (neuro)endocrine cells present in the lamina propria of the mucosa and submucosa as part of he 'subepithelial neuroendocrine complex,' together with Schwann cells and occasional neurons.[26–29] Actually, when these cells are detected, they are often misinterpreted as evidence of perineural invasion by the tumor. Similarly, only a few authors in recent times have realized that most 'obliterated tips' or 'fibrous obliterations' of the appendix are in reality neural proliferations containing (neuro)endocrine cells, and that appendiceal carcinoid tumors probably arise from these intraneural (neuro)endocrine cells. We view the situation with gangliocytic paraganglioma as being analogous. Here too, the neural spindle cells try to reach the mucosa, perhaps in order to direct there the colonization of (neuro)endocrine cells, only to find that such mucosa has disappeared in the course of evolution, the result being the mishmash of neural cells, ganglion cells and (neuro)endocrine cells that characterizes gangliocytic paraganglioma.

At first sight it could seem odd to include the melanocytic system and the compound/intradermal melanocytic nevi in this grand scheme, yet they possess all of its components, as masterfully shown by Masson.[30,31] Specifically, they have neural Schwann-like spindle cells in the deep dermis, which in its most superficial sprouts generate the small round cells that are called nevus cells or nevocytes, and which are very different from the heavily pigmented melanocytes located along the dermo-epidermal junction.[32] The resemblance of these nevi with the carcinoid tumors arising in appendiceal obliterated tips can be so striking as to have led some early authors to refer to the latter as appendiceal nevi.

Admittedly, all of the above conjectures are purely hypothetical.[33] Yet, on the whole, I would like to believe that they point toward an important biologic theme, the existence of which is suggested by static morphologic observations on normal and diseased human tissue, and which hopefully will be investigated in the future with better means by better minds.

Disclosure/conflict of interest

The author declares no conflict of interest.

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