Tumour Pathology of the Neuron-Paraneuron System and its Evolutionary Background, with Special Attention to Neuro-Endocrine Differentiation in Prostate and Mammary Carcinomas*

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Summary. From results of phylogenetical investigations of the neuron-paraneuron or neuroendocrine system it is known that cells producing hormonal peptides appear first in the nervous system, then also as disseminated cells of an open or closed type in the mucosa of the alimentary tract, and, lastly, as the parenchyma of the classical endocrine glands. In all these three parts of the neuroendocrine system the parenchymal cells can undergo neoplastic transformation, expressing "eutopic" and/or "ectopic" peptide hormones and/or biogenic amines. The neuro-paraneuronal tumours arising in the central or peripheral nervous system in man are rare. In contrast, they are more common when they originate from the disseminated neuroendocrine cells in the gut mucosa, and when they arise in the endocrine glands. Here, a broad spectrum of histopathologically benign or malignant neoplasms, hyperplastic nodules, and other tumour-like lesions occur, some of which are associated with well-known clinicopathological entities. Typical examples are the gastrointestinal carcinoids, pancreatic insulomas, pituitary adenomas, medullary thyroid carcinomas, and pheochromocytomas.

The fact that neoplastically transformed cells of some traditionally non-endocrine tumours, e. g., mammary and prostatic carcinomas, can undergo neuroendocrine differentiation has recently become well established. The "malignancy potential" of neuroendocrine carcinomas and that of the paraneuronally differentiated cells of prostatic and mammary carcinomas have both been studied by means of cytochemical DNA assessments of the nuclei of the tumour cells, applying a recently developed deparaffinisation-disintegration procedure using archival material. So far, it seems as if about 1/4

of common gut carcinoids show a DNA distribution pattern of the "aneuploid" type, whereas the other 3/4 are "euploid" i.e., show a "diploid" or "diploid/tetraploid" DNA distribution pattern. The cell nuclei in areas in prostatic and mammary carcinomas with high amounts of paraneuronally differentiated cells displayed to a greater extent "euploid" DNA patterns than those in areas of the same carcinomas without such paraneuronal cells. Thus, the "malignancy potential" of the neoplasms of neuro-paraneuronal cells seems to be rather low.

Parenchymal cells of all three parts of the neuroendocrine (or neuronal-paraneuronal) system (see below) can undergo neoplastic transformation, although to a markedly different extent (cf. CHEJFEC et al., 1988). Thus, the neuronal cells only rarely give rise to tumours; best known are the small benign gangliogliomas and the highly malignant neuroblastomas. In contrast, the carcinoid tumours of the diffusely disseminated paraneurons in the gut and bronchi are well-known entities, as are also the adenomas and carcinomas of the classical endocrine glands.

This review gives a brief account of some recent work in our laboratories. (Consequently — and in order to save space — the list of references is to a great extent limited to works published from our own laboratories; original references to works from other laboratories are given in the list of references of our cited work.) to use modern cytochemical and immunohistochemical techniques to obtain new in-

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Fig. 1. Hypothetical relationship between the three major parts of the neuroendocrine system, viz. **a)** the neurons in the central and peripheral nervous system, **b)** the disseminated paraneurons of the open or closed type in the mucosa of hollow organs, and **c)** the parenchymal cells of the classical endocrine glands. This working hypothesis is based on the results of onto- and phylogenetical investigations, mainly on the evolution of the pancreatic islet parenchyma (cf. FALKMER and ASKENSTEN, 1988).

sights into the biological properties of paraneuronal neoplasms—in particular their potency of malignancy—and into those of paraneuronal cells appearing in the parenchyma of traditionally non-endocrine tumours. As has recently been most cogently remarked concerning the neuroendocrine tumours or paraneuromas, "the distinction between benignity and malignancy is not always clear" (FUJITA et al., 1988).

EVOLUTIONARY BACKGROUNDS

The evolutionary backgrounds of the classical endocrine organs, where the messenger substances are peptide hormones and/or biogenic amines, such as the adenohypophysis, the islets of Langerhans, the thyroid, the parathyroid, the adrenal medulla, and the paraganglia, are all equipped with parenchymal cells fulfilling the criteria of a paraneuron (cf. FUJITA et al., 1988). The same holds true for the paracrine and endocrine cells of the open or closed type, constituting Feyrter's Helle-Zellen-System in the mucosa of most hollow organs, notably that of the gastrointestinal tract and the bronchial mucosa. Phylogenetical and ontogenetical investigations have shown that a close relationship exists between these paraneuronal cells of the endocrine glands and those of the disseminated endocrine system (FALKMER et al., 1984; FALKMER and ASKENSTEN, 1988). Together, they form the two most recent components of the large neuroendocrine system, where its third and most original (see below) part comprises the neuronal cells of the central and peripheral nervous system, including also their nerve fibres and nerves, constituting the large peptidergic autonomous nervous system (FALKMER et al., 1984; FALKMER and WILANDER, 1989).

That a close relationship exists also between the neuronal cells of the brain and the paraneurons of the gut is illustrated by the well-known concept "the brain-gut axis", based on the fact that several of the peptide hormones are produced both by the central neurons and the peripheral paraneurons (cf. FALKMER et al., 1984). A hypothetical schematic outline of the histogenetic interrelationship between the neurons and paraneurons of the three main parts of the neuroendocrine system has previously been given (Fig. 1); it is based on the results of recent onto- and phylogenetical investigations, in particular of the evolution of the islets of Langerhans (FALKMER and ASKENSTEN, 1988). Briefly summarizing, it seems as if the most original location of neurohormonal peptide-producing cells, for instance, the insulin-producing cells (cf. CONLON et al., 1988), is the nervous system; then, in the next step of evolution they appear both in the "brain" and as disseminated cells in the "gut" mucosa; and, in the ultimate stage (i.e., in vertebrates) they can also bud out from the epithelium of the mucosa of various parts of the gastro-intestinal tract to form a separate endocrine gland (Fig. 1). Typical examples of this hypothetical sequence of events in the evolution of an endocrine gland are not only the islets of Langerhans (cf. FALKMER, 1985) but

also the development of the anterior pituitary from Rathke's pouch and the thyroid from the endostyle (GORBMAN et al., 1983).

SPECTRUM OF HYPERPLASIA AND NEOPLASIA

In the classical endocrine glands there is often some clinical confusion about the implications of the "tumour" concept. Thus, it is important to recognise the fundamental differences between nodular hyperplasias, genuine adenomas, and clear-cut carcinomas, since all these lesions can, from their gross appearance, be difficult to discriminate from each other. As recently accounted for in a monograph on neuroendocrine tumours (NESLAND et al., 1988a), there would seem to be a general developmental spectrum of events that occurs in the pathogenesis of paraneuronal neoplasms, both in the parenchyma of the endocrine glands and in the endocrine/paracrine disseminated cells in the gastrointestinal tract and bronchial mucosa (SOLCIA et al., 1988) (Fig. 2). This sequence of events has become most obvious in the experimental model inducing gastric ECL(enterochromaffin-like)-cell hyperplasias and carcinoids in the gastric mucosa of aged female rats by means of long-lasting hypergastrinaemia, this being evoked by means of the administration of high doses of the proton-pump-blocker (and, thus, HCl-secretion inhibitor) omeprazol (HAVU, 1986) or via partial fundectomy (HAVU et al., 1988). It was clearly shown that gastrin-induced hyperplasias (at least diffuse ones) were reversible when hypergastrinaemia was abolished, whereas genuine neoplasms were irreversible.

IMMUNOHISTOCHEMICAL FEATURES: NEUROENDOCRINE TUMOUR MARKERS

Immunohistochemical investigations, both light- and electron-microscopically, have shown that all the paraneuronal cells — normal, hyperplastic, or neoplastic — are equipped with secretion granules containing peptide hormones and biogenic amines; co-localisation may occur in these secretion granules, not only for peptide hormones and biogenic amines but also for different kinds of peptide hormones (cf. CHEJFEC et al., 1988). The spectrum of peptide hormones and biogenic amines in the most common of these endocrine neoplasms, namely gut carcinoids, islet-cell tumours of the pancreas, medullary carcinomas of thyroid, pituitary adenomas, and phaeo-

Fig. 2. Crude diagram showing parts of the spectrum of hyperplastic and even neoplastic lesions in paraneurons (and perhaps their sequence of events in the evolution?) of, e.g., gastric carcinoids. The scheme is mainly based on the results of experimental studies on the effect of prolonged hypergastrinaemia on rat gastric mucosa (cf. HAVU, 1986; HAVU et al., 1988). The nomenclature is the result of a recent "consensus" among some endocrine pathologists (cf. SOLCIA et al., 1988).

chromocytomas, is often most variegated, but can also be rather characteristic. This fact can aid the practising pathologist in his daily diagnostic work, e.g., when dealing with a metastatic lesion from an unknown primary tumour. As an example, ileal carcinoids — in contrast to fore-gut and hind-gut carcinoids — are characterized immunohistochemically by an astonishingly narrow spectrum of peptide hormones and biogenic amines, essentially restricted to substance P and serotonin (CHEJFEC et al., 1988).

As regards the possibility of distinguishing between malignant and benign neoplasms of the neuro-paraneuronal system, immunohistochemical investigations have proved of limited value (CHEJFEC et al., 1988).

Of practical importance for a pathologist is a tool that enables him to trace neuro-paraneuronal features in a given neoplasm, perhaps one originally diagnosed as a conventional carcinoma. Several such neuro-paraneuronal tumour markers are known: some detect the presence of secretion granules; others specific enzymes common to neurons and paraneurons. The most commonly used is the detection of the presence of argyrophil cells by means of the silver nitrate procedure (GRIMELIUS and WILANDER, 1980). The argyrophilia is located to the secretory granules. Here, it has recently been shown that the argyrophil reaction is closely linked to the presence of chromogranins in the secretion granules (RINDI et al., 1986). After the advent of commercially

available antisera against one of the chromogranins, namely chromogranin A, this peptide has also become a useful neuro-paraneuronal marker in visualizing endocrine secretion granules (CHEJFEC et al., 1987; ABRAHAMSSON et al., 1989).

When the secretion granules are few, the silver nitrate technique and the chromogranin immunoreactivity may give too weak reactions. In such cells a good neuro-paraneuronal tumour marker is the immunohistochemical detection of the presence of neuron-specific enolase (NSE) in the cytoplasm, since this enzyme, common to many neurons and paraneurons, is not localized to secretion granules but to the cytosol (cf. NESLAND et al., 1986a). The NSE immunoreactivity has, however, gained the reputation of being somewhat non-specific (CHEJFEC et al., 1987); consequently, a supplementary ultrastructural examination for the presence of secretory granules of the "endocrine" type is sometimes needed (NESLAND et al., 1986a, b, 1988b).

Fig. 3. Electron micrograph, showing part of a human glucagon cell after incubation with an antiserum raised against synaptophysin, and visualising the immunoreactivity by means of the immunogold technique. The synaptophysin immunoreactivity seems to be localised to the secretion granules, if not exclusively. $\times 50,000$ (Courtesy of Prof. Lars GRIMELIUS; Department of Pathology, University of Uppsala.)

In these last few years, a fourth neuroendocrine marker, synaptophysin, has been used, e.g., in detecting carcinoid tumours and islet-cell neoplasms (NESLAND *et al.*, 1988a). In one of our laboratories the ability of synaptophysin in detecting the paraneuronal features of pancreatic tumours has recently been compared with that of silver nitrate argyrophilia, chromogranin A, and NSE; synaptophysin was found to be most sensitive (*cf.* CHEJFEC *et al.*, 1987). Although originally looked upon as a glycoprotein marker for presynaptic microvesicles, some evidence has recently been obtained that synaptophysin is also localized to the neuroendocrine secretory granules (Fig. 3) (GRIMELIUS, personal communication, 1988). Its main draw back is that some batches of the commercially available antisera seem to have been of poor quality; part of the failure here can probably be ascribed to less suitable fixations, as found in a recent study from one of our laboratories (HÖÖG *et al.*, 1988).

CYTOCHEMICAL ASSESSMENTS OF THE DNA PLOIDY

The nuclear DNA ploidy pattern in the parenchymal cells of various human neoplasms has during the last five to ten years been found to give both diagnostic and prognostic information (*cf.* ASKENSTEN, 1988). Tumours with a cytochemically assessed DNA distribution pattern of the "diploid" or "diploid/tetraploid" type have, in general, been found to show a clinically more benign course than those with an "aneuploid" DNA pattern. When this tool for malignancy assessments, using a newly modified deparaffinisation-disintegration technique for paraffin-embedded tumour specimens, combined with image or flow cytometry (ASKENSTEN, 1988) (Fig. 4), was employed in a current study of a number of carcinoids, about 1/4 of them were found to show a DNA distribution pattern of the "aneuploid" type (CHEJFEC *et al.*, 1988). Preceding investigations including some of the same specimens, using an old, coarser microphotometric technique, had shown that practically all the carcinoids showed either a DNA distribution pattern of a "diploid" type or of a "non-diploid" — but not clearly an "aneuploid" — type (FALKMER *et al.*, 1986). The reasons for this discrepancy in the results are currently being examined. It can, however, now be concluded that most of the gut neuro-paraneuronal neoplasms show an "euploid" (*i.e.*, a "diploid" or a "diploid/tetraploid") DNA distribution pattern, indicating a low or only moderate degree of malignancy.

NEUROENDOCRINE DIFFERENTIATION IN TRADITIONALLY NON-ENDOCRINE NEOPLASMS

During the last decade it has become evident that several traditionally non-endocrine carcinomas can harbour parenchymal cells displaying paraneuronal features (*cf.* BOSMAN *et al.*, 1985), such as NSE activity and argyrophil secretion granules showing immunoreactivity with antisera raised against chromogranin A and synaptophysin, *i.e.*, all the neuro-

Fig. 4. Overview of the major steps in the modified deparaffinisation/disintegration technique used in one of our laboratories (ASKENSTEN, 1988) for image cytometry of the DNA distribution pattern in enriched Feulgen-stained nuclei of the parenchymal cells of conventionally formalin-fixed and paraffin-embedded specimens of human neoplasms.

Fig. 5. High-power photomicrograph of a mammary carcinoma showing paraneuronal differentiation with NSE immunoreactivity in the cytosol and ultrastructurally verified secretory granules of the "endocrine" type. At this magnification, these secretion granules appear as a fine granularity of the cytoplasm of the cancer cells. $\times 720$

paraneuronal tumour markers (NESLAND et al., 1988) (see above). In our laboratories, we have particularly studied carcinomas of the prostate (ABRAHAMSSON et al., 1987, 1989) and mammary glands (NESLAND et al., 1986a, b, 1988b) (Fig. 5) in this respect.

As a matter of fact, when a comprehensive and systematic investigation of properly fixed biopsy specimens of hyperplastic and neoplastic prostatic parenchyma was made, almost all the prostate glands examined contained at least some parenchymal cells with paraneuronal features (ABRAHAMSSON et al., 1986). The number of such cells increased markedly with increasing degrees of anaplasia of the carcinoma and as the carcinoma progressed (ABRAHAMSSON et al., 1987, 1989). The results of analogous studies of mammary carcinomas, using mainly conventionally formalin-fixed, paraffin-embedded, surgical specimens, showed that about 1/3 of all the tumours contained neoplastic cells with NSE im-

munoreactivity and ultrastructurally detected secretion granules (NESLAND et al., 1986a, b, 1988b).

The fact that these cells with paraneuronal features are actually genuine, neoplastic cells was established by the finding that they did not only appear in the primary tumours but also in their metastases (ABRAHAMSSON et al., 1987). In the prostatic carcinomas, this paraneuronal phenotype of the carcinoma often showed a rather patchy distribution with a fairly characteristic spectrum of messenger substances, consisting mainly of the biogenic amine serotonin and a hormonal peptide closely related to TSH (thyroid stimulating hormone) (ABRAHAMSSON and LILJA, 1989). In addition, calcitonin and somatostatin immunoreactive cells occurred, as well as — in the anaplastic carcinoma — cells immunoreactive for several additional hormonal peptides of the so-called fore-gut type (ABRAHAMSSON et al., 1987). Only exceptionally, however, did these paraneuronal cells give rise to clinical signs of hormone overproduction; we observed only one such case, namely a patient with Cushing's syndrome due to paraneuronal cells in the prostate producing CRH (corticotropin-releasing hormone) (FJELLESTAD-POULSEN et al., 1988). In the mammary carcinomas the peptide hormones detected in the paraneuronal cells were also mainly of the fore-gut type (NESLAND et al., 1988b).

In order to attain an idea of the potency of the malignancy of the neuroendocrine phenotype of prostatic and mammary carcinomas, their nuclear DNA ploidy pattern was assessed cytochemically, using the recently described technique (see above), where formalin-fixed, paraffin-embedded, surgical specimens were disintegrated and analysed by means of image cytometry (ASKENSTEN, 1988). Areas rich in neoplastic cells with neuro-paraneuronal differentiation (Fig. 5) and areas lacking such cells were selected and analysed.

In the prostatic carcinomas it seemed as if the paraneuronally differentiated neoplastic cells showed essentially an "euploid" DNA distribution pattern, whereas the cells of the non-endocrine phenotype of the carcinoma displayed to a great extent an "aneuploid" DNA ploidy pattern (FALKMER et al., 1989). Thus, despite the fact that the neuroendocrine phenotype appears more abundantly in anaplastic prostatic carcinomas, it does not seem to belong to those neoplastic cell clones that have the highest potency of malignancy.

In mammary adenocarcinomas rather similar observations have been made (Table 1). Whereas in a large series of unselected breast adenocarcinomas not less than every second case displayed a DNA

Table 1. Overview of the results of cytochemical assessments of the nuclear DNA distribution pattern of the neoplastic parenchymal cells in two major groups of mammary carcinomas, using image cytometry on material obtained by means of the deparaffinisation-disintegration procedure outlined in Figure 4

Kind of mammary carcinoma	Number of tumours with a cytochemically assessed DNA distribution pattern			
	Total number	"Euploid" type		"Aneuploid" type
		"Diploid"	"Tetraploid"	
Unselected tumours	831 (=100%)	338 (40%)	80 (10%)	413 (50%)
Tumours with areas of paraneuronal differentiation	42 (=100%)	21 (50%)	7 (17%)	14 (33%)

ploidy pattern of "aneuploid" type, only 1/3 of those mammary carcinomas with paraneuronal differentiation showed an "aneuploid" nuclear DNA distribution pattern. Thus, in mammary carcinomas as well, it seems as if the neuroendocrine phenotype might be a neoplastic cell clone with a lower potency of malignancy than that of the non-paraneuronal cell clones.

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