Secretory Vesicle and Cell Surface Markers for Human Endocrine Pancreatic and Pituitary Tumors

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The pancreatic islet and the adenohypophysis are endocrine tissues sharing several properties. These organs are both composed of different endocrine cells secreting a variety of hormones. These are stored within the cells in dense core secretory vesicles and are released by appropriate stimuli. In addition to dense core secretory vesicles, endocrine pancreatic and adenohypophyseal cells contain small translucent vesicles, which are very similar to synaptic vesicles not only in their morphological appearance but also with regard to their protein constituents [31, 64]. Indeed, several lines of evidence suggest that pancreatic and adenohypophyseal endocrine cells have an additional secretory pathway for neurotransmitters besides the classic exocytotic pathway for hormone release [31, 54, 60]. Proteins present in the dense core vesicles containing hormones, such as chromogranins and secretogranins, have also been detected in their counterparts within neurons (for refs. see [30, 37, 38, 61]). This indicates that neurons and endocrine cells are very similar with respect to the composition of their characteristic small secretory vesicles (SSV) and large secretory vesicles (LSV) besides having a variety of molecular, biochemical, and functional similarities [25].

Neurons and endocrine cells also share proteins exposed on the external surface. The best documented proteins of this class are members of the family of neural cell adhesion molecules (NCAMs), which are present in endocrine cells of the adult, including pancreatic islet and adenohypophyseal cells [2, 39–41]. While tumors derived from the endocrine pancreas are rare [35], pituitary tumors are frequently observed [27, 35, 73]. Besides symptoms produced locally by the tumors, overproduction of their hormones and their subsequent effects in the body often permit a straightforward diagnosis. However, a significant percentage of endocrine pancreatic and pituitary tumors do not cause elevated serum hormone levels and thus are hormonally inactive [27, 29, 73]. In such cases additional characteristic constituents of endocrine cells are critical in providing information on the nature, location, and distribution of endocrine tumor cells in the body. Here we summarize recent findings concerning the expression of membrane proteins of SSV and of the cell surface antigen NCAM by normal and neoplastic endocrine cells.

Membrane Proteins of Small Secretory Vesicles and the Cell Surface

Studies on the composition of synaptic vesicles of neurons have provided detailed information on the composition of their membranes [6, 31, 64]. Although many of these membrane proteins are also present in endocrine cells, few have been shown to be present in the SSVs of normal pancreatic islet or adenohypophyseal endocrine cells or of pituitary and pancreatic endocrine tumors. Thus the application of analysis of membrane SSV antigens in pathology is largely restricted to the analysis of synaptophysin, which, like the neural cell adhesion molecule NCAM, has been detected by at least three independent research groups [9, 32, 69].

Synaptophysin is a highly conserved protein consisting of four transmembrane domains in addition to a short N-terminal and an extended C-terminal cytoplasmic domain [6, 31, 64]. Recently discovered homologs like synaptoporin and their avian

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counterparts have a similar structure [8, 36]. These and other synaptic vesicle membrane proteins, termed synaptotagmin and vesicle associated membrane protein (VAMP), have closely related isoforms that are differentially distributed in the central nervous system [22, 46, 47, 67]. It is not yet known whether the isoforms of synaptic vesicle membrane proteins are also differentially distributed between endocrine cells. In addition, reports from several groups suggest that some membrane proteins or their isoforms may be shared by SSV and LSVs [22, 31, 45, 52, 58, 59]. In neurons, the membrane proteins of synaptic vesicles are involved in transmitter uptake, exocytosis, and membrane recycling [7, 10, 64]. The SSVs of endocrine cells, which bear the same antigens as synaptic vesicles, may fulfil similar roles. However, data concerning the biosynthesis of neurotransmitters, their uptake into SSVs, and their release from endocrine cells such as the pancreatic islet or adenohypophyseal cells are still fragmentary [54, 60]. However, the same processes that occur during hormone release from LSVs are well understood.

Intercellular contacts via cell adhesion molecules are critical during embryonic development. Neural cell adhesion molecules, including NCAM, NgCAM, and N-cadherins, have been shown to participate in cell sorting, cell assembly, and tissue reorganization [11, 16, 56]. Also, in the adult, NCAMs persist in strategic locations, suggesting their importance in the maintenance of tissue architecture. Endocrine cells of the adenohypophysis, the pancreatic islets, and the gonads adhere to each other by means of NCAMs [2, 39–41, 48, 49].

The different members of the NCAM family are translation products of different messenger RNAs generated by alternative splicing and differential polyadenylation from a single gene consisting of at least 24 exons. The three major NCAM isoforms have similar extracellular domains, and they differ primarily in their plasma membrane associated domains and the lengths of their intracellular domains. For example, NCAM 180 (the isoform with a molecular weight of 180 kD) has a cytoplasmic domain that is larger than that of NCAM 140, whereas NCAM 120 consists only of the extracellular domain, which is anchored to the plasma membrane by phosphatidylinositol [11, 16, 41, 51, 56]. In addition to the production of the different membrane associated and cytoplasmic domains of the three major NCAM isoforms, alternative splicing of minor exons gives rise to variations in those regions of the extracellular NCAM domains that mediate cell adhesion. For example, at the exon 12/13 junction, coding for the presumptive hinge region of the molecule, smaller additional alternative exons of different lengths were present, which are inserted by alternative splicing. In addition, at the exon 7/8 junction, an exon coding for a further 10 amino acids has been detected in several cell types (for refs. see [3]). This modification within the fourth immunoglobulin-like extracellular domain of NCAM down-regulates neurite growth-promoting activity of NCAM 140 [15]. Posttranslational modifications involving addition of polysialic acid (PSA) homopolymers to the extracellular domain of NCAM can occur. The polysialylation of NCAM decreases cell-cell adhesion and is thus of functional importance [1, 55].

Expression of Synaptophysin and NCAM in Human Pancreatic Islets and Endocrine Pancreatic Tumors

In all species examined including humans, synaptophysin has been detected in all endocrine pancreatic cell types [50, 53, 70, 71]. Since synaptophysin has also been found in all pancreatic endocrine tumors examined [24, 70–72], this protein can be regarded as a general marker for these tumors. Insulinomas are the most frequent of the relatively rare endocrine pancreatic tumors (for refs. see [35]). They have been reported to contain synaptophysin [24, 30, 70, 71]. Figures 1a and 1b show a typical insulinoma exhibiting immunoreactivity for insulin and synaptophysin. The strong hybridization signal for synaptophysin mRNA observed in the same tumor (Fig. 1c) indicates that both transcription and translation of synaptophysin are typical features of such tumors. Other marker proteins such as chromogranin A, which is present in all endocrine cell types in bovine pancreatic islets [17, 18], is present in A cells in humans and at lower levels in human B cells, but is apparently absent from D and PP cells [26]. Interestingly, endocrine pancreatic tumors that are immunoreactive for soma-

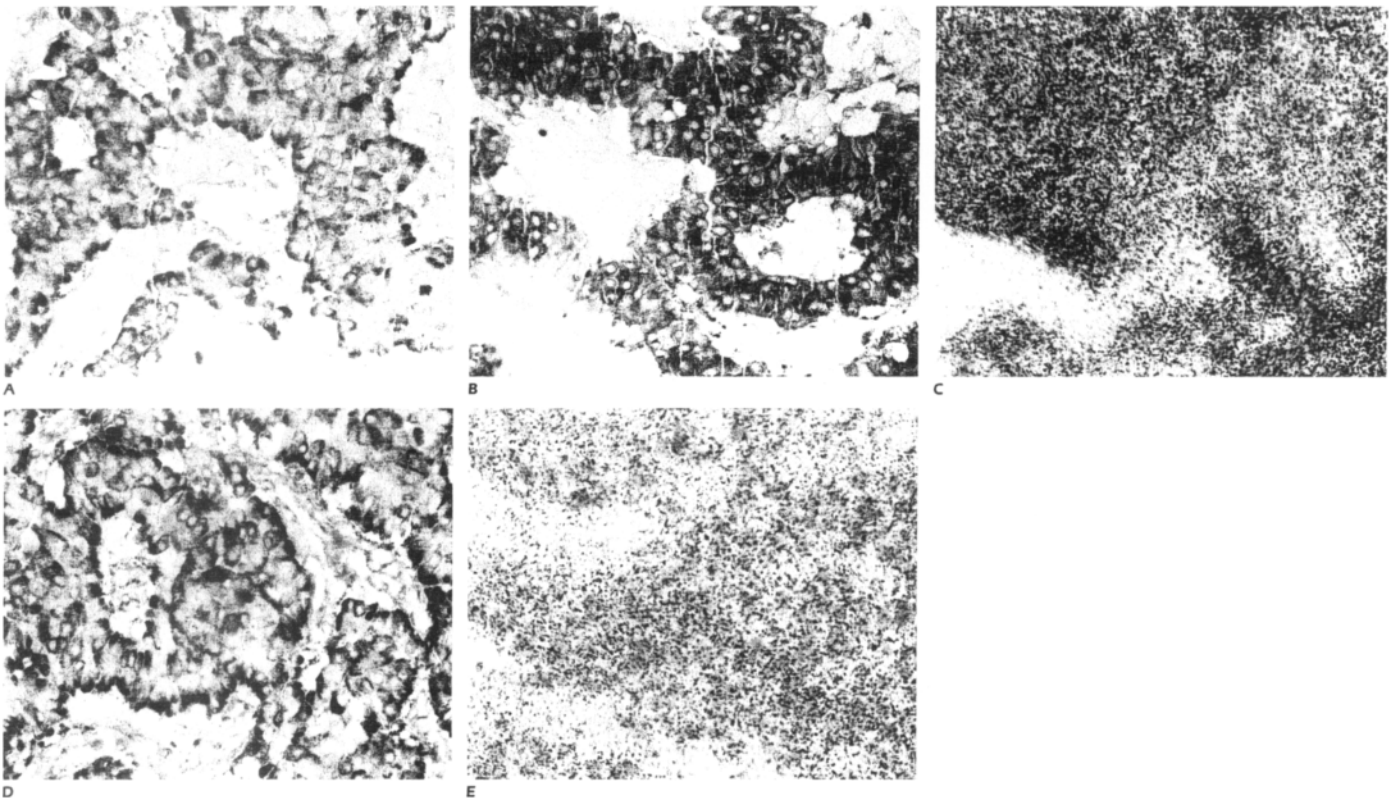


Figure 1. Human insulinoma expressing synaptophysin, chromogranin A, and NCAM. Immunolocalization of insulin (a) and synaptophysin (b) in a human insulinoma. A strong hybridization signal for synaptophysin mRNA was observed in sections of the same tumor (c). The insulinoma was also immunostained for chromogranin A (d) and showed a positive hybridization signal for NCAM mRNA (e). Synaptophysin mRNA was visualized by in situ hybridization [5] with a cRNA directed against the sequence coding for the C-terminal cytoplasmic tail of the molecule [42]. For NCAM mRNA detection we used a cRNA probe directed against parts (exon 13/14) of the extracellular domain of NCAM [23]. (×340).

tostatin or pancreatic polypeptide have been reported to contain chromogranin A (see refs. [30]), which may indicate that, compared to normal counterparts, chromogranin A expression is up-regulated in D- and PP-secreting tumors. Tissue-specific expression of chromogranin A appears to be regulated by several transcriptional control elements. A cyclic AMP responsive element has recently been detected in the chromogranin A promoter region [74]. Its activation may also occur in insulinomas which contain elevated levels of chromogranin A as compared to the weak immunostaining of human pancreatic B cells [26]. Prominent immunostaining for chromogranin A in a human insulinoma is shown in Fig. 1d.

Recently it was observed that expression of the neural cell adhesion molecule NCAM not only is a general feature of the endocrine cell types constituting the rat pancreatic islet, but also is characteristic of a rat insulinoma cell line [39, 40]. NCAM 140 is the prevalent isoform in normal and neoplastic endocrine pancreatic cells. NCAM transcripts have also been detected in one human insulinoma [33]. Moreover, the insulinoma immunostained for chromogranin A and synaptophysin shown here exhibited hybridization signals not only for synaptophysin but also for NCAM mRNA (Fig. 1e). Thus it can be concluded that the expression of synaptophysin, chromogranin A, and NCAM is a typical feature of human insulinomas.

Cell surface components as marker substances present several advantages compared with intracellular molecules. They are accessible to external molecules and could be used for immunoscintigraphy. It is conceivable that antibodies could be used to target drugs to tumors expressing NCAM. Surface markers such as NCAM could also be valuable for cytological diagnosis on biopsies or bone marrow aspirates. Besides NCAM, only

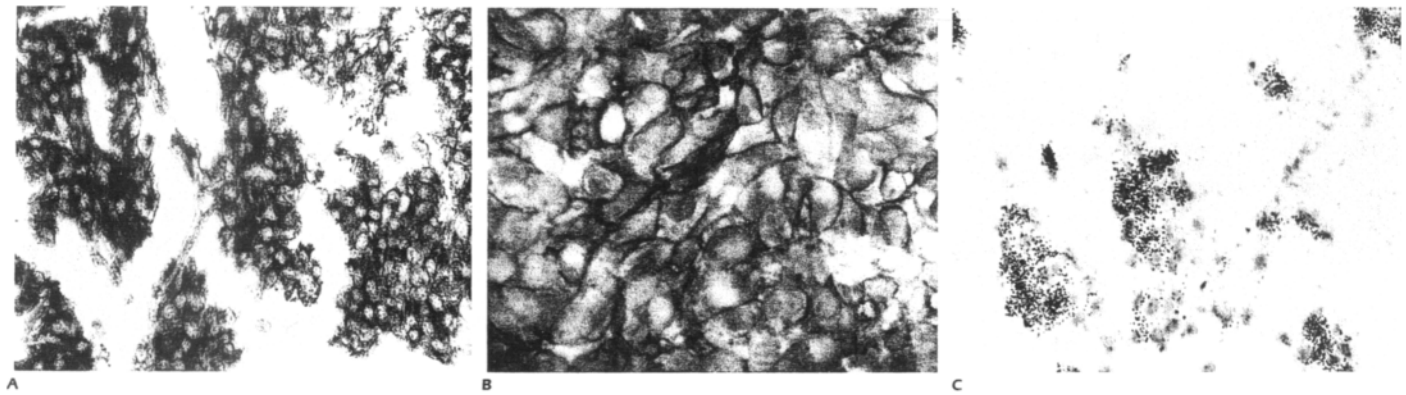
tetanus toxin binding sites, which are present at the surface of pancreatic islet cells and human insulinoma cells [19], could be used for similar purposes. However, tetanus toxin binding occurs only initially at the cell surface and is followed by internalization of the toxin and its action within the cells. Thus not only the receptors of tetanus toxin at the cell surface but also its target inside the cells, which is present in endocrine but not in exocrine cells (cf refs. [62]), may provide further marker molecules for endocrine cells.

Expression of Synaptophysin and NCAM in Human Adenohypophyses and Pituitary Tumors

Following the first report on the presence of synaptophysin in rodent adenohypophyses [50] several articles published in 1987–88 documented its presence also in normal and neoplastic pituitary cells of humans (cf refs. [71, 2, 24, 63]). All reports agree that the SSV antigen exists in all these cells, indicating that synaptophysin can be used as a general marker for pituitary cells (Fig. 2a), as is the case for endocrine pancreatic cells (see above).

In contrast, the distribution of other markers in normal and neoplastic hypophyseal cells is not so homogeneous. For example, most investigators agree that chromogranin A is not present either in normal prolactin cells or in prolactinomas, whereas chromogranin A has been frequently detected in human GH adenomas [14, 30, 43, 44, 63]. Acidophil pituitary adenomas produce growth hormone (GH), prolactin, or both (cf refs. [29]). Thus acidophil adenoma cells may be distinguished by the prevalence of the hormones expressed and be regarded as a bihormonal cell type, in which the transcription and translation of hormones and other characteristic constituents is regulated by hypothalamic, gonadal, and adrenal steroids in addition to other factors (cf refs. [21]). In this context it is interesting to note that the chromogranin A and prolactin genes share similar promoter/enhancer elements [21, 74], which would allow parallel regulation of both substances (e.g., via dopamine and cAMP) in normal and neoplastic cells. In both promoter regions there is a *cis*-acting cyclic AMP responsive element termed CRE [34]. CRE is a target site for the transcription factor CREB (cyclic AMP responsive element binding protein), which shares many features with the fos-jun family of transcription factors [75]. Steroid hormones can also act as transcriptional activators by binding of the hormone-receptor complex in the promoter region of the gene. Estrogen receptors are clearly involved in enhancement of prolactin gene expression [21], and there is evidence that chromogranin A expression is also affected by estrogen [20]. While hormone and chromogranin levels in the pituitary acidophils can be varied in this way, it is not entirely clear whether growth hormone,

Figure 2. Human GH adenoma expressing synaptophysin and NCAM. Apparently all cells of the human GH adenoma exhibited intracellular immunolabeling for synaptophysin (a) while their surfaces were immunolabeled for NCAM (b). In situ hybridization histochemistry revealed that only some cells of the adenoma expressed NCAM mRNA (c). (×340). The nature of the NCAM probe is described in legend to Fig. 1.



prolactin, and chromogranins are stored together in the same or in different secretory vesicles [4]. In the bihormonal FSH/LH cells chromogranin A is confined exclusively to the large dense core secretory vesicles [68].

Like chromogranin A, neural cell adhesion molecules are differentially expressed in human pituitary tumors [2, 25]. While GH adenomas and inactive adenomas express NCAM 140 at levels that permit easy identification in immunoblots, NCAM could not be detected by such techniques in prolactinomas. Its presence could be confirmed by immunoelectron microscopy [2] and also in one prolactinoma by immunocytochemistry and in situ hybridization histochemistry [33]. These data are indicative of low levels of NCAM in prolactinomas and higher levels in GH adenomas. Intense immunocytochemical staining for NCAM of a GH adenoma is shown in Fig. 2b. In situ hybridization histochemistry (see Fig. 2c) revealed that only some groups of cells express NCAM mRNA at a given time, whereas the translated protein appears to be more evenly distributed in the tumor cells (Fig. 2b).

With one exception NCAM 140 was detectable in hormonally inactive adenomas [2]. It seems likely that levels of prolactin were below the sensitivity limits of immunocytochemistry in the inactive adenoma, which appeared negative for NCAM. Such adenomas have recently been reported to contain prolactin mRNA [57]. The presence of NCAM in most inactive adenomas has recently been confirmed [33]. Clearly there now exists a variety of diagnostic markers for inactive adenomas, which are frequent and by definition cannot be identified by hormone analyses. Besides cytoplasmic neuron-specific enolase, NCAM, and synaptophysin, they produce and release chromogranin A, which can be analyzed not only by immunocytochemistry but also in serum [12, 13, 65, 66]. The differences in the expression of hormones, chromogranin A, and NCAM between pituitary acidophil adenomas, which have been reported, are of particular interest. The pattern of expression of these proteins could be explained by the fact that their transcription is regulated by closely related transcription factors [21, 28, 74].

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