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INVITED NEL REVIEW

The neuroendocrine phenotype, cellular plasticity, and the search for genetic switches: Redefining the diffuse neuroendocrine system

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Abstract The term neuroendocrine has been used to define cells that secrete their products in a regulated manner, in response to a specific stimulus. The neuroendocrine system includes neurons and endocrine cells sharing a common phenotypic program characterized by the expression of markers such as neuropeptides, chromogranins, neuropeptide processing enzymes SPC2 and SPC3 (subtilase-like pro-protein convertases) or dense core secretory granules. Various theories such as the APUD (amine precursor uptake decarboxylation) concept, the diffuse neuroendocrine system (DNES) or the paraneuron concept have been put forth to classify neuroendocrine cells as a cohesive group. Neuroendocrine characteristics have been used as evidence of a common embryological origin for normal and neoplastic cells. However, it is now recognized that neuroendocrine characteristics can be observed in various cell types, such as immunocytes, that are not of a common embryological origin with either neurons or endocrine cells. We propose to redefine previous "neuroendocrine" concepts to include the notion that activation of specific genetic switches can lead to the expression of a partial or full neuroendocrine phenotype in a variety of cell types, including immune cells.

We can no longer define neuroendocrine cells simply based on their content of neuropeptides or chromogranins. When appropriately stimulated, cells that are neither neural or endocrine can express either a neuroendocrine phenotype or an immune cell phenotype. These data provide evidence that genotypic switches are present in cells that were previously thought to be irrevocably differentiated.

Claude Bernard (1813–1878) originally recognized the importance of the 'internal environment' of the organism and the 'internal secretion' in relation to the maintenance of homeostasis. The origin of these internal secretions was later understood through the studies of the pancreas by Paul Langerhans (1869) and of the gut mucosa by Heidenhain (1870) and Kultschitzky (1897). The demonstration of silver-staining granules in chromaffin cells led to the discovery of other such cells, which were found in dispersed patterns throughout different tissues. Friedrich Feyrter (1938) reported the presence of rather pale cells (*helle Zellen*) distributed widely throughout the body using staining techniques, which led to the concept of a 'diffuse endocrine system'. It was not till much later that these basic observations were crystallized into a unique concept. It was suggested that these cells secrete chemical messengers acting in a paracrine or endocrine manner. Everson Pearse developed the APUD concept (amine precursor uptake and decarboxylation) based on the finding of identical biogenic amines and peptides hormones in neurons and in the dispersed endocrine cells located in different organs [1,2.]. Based on histological and biochemical features, Pearse grouped into a single entity widely separated cells present either in classic endocrine organs or isolated in sites dispersed throughout the body, whose function was to act as a diffuse neuroendocrine system (DNES) [3]. The major features included the production of polypeptide hormones as well as the presence of a set of cytochemical and ultrastructural characteristics. Pearse went further by suggesting that all cells comprising this system shared a common embryological origin, namely the neural crest [1]. An important conceptual notion was advanced that these cells were functionally coordinated in their actions, providing communication with the nervous system. Coordinated actions of the DNES with autonomic and somatic nervous systems would affect or control the functions of internal organs. This original and very useful concept was expanded with the advent of improved staining methodologies, the advancement of molecular biological techniques and the growing list of neuropeptides and cell markers that appeared to be particular to neural or endocrine cells [4–6]. These markers were studied extensively especially to establish the origins of various tumors. The presence of a marker, such as chromogranin A, was viewed as evidence of the neuroendocrine origin of the tumor cells. Other postulates, such as that of the 'paraneuron' concept, introduced by Fujita, re-enforced the notion of a common embryological origin of all neuroendocrine cells, namely the neuroectoderm [7].

Embryonic origin and the DNES today

The current concept of a diffuse neuroendocrine cell no longer holds steadfast to the notion that all neuroendocrine cells have a common embryological origin because many different cell types with neuroendocrine characteristics have been shown to derive from other regions than the neuroectoderm [8–11]. Based on the original criteria of Pearse and Fujita, it is now generally believed that neuroendocrine cells follow the following criteria [4]. First, neuroendocrine cells produce a neurotransmitter and/or neuromodulator or a neuropeptide hormone. Second, these substances are contained within membrane-bound granules or vesicles from which they are released by a process of regulated exocytosis in response to external (neural) stimuli. Third, neuroendocrine cells differ from neurons by the absence of axons and specialized nerve terminals (i.e. their mode of transmission is endocrine or paracrine rather than synaptic). And, finally, different types of neuroendocrine cell share many specific properties and express several proteins in common, but the expression of any one marker protein is not an absolute criterion.

Today the definition of a neuroendocrine cell is still based on molecular markers or particular cellular characteristics. Neuropeptides are important components of neuroendocrine cells because they are vital in cell-cell communication. It is now known that neuropeptides originate from protein precursors that require enzymatic cleavage to release their biologically active neuropeptides. An important family of enzymes, the subtilase proprotein convertase (SPCs), has been shown to be important in the activation process, namely via cleavage of specific single or paired residues within the neuropeptide protein precursor [12]. Two of these enzymes SPC2 and SPC3 are considered to be excellent markers of neuroendocrine cells [12].

Neuropeptides are not expressed uniquely in neuroendocrine cells

Neuropeptide synthesis can no longer be considered a criterion for a neuroendocrine cell. Neuropeptides have been found in several different cell types, including immune cells [13–17] and cardiomyoctes [18–19]. So-called markers of neuroendocrine cells, such as chromogranins, have also been detected in immune cells [20]. The hematopoetic origin of these cells would not fit with the original concept of APUD or paraneuron. With the advent of molecular methodologies, such as *in situ* hybridization histochemistry, neuropeptide expression has been shown in cells that lack secretory granules. For example, prodynorphin is expressed in adrenal corticosteroid-producing cells [21] and proenkephalin is synthesized in ventricular cardiomyocytes [19]. In either case, the expression of a neuropeptide mRNA does not re-define these cells as neuroendocrine. Similarly, the presence of chromogranins in some immunocytes does not imply that the immunocytes is a neuroendocrine cell or is neuroendocrine in origin [22].

Similarly, some immune molecules, such as antimicrobial peptides, can be defined as signaling molecules [23,24] that can be produced by either immunocytes [23] or neurons [25], however, this does not necessarily imply that leukocytes are neuroendocrine cells. Interestingly, neuroendocrine cell characteristics can be expressed in immune cells [20,26] under basal conditions although is most common during a challenge of the immune system [20, 26–28]. Thus, expression of chromogranins, enkephalin or SPCs can be induced or up-regulated during immune challenge [20, 26–28]. Does this mean that some immunocytes should be classified as a sub-group of neuroendocrine cells?

Cellular plasticity and gene switches

Cellular plasticity explains observations of expression of neuropeptides, chromogranins or even secretory granules in immune cells. Each of these elements is dependent on various nuclear factors that direct their expression. These factors are not necessarily the same among all neuropeptides, since we know that different neurons express different neuropeptides. Similarly, the expression of chromogranins or the regulation of secretory granule biogenesis is controlled by independent nuclear factors. Neurons have also been shown to express cytokines [29–32] and antimicrobial substances [25]. This cross-over of phenotypic differentiation is involved in precise functions, such as cell-cell communication, that is communication between neuroendocrine and immune systems via a common set of messenger molecules. In terms of embryological origin, it is known that immune cells are derived from different precursor cells than are neuroendocrine cells, but still have evolved a parallel system of neuropeptide production. However, the same nuclear factors would be required to express specific neuropeptides in immune cells as in neuroendocrine cells. It thus seems that various cells can acquire at least partial neuroendocrine phenotype characteristics that are traditionally ascribed to neuroendocrine cells. Similarly, Ectopic expression of cytokines and other immune factors in neurons are a reflection of the presence of a partial immune phenotype. These expressions pattern are due to the complex interactions of nuclear factors that result ultimately in the expression of that phenotype. The challenge is to define functionally which factors are implicated in the expression of these neuroendocrine elements. The identification of such 'genetic switches' is the object of much current research, which has been expanded thanks to the high throughput screen techniques, such as the use of DNA chips.

Neoplastic neuroendocrine cells

Chromogranin A, B and C (secretogranin II) are acidic soluble proteins that are located typically in the secretory granules of many neuroendocrine cells. Chromogranin A has been considered as a powerful universal marker of neuroendocrine tissues and tumors. However, other markers that are not associated with neuroendocrine cells are sometimes found within the same tumor cells. It is thus possible that expression of a neuropeptide/chromogranin in a tumor reflects the fact that the cell type has acquired a neuroendocrine phenotype rather than being of neuroendocrine origin. Increased expression of these markers in tumors could be due to stimulation of specific factors or genetic switches that control neuropeptide/chromogranin/expression.

Redefining the neuroendocrine concept

Whereas the original attempts to categorize cells within a DNES has had much value, it no longer fits with our current concept of functional genomics, and the current definitions are causing more confusion. For example, a recent review proposes an expansion of the DNES to take into account the expression of neuroendocrine markers in immune cells [33] and is called the diffuse neuroimmunoendocrine system (DNIES) [33]. This leads us to question the necessity or usefulness defining cells with only some common properties into a single category. Just because two cells express a neuropeptide does not necessarily mean that they have a common embryological origin or that they should be classified as part of a coordinated system of cells. Furthermore, differential functions within a single neuropeptide precursor are the rule rather than the exception. For example, neurons expressing proenkephalin will process this precursor into opioid-like peptides that have specific inhibitory functions via opioid receptors. However, proenkephalin is also expressed in immunocytes that could also yield peptides with antibacterial function. Should we then categorize the immunocyte as a neuroendocrine cell, or even consider it to be part of a DNIES? Defining cells as an expanded DNES seems to lead to more conceptual problems.

We need a more fluid definition that can reconcile the embryological origin of a cell and its functional characteristics. We propose that cells be categorized solely on their experimentally determined embryological origin. [This is a classic concept that has enabled researches to group various neurons, endocrine, immune, cardiac cells etc.] The fully differentiated cell expresses a phenotype that is based on the proteins synthesized with that cell. However, we must now recognize that, even in a state of terminal differentiation, there can be varying levels of cellular plasticity. This plasticity can lead to the expression of, for example, neuropeptides in immune cells or of cytokines in neurons. An immune cell could acquire a partial 'neuroendocrine phenotype' (NEP) if it expresses neuropeptides, chromogranins or SPC2 and/or SPC3. The acquisition of NEP would be the result of a genetic switch that induces expression or inhibits repressors that prevent the expression of those so-called neuroendocrine markers. In the immune system, expression of an NEP can occur under basal conditions, but, interestingly, they appear predominantly when the cell is challenged, such as in the innate immune response. The potential functional significance of the ability of a cell

to express a partial NEP could be directly related to the need for a common currency of signals for cell-cell communication, as for example in immune cell-neuron communication. In a similar manner, we can envisage that neurons could express an 'immune cell phenotype' or ICP. These specific examples lead us to suggest that these effects should be discussed more in terms of genetic plasticity rather than attempting an impossible task of re-grouping cells in systems such as the DNES or the DNEIS. Cells types have already been extensively defined based on molecular properties and phenotypic characteristics. Extensive data are available from embryological studies that reveal the primordial origin of cells. However, embryological origin is not affected by the functional and plastic effects that a cell undergoes once it is in its final environment. This is certainly the case in a state of challenge as in the innate immune response for immune cells, but could also be applicable to neoplastic cells. In this extreme state, cells can acquire several phenotypes that have lead some researches to speculate on the origin of those cells, sometimes incorrectly. For example, that a cell expresses chromogranin does not in any way imply that the cell has a neuroendocrine origin. It does imply that the cell has now acquired a NEP. The actual cell origin is just as likely to be neuroendocrine as it could be immune or even epithelial.

It now remains for us to define the specific characteristics that we wish to categorize as part of the NEP. We suggest that the presence of classic neuropeptides should constitute one of these criteria. A cell that expresses one or more neuropeptides would have a NEP. A second potential criterion would be the presence of classic dense-core secretory granules, which are functionally regulated to release their contents into the external media upon a specific stimulus. These characteristics are independent of each other for example the expression of prodynorphin in corticosteroid cells, which do not contain secretory granules. We could therefore state that adrenal cortical cells have a partial NEP because they can express neuropeptides. It should be noted that each of these proposed criteria should remain fluid based on our future knowledge of what constitutes a neuron or an endocrine cell. These definitions will be further elaborated as experimental evidence is provided. Similarly, the ICP can be defined by the presence of specific molecules that are generally expressed in immunocytes, such as cyokines or defensins.

Conclusions

We believe that defining cells to reflect their ability for cellular plasticity can focus present efforts to identify phenotypic gene switches. These include switches for the expression of neuropeptides, secretory granule biogenesis or to obtain a regulated secretory pathway. Knowledge of these switches (which are most likely to be nuclear factors) could help us to develop the ability to directly control stem cell differentiation and to create specific terminally differentiated cells. Such cells are important for treatment of diseases in which loss of a specific cell type is observed, such as in neurodegenerative diseases, e.g., Parkinson's disease or type I diabetes. Knowledge of genes that control the NEP could also result in our ability to block the proliferation and expansion of certain types of neoplastic cell, such as those that have obvious NEP characteristics, including small cell lung carcinomas. These types of cancer cell have devastating effects, because they secrete large amounts of neuropeptides, many of which have known growth factor function. A global block of the expression of these neuropeptides could have important repercussions in terms of the delay of the onset of further metastatic and clonal development of these tumors.

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