RECENT PROGRESS AND PATHOLOGICAL APPLICATION OF HORMONE IMMUNOHISTOCHEMISTRY

AKIRA KAWAOI

*Department of Pathology, Nihon University School of Medicine,
Ohyaguchi, Kamimachi, Itabashi-ku, Tokyo 173*

The antigenic structure of a hormone molecule, like its biological activity, is peculiar to each hormone. Today, it is possible to prepare an antiserum for almost all hormones. As a result, hormone immunohistochemistry has made great progress.

One of the earliest achievements in immunohistochemical demonstration of the hormone was the observation of ACTH in the pituitary gland of the pig made by Marshall (10). Since then, research on the localization of the hormone of the adenohypophysis has made rapid progress, starting from the beginning of the 1960s. Owing largely to development of the enzyme-labeled antibody method (13), it may be said that most of the problems regarding the localization of the hormones of the anterior pituitary gland have been solved as far as mammals are concerned (21). Likewise, considerable knowledge has been obtained about the localization of the islet hormones between 1960 and the 1970s and about gut hormones since early 1970 up to the present (19, 20). And spectacular progress is now being made in research into the localization and distribution of various peptide hormones in the nervous system (15, 22).

Regarding hormones other than protein hormones, such as thyroid hormones T_4 and T_3 , steroid hormones, bioactive amines and prostaglandins, on the other hand, some attempts to clarify their localizations have been made, but research into their localization or distribution is still in the developmental stage (1, 6, 25).

Unquestionably, application of the enzyme-labeled antibody method by Nakane *et al.* has rendered a great contribution to the subcellular localization of a hormone antigen (12, 13). Figure 1 shows the localization of ACTH in the rat anterior pituitary detected by employing a new methacrylate resin (16), in which development we took part, as an embedding material and by staining on ultra-thin sections of the specimen. As shown in this figure, it has been demonstrated that this peptide hormone is contained in the secretory granules.

Important in detecting a hormone antigen is the basic question of how its antigenic activity is affected by the fixative solutions. Figure 2 shows the results of treatment of the placental hormone, hCG and its subunits with various fixatives and examining the changes in their antigenic activities by means of RIA (18). Evidently, different fixative solutions affected hCG in different ways, and the effect differed in different subunits. What is important, however, is that, contrary to our expectations, this glycoprotein hormone produced almost no sedimentation regardless of the fixative solution employed. This indicates that these fixative solutions have little ability to make hCG insoluble and fix it. Therefore, the hCG of the chorionic villi of the placenta whose existence is verified immunohistochemically is in the state

FIG. 1. Ultrastructural localization of ACTH on methacrylate resin-embedded ultrathin section of the rat adenohypophysis. $\times 6,000$

FIG. 2. Effects of fixatives on hCG and its subunit immunoreactivity (18).

of binding with an insoluble protein by some working mechanism, and it can be inferred that the hCG in a soluble or free state was washed away during the process of preparing a tissue specimen. In other words, if any hormone is detected by an immunohistochemical method, it is only part of the hormone which existed. This can be said not only in regard to hCG but also of other protein hormones.

Steroid hormone is also easily washed away from the tissue. Probably for this reason, it is difficult to detect its localization in the ordinary steroid-producing tissues. We attempted to prove the existence of corticosteroids (CS) in cultured cells (Y-1) originating in adrenocortical adenoma of a mouse. Figure 3 shows that the amount of CS in the cultured Y-1 cells and culture medium as a result of round-up of the cells due to stimulus of c-AMP increases with the lapse of time (5). Figure 4 shows the Y-1 cells in partial round-up, and Figure 5 in the complete round-up both verifying the localization of progesterone by the enzyme-labeled antibody method. Thus, the morphological change of the cells, the amount of hormone in the cells and the immune stainability correlated well with each other, proving the result of staining specific (6).

If tissue sections are to be investigated, however, the detection of CS succeeds only when it exists in large quantities in the tissue, as in the case of a CS-producing tumor. Figure 6 shows the localization of cortisol containing cells in the tissue of adrenocortical adenoma accompanied by Cushing's syndrome. A positive staining was seen in the compact cells, which are thought to be highly functional in steroid biosynthesis.

Probably the first immunohistochemical localization of thyroid hormones T_4 and T_3 was the one reported by Wilson *et al.* in 1978 employing the fluorescent antibody method on frozen sections of the rat thyroid gland (25). We clarified that it is possible to detect T_4 and T_3 by using thyroid tissue fixed with formalin and embedded in paraffin (7, 8). Figure 7 shows the localization of T_4 in non-functioning adenoma of the thyroid gland. Likewise, the localizations of T_4 and T_3 were detected in most of the thyroid tumors, whether benign or malignant, and it has become clear that they are functional, though subclinical.

The fluorescent method has been established to prove the localization of bio-

FIG. 3. The time course response of Y-1 cells to 1 mM dbcAMP(5).

- : Fluorogenic steroids in medium (ng/10⁴ cells),
- : Fluorogenic steroids in cells (ng/10⁵ cells),
- : Percentage of round cells.

FIG. 4. Localization of progesterone in the Y-1 cells stimulated by ACTH. $\times 300$

FIG. 5. Localization of progesterone in the Y-1 cells stimulated by dbcAMP. $\times 300$

FIG. 6. Demonstration of cortisol positive cells in the adenoma of Cushing's syndrome. $\times 360$

FIG. 7. Localization of thyroxine (T_4) in the follicular adenoma of the thyroid gland. $\times 360$

FIG. 8. Localization of serotonin in the rectal adenoma. $\times 360$

active amines (2), but reports concerning their immunohistochemical verification are so far very few (1). We are attempting to detect immunohistochemically the localization of serotonin by preparing a specific antiserum against it (14). Figure 8 shows that some of the cells which constitute the adenoma of the colon contain

serotonin. These serotonin-positive cells were confirmed to be argyrophil cells by staining the adjacent section with Grimelius' method. Thus, the immune staining of serotonin will be helpful in diagnosing the function of carcinoid tumors.

Unfortunately a fixative solution which is effective in making steroid hormone, thyroid hormone, serotonin, etc. insoluble and of fixing them is not available to us. Despite that, we succeeded in detecting them because, we inferred, steroid hormone, T_4 and T_3 and serotonin remained in small quantities, steroid hormone in the form of a binding with synthesizing enzymes, etc., T_4 and T_3 by being integrated in thyroglobulin molecules and serotonin in the form of condensation with a protein constituent. In order to establish a method of immunohistochemical verification of the localization of these hormones, we should further improve the sensitivity of detection techniques and develop an epochal new fixing method.

Many protein hormones retain their antigenic activity even if they are fixed by formalin. This helps us detect such hormones in histopathological materials, in particular, in diagnosing the function of pituitary adenoma (11), confirming the existence of prolactin-producing cells in chromophobe adenoma accompanied by hyperprolactinemia (9) and ascertaining simultaneous production of two or more hormones and ectopic hormone production in islet cell tumors (4).

It has been clarified by the immune staining of the pathological specimens that hCG, a placenta hormone, is produced in no small number of tumors, both trophoblastic and non-trophoblastic (17). In 51 (18.6%) out of the 274 cases of various tumors, benign or malignant, studied so far, hCG or its subunit was detected. In particular, the frequency was high in germ cell tumors. Moreover, simultaneous production of AFP or CEA was detected in no small number of the cases. Figure 9 shows a case of malignant teratoma which showed production of AFP and CEA, and, in some of them, hCG was detected.

Thus, hCG was frequently detected in various kinds of tumors, particularly in germ cell tumors, without clinical signs of excessive secretion, at the same time as AFP and CEA. Further, it is detected in the liver, pancreas and the alimentary tract of human fetuses. From these facts, it seems more reasonable to regard hCG

Fig. 9. Demonstration of hCG- β in the malignant teratoma of the mediastinum. $\times 720$

detected immunohistochemically, beyond the ordinary concept of "hormone", as an onco-feto-placental antigen closely related to AFP and CEA. Progress in the biochemical clarification of the substance which causes the immunoreactivity of hCG is strongly expected (24).

It would be possible to use immunohistochemistry to verify the location of where hormones work and to clarify their acting mechanisms. But no significant progress has been made yet in this regard because of various technical difficulties (3, 23). As morphological approaches to the mechanism of protein hormone action, we can mention; 1. the verification of the location where a labeled hormone is bound, 2. an immunohistochemical demonstration of a hormone which is bound with a target cell, 3 the localization of a receptor by an antireceptor antibody, 4. detection of the activities of adenyl cyclase and guanyl cyclase, 5. an immunohistochemical demonstration of cAMP, and so forth. We attempted to demonstrate the localization of FSH and LH in a rat ovarian tissue by immune staining (3). Figure 10 shows the localization of FSH in ovarian follicles in the proestrus phase. The follicular fluid and some follicular epithelia showed a positive staining. We could also detect LH in the same manner. Summing up, it was shown that FSH and LH revealed relatively similar distributions except that FSH and LH were stainable rather conspicuously in follicles and in interstitial cells, respectively, and that both persistently exist in each stage of the estrus cycle though with some difference in concentration. The localization of hormone, however, does not necessarily mean their working at that location, and it is a problem for the future to interpret the results of immune staining.

In conclusion, in the absence of any effective method to detect *in situ* the bio-activity of a hormone, immunohistochemistry is the only specific method to verify

FIG. 10. Localization of FSH in the rat ovary in the proestrus phase. $\times 300$

the localization of hormones. However, the localization of a hormone detected by this method must not hastily be regarded as identical with that *in vivo*. It should be necessary to take a comprehensive approach including the correlation of the immunoreactivity of hormones with the bioactivity and localization of enzymes which take part in its biosynthesis in order to deepen understanding of the endocrine phenomenon.

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