

Intranuclear Cytoplasmic Pseudoinclusions in Pituitary Adenomas

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Intranuclear pseudoinclusions are well known in papillary carcinomas of the thyroid gland, hepatocellular carcinomas, meningiomas, paragangliomas, pheochromocytomas, and melanomas. Only two papers on the intranuclear inclusions of adenohypophyseal cells in humans have been reported. This study found that intranuclear cytoplasmic pseudoinclusions occur frequently in pituitary adenoma cases (70.3%, 97 of 138 pituitary adenomas) and are uncommon in normal pituitary tissue (11.1%, 1 of 9 normal pituitary tissues). In addition, the frequency of intranuclear cytoplasmic pseudoinclusions between the functional and non-functional pituitary adenomas was found to be similar. Electron microscopy and immunostaining was used to reveal the entity of the intranuclear inclusion. These intranuclear inclusions are due to cytoplasmic invagination because 1) the inclusions are continuous with the cytoplasm, 2) all cytoplasmic organelles, such as the endoplasmic reticulum, the Golgi apparatus, and the secretory granules are found in the inclusions, 3) immunoreactivity of the intranuclear inclusion is the same as that of the cytoplasm. In conclusion, intranuclear cytoplasmic pseudoinclusions in pituitary adenomas occur frequently (70.3%) and are formed by cytoplasmic invagination. This study suggests that pituitary intranuclear inclusions caused by cytoplasmic invagination be called "intranuclear cytoplasmic pseudoinclusions".

Key Words: Inclusion bodies, nuclear inclusion, pituitary neoplasms, pituitary adenoma, ultrastructure

INTRODUCTION

Intranuclear pseudoinclusions are found in

meningiomas,¹ paragangliomas,² pheochromocytomas,² hepatocellular carcinomas,³ adrenal cytomegaly,⁴ adenohypophyseal cells,^{5,6} and papillary carcinomas of thyroid.⁷ Mosca and Bertoli previously reported the intranuclear inclusions of adenohypophyseal cells in human in 1969.⁵ The authors concluded that the human pituitary nuclear inclusions were likely to be true inclusions rather than the result of cytoplasmic invagination, possibly due to the lack of a confirmative ultrastructural study. Since then, Ryder, et al.⁶ in 1979 described three types of nuclear inclusions based on electron microscopic findings as; a simple nuclear body, a complex nuclear body, and a filamentous aggregate, which were not related to hormonal hyperactivity in the human adenohypophysis. This report includes the light microscopic and electron microscopic findings of the intranuclear inclusions, and illustrates new aspects of the pituitary intranuclear inclusion structure. This study also investigated the relationship between the occurrence of nuclear inclusions and the functional hormone status of a pituitary adenoma.

MATERIALS AND METHODS

One hundred and forty seven human pituitary glands were examined in this study. Representative paraffin sections of the pituitary adenomas were selected from the archives in the Department of Pathology at both Yonsei University College of Medicine and Yonsei University Wonju Medical College. One hundred and thirty eight cases of

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surgically removed pituitary adenomas and nine normal pituitary tissues from autopsies with an unrelated cause of death were reviewed. The normal pituitary tissue from autopsies was used as the normal control. The hematoxylin and eosin (H&E) stained sections of the specimens were reviewed. Frozen sections and squash preparation slides were also reviewed where possible. Only the pituitary adenomas that measured more than 5 mm² were included.

The functional hormonal status of the pituitary adenomas was determined by the serum hormonal level from a chart review. Of the 28 cases previously fixed in a glutaraldehyde solution, 7 cases had enough inclusions in the H&E sections to be processed for both the electron microscopic examination and immunostaining. These were embedded in Epon epoxy resin. The semi-thin sections stained with toluidine blue were then examined to confirm the presence of nuclear inclusions. Finally, ultrathin sections were stained with both uranyl acetate and lead citrate.

The relationship between the presence of nuclear inclusions and hormonal activity was analyzed using a chi-square test in two respects; for the activity of GH, prolactin, ACTH, TSH, FSH and LH, and for the difference between the functional adenoma and non-functional adenoma. A *p*-value of <0.05 was considered significant.

RESULTS

Patients

The mean age of the 138 patients with the pituitary adenomas was 43.1 years (range: 15 - 71). 58 patients (42.0%) were male and 80 (58.0%) were

female. Seventy five (54.3%) had non-functional adenomas and sixty three (45.7%) had functional adenomas. Among the functioning pituitary adenomas, the prolactin secreting pituitary adenomas were the most frequent (21.0% out of the total and 46.0% of the functioning pituitary adenomas, Table 1).

Light microscopic findings

Ninety seven (70.3%) of the 138 pituitary adenomas and one from the 9 normal pituitary tissues (11.1%) had intranuclear inclusions in the H&E sections (Table 1). Although the number and distribution of the inclusions varied from case to case and from area to area, ranging from 1/10HPF to 7/10HPF, the number of inclusions of all the adenomas was significantly higher than those of the normal pituitary tissue. Intranuclear inclusions were amphophilic or weakly eosinophilic and showed round contours with well-defined borders (Fig. 1). These intranuclear inclusions were found in the frozen sections (Fig. 1A), the paraffin sections (Fig. 1B and 1C), and in squash preparation (Fig. 1D and 1E). Their immunoreactivity to the pituitary hormone was similar to that of the cytoplasm (Fig. 1F).

Electron microscopic findings

The membrane surrounding the intranuclear inclusion was an extension of the nuclear membrane. The inner contents of the intranuclear inclusions were the cytoplasmic organelles such as the rough endoplasmic reticulum (RER), the Golgi apparatus, and the secretory granules, all of which were similar to those of the cytoplasm (Fig. 2 and 3A). The secretory granules of the intranuclear

Table 1. Hormonal Activity and Frequency of Intranuclear Inclusions in Pituitary Adenomas

	Hormonal Activity						Total
	PRL	GH	GH & PRL	ACTH	TSH	NFT	
Case Number (%)	29 (21.0)	21 (15.2)	8 (5.8)	3 (2.2)	2 (1.4)	75 (54.3)	138
Inclusion	21	17	6	3	2	48	97
Inclusion %	72.4	81	75	100	100	64	70.3

PRL, prolactin; GH, growth hormone; ACTH, adrenocorticotrophic hormone; TSH, thyrotropin; NFT, non-functional tumor.

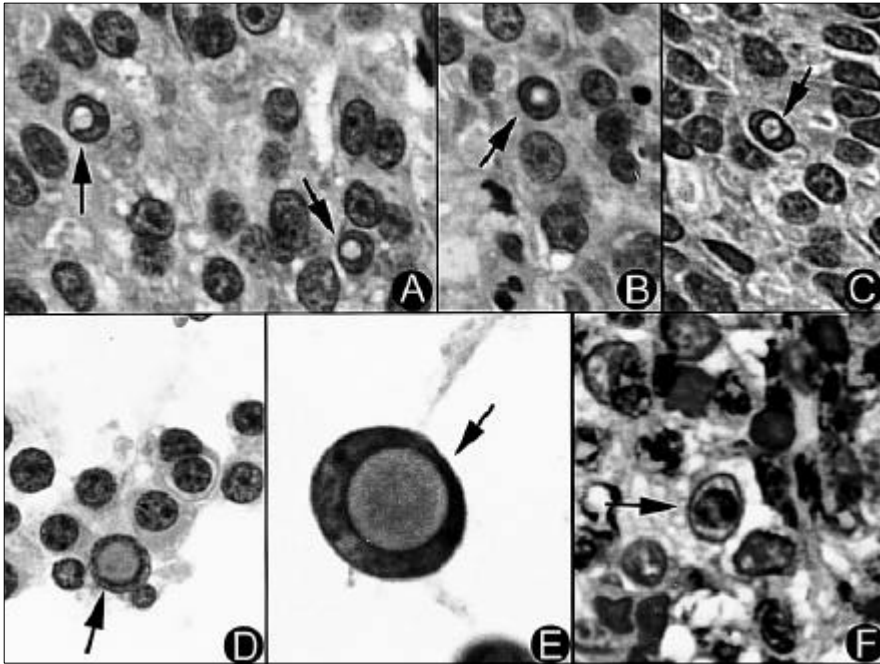


Fig. 1. Light microscopic findings: intranuclear pseudoinclusions of pituitary adenoma tumor cells from the paraffin sections (A, B, & C) and from the squash preparations (D & E). The intranuclear inclusions are amphophilic to weakly eosinophilic. Immunostaining for prolactin shows intranuclear immunoreactivity (F). (A, B, C, D, F; $\times 200$, E; $\times 400$).

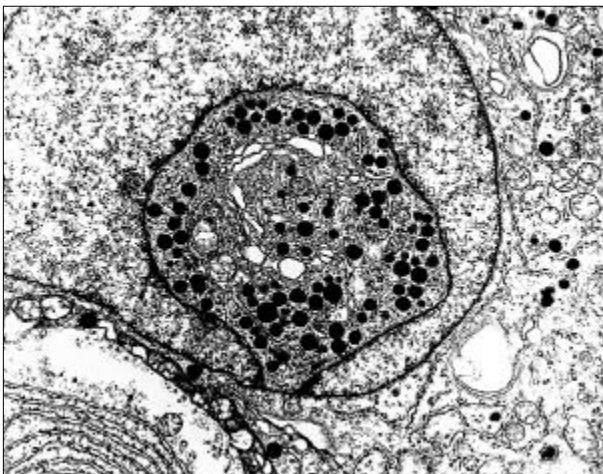


Fig. 2. Electron microscopic findings: The surrounding membrane of the intranuclear inclusion is part of the adjacent continuous nuclear membrane. The surrounding membrane of the inclusion is covered by coarse chromatin, which are present along the inner surface of the nuclear membrane. The intranuclear cytoplasmic pseudoinclusions contain RER and secretory granules. (Original magnification; $\times 13,100$).

inclusions were morphologically the same as those of the cytoplasmic secretory granules. Of the 7 adenomas, one had intranuclear homogeneous material, which was arranged in a concentric or whorl-like formation, surrounded by an electron-lucent halo (simple nuclear body,⁶ Fig. 3B).

Statistical analysis

The ACTH and TSH secreting adenoma cases were excluded from the relational analysis of hormonal activity and the occurrence of nuclear inclusions, because the expected counts were ≤ 5 . The chi-square test results revealed that there were no statistically significant differences in the frequency of inclusions between the functional and non-functional adenomas (Table 2).

DISCUSSION

The results of this study are in agreement with those reported by Mosca and Bertoli⁵ in terms of the morphological features of intranuclear inclusions by H&E staining. This study analyzed the electron microscopic structures of the nuclear inclusions in terms of the surrounding membrane and the inner contents. The surrounding membrane of the intranuclear inclusion was continuous with the nuclear membrane, and they contained RER and electron dense secretory granules, which were identical to those within the cytoplasm. These findings strongly suggest that the intranuclear inclusions are formed by cytoplasmic invagination. The ultrastructural features

of the inclusions described above are similar to those of paragangliomas,² pheochromocytomas,² adrenal cytomegaly,⁴ and thyroid papillary carcinomas,⁷ which are generally accepted as intranu-

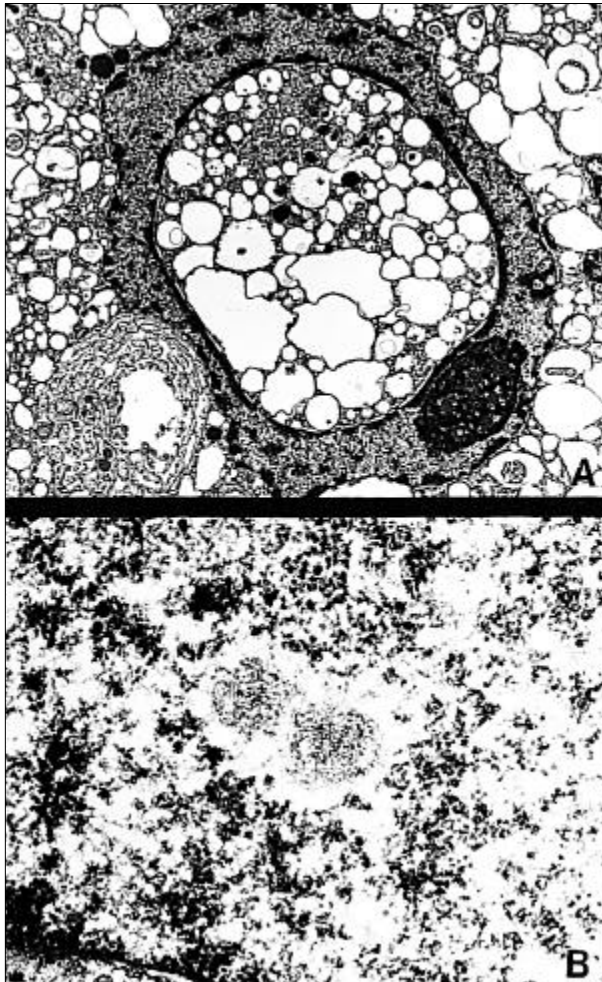


Fig. 3. Electron microscopic findings: Intranuclear cytoplasmic pseudoinclusion and paranuclear parallel stacks of rough endoplasmic reticulum of non-functional pituitary adenoma. The contents of the intranuclear cytoplasmic pseudoinclusions are similar to those of the cytoplasm (A). Intranuclear homogeneous material, arranged in a concentric or whorl-like formation, surrounded by an electron-lucent halo (simple nuclear body, B). (Original magnification: A; $\times 13,100$, B; $\times 31,000$)

clear cytoplasmic invagination. To the best of the authors' knowledge, this report is the first in the literature to show in detail the cytoplasmic origin of the intranuclear inclusions of the human pituitary adenomas based on light microscopic and electron microscopic features. Different kinds of pituitary nuclear inclusions were previously reported by Ryder, et al.,⁶ which were described as a simple nuclear body, a complex nuclear body, and a filamentous aggregate. Only one of the complex nuclear bodies described in this study had a vesicular core, for which the authors suggested cytoplasmic invagination as the pathogenesis. The rest of the nuclear inclusions were described as being composed of homogenous or filamentous structures. Only one homogenous nuclear body (simple body) was found in this study. In terms of the pathogenesis of the nuclear inclusions, DeLellis, et al.² in 1980 suggested that the intranuclear inclusions in pheochromocytomas were a reflection of the high degree of nuclear angulation and lobulation that is commonly seen in these tumors. However, this study observed that the nuclear contours were relatively round in the pituitary adenomas. It is believed that these inclusions may reflect a higher amount of RER and secretory granules in the confined cytoplasmic spaces, which pushes the nuclear membrane inward to form intranuclear inclusions. Increased RER and secretory granules might be related to the functional hormonal status of a pituitary adenoma. However, in this study, there were no statistically significant differences in the frequency of the inclusions between the functional and non-functional adenomas.

In summary, the intranuclear inclusions in pituitary adenomas consist of the surrounding membrane continuous with the nuclear membrane, the RER, and the secretory granules, which are identical to those of the cytoplasm. This suggests that the inclusions are formed by cytoplasmic

Table 2. Results of the Chi-square Test on the Hormone Functional Status and the Occurrence of Nuclear Inclusions

	Hormonal Status		X ²	p-value
	Functioning	Non-functioning		
Number (%)	63 (45.7)	75 (54.3)		
Inclusion (%)	49 (77.8)	48 (64.0)	2.488	0.115

invagination. These inclusions may reflect increased number of cytoplasmic organelles in the confined cytoplasmic spaces, which push the nuclear membrane inward to form an intranuclear inclusion. This study suggests that intranuclear inclusions caused by cytoplasmic invagination be called "intranuclear cytoplasmic pseudoinclusions".

In terms of the relationship between the occurrence of nuclear inclusions and the functional hormonal status of the pituitary adenoma, this study found no differences in the frequency of intranuclear pseudoinclusions between the functional and non-functional pituitary adenomas.

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