Adrenocorticotropic hormone production in breast cancer

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Presence of adrenocorticotropic hormone (ACTH) was investigated in tissues from 150 cases of primary breast cancer. ACTH peptides were detected in 16.7% cases and ACTH expression was higher in post-menopausal cancers. A significant association was noticed between the presence of ACTH and the positive estrogen receptor (ER) status of tumors. The study indicated a probable role of these ectopic ACTH peptides in steroid hormone related pathology of breast cancer.

Keywords: Adrenocorticotropic hormone, Breast cancer, Cancer, Estrogen receptor

Neuroendocrine cells are found in different tumors. These cells produce various neuropeptides, which may be involved in autocrine/paracrine pathways of regulation of growth, differentiation, secretion and immune interactions. However, ectopic ACTH secretion from breast cancer is a peculiar phenomenon and perhaps influences the pathological course of the disease. It is well-known that breast cancer is a steroid hormone dependent malignancy and ACTH stimulates the initial phase of steroid hormone biosynthesis (i.e., cholesterol to pregnenolone) in adrenal cortex. ACTH, a 39-amino acids peptide, is an anterior pituitary hormone and is processed from the precursor pro-opiomelanocortin (POMC) molecule along with other peptides such as melanocyte stimulating hormone (MSH), endorphins, \( \beta \)-lipotropin (\( \beta \)-LPH), etc. POMC/ACTH has a wide range of activities in various physiological as well as pathological conditions. For instance, there is a close association between the functions of POMC-derived peptides and leptin, an adipocyte-secreted hormone. Recently, leptin and leptin receptors have been found to be involved in the pathological process of breast cancer. Similarly, cytokines such as interleukin-1 (IL-1) and IL-6 are intricately related with POMC/ACTH production.

In non-pituitary tumors, Meador-Liddle and their colleagues first demonstrated the production of ACTH. The POMC gene is located on chromosome 2 and exists as a single copy. POMC gene expression is normally restricted to the pituitary corticotroph cells, the arcuate nucleus of the hypothalamus and melanocytes. Recent progress in the understanding of the tissue specific regulation of POMC gene expression and new insights into the processing of POMC peptide in non-pituitary tissues has helped elucidate some of the molecular events leading to its ectopic expression. Nevertheless, by contrast to the mechanisms of POMC gene expression in pituitary corticotrophs, those in non-pituitary tumors remain largely unknown. Initially, POMC is processed to form pro-ACTH and then ACTH. Depending on the degree of processing in the tissue or tumour, there is the possibility for a number of ACTH-related peptides to be secreted from POMC expressing cells. Like other peptide hormones, ACTH binds to its membrane receptor (melanocortin receptor \( \text{MC}2\text{R} \)) and within a few seconds of this interaction, intracellular cyclic AMP levels increase markedly.

Breast cancer is a leading cancer amongst females worldwide. In developed countries, it is the commonest cancer in women. Breast cancer is the second most common cancer among Indian women; however, the disease ranks first in Mumbai and Kolkata, and an increasing trend in its incidence has been noticed in most of the metropolitan cities. There are substantial clinical, epidemiological and experimental evidences, which show that the pathological process of breast cancer is influenced by endogenous steroid hormones (particularly estrogens). Incidence of breast cancer is strongly related with age,
two-third cases occur during the post-menopausal period. After menopause, most of the circulating estrogen is produced by the conversion of androgens in peripheral tissues (aromatization); and the androgens come mainly from the adrenal cortex in post-menopausal women. Therefore, the hypothalamic-pituitary-adrenal (HPA)-axis and related ACTH has an important role in the pathology of breast cancer, particularly post-menopausal cases. The aim of the present study was to search the existence of ACTH producing cells in breast cancer and their relation (if any) with some common clinical parameters such as menopausal status, metastasis to lymph node and ER status.

Materials and Methods

Patients—The study was carried out on the mastectomy specimen of breast cancer tissues, collected from Maulana Azad Medical College, New Delhi. Tissue sections from 150 cases of histopathologically diagnosed primary infiltrating duct carcinoma of the breast were analyzed in this study. Moreover, tissue sections from 3 cases of fibroadenoma of the breast were used as negative control and 1 adrenal tumour was used as positive control for the immunohistochemical staining of ACTH. Relevant social and clinical histories of the patients were obtained. All patients in this study were married and 82 were post-menopausal. Only 3 patients had a family history of the disease. No patient presented the clinical features of Cushing’s syndrome/ectopic ACTH syndrome.

Immunohistochemistry—On poly-L-lysine coated slides, 5 μm thick formalin-fixed paraffin-embedded tissue sections were used for immunostaining. Tissue sections were deparaffinized by successive changes of xylene and subsequently subjected to hydration through descending grades of ethyl alcohol. Antigen retrieval was done by immersing sections into pre-heated 10 mM sodium citrate buffer (pH 6.0) for 30 min within water-bath (95°C). Then, the containers with citrate buffer and slides were taken out from water-bath and allowed to cool for 20 min at room temperature, and the slides were rinsed with phosphate buffered saline (PBS, pH 7.2) for 3 times. Subsequently, tissue sections were incubated with freshly prepared solution of methanol and hydrogen peroxide (3%) to block endogenous peroxidase activity. The sections were incubated with 1.5% normal blocking serum in a humid chamber at room temperature for 1 hr. Afterwards, optimally diluted primary antibody was applied to tissue section: mouse monoclonal antibody against ER and rabbit antibody against ACTH (Sigma, USA). Sections were incubated with above-mentioned primary antibody diluted in PBS with 1.5% normal blocking serum, for 1 hr at room temperature in a humid chamber. The slides were then rinsed in PBS and subsequently, the tissue sections were incubated with secondary antibody (1:100 dilution in PBS with 1.5% normal blocking serum) for 30 min in a similar condition like above. After 3 washes with PBS containing Tween 20 (0.005%), the tissue sections were again incubated with peroxidase-antiperoxidase complex for 1 hr at room temperature. Then, the sections were washed thrice in PBS with Tween 20, and the reaction was visualized by substrate diaminobenzidine hydrochloride (DAB, 0.1% freshly prepared solution in PBS with 0.05% hydrogen peroxide). After the development of proper colour, the slides were washed with double distilled water and counter stained with haematoxylin. Subsequently, the sections were dehydrated gradually through graded alcohol and then mounted with DPX. Afterwards, the slides were assessed under microscope.

Immunohistochemistry or immunocytochemistry is an immunoenzymatic staining method for in situ detection of cellular antigen with optimal specificity and sensitivity. In this study, several steps were taken to reduce false positivity such as heating during antigen retrieval and subsequent incubation with hydrogen peroxide to quench endogenous peroxidase enzyme activity. Further, normal serum was used to block the non-specific reacting sites in the tissue in order to improve specificity. Also, during assessment of tissue sections under microscope, non-specific staining and doubtful positivity (if any) were not considered.

Statistical analysis—The data were subjected to MS EXCEL 2000 and analyzed by Epi info version 6 software. ACTH expression was calculated according to each level of the different factors. Odds ratio (OR) and 95% confidence intervals (CI) were estimated. To test the association between ACTH and various factors, chi-square test ($\chi^2$) was applied. $P$ value at $>0.05$ was considered as a significant level.

Results

In the present study, out of 150 cases of primary breast cancer, 25 cases (16.7%) showed the
immunoreactivity for ACTH (Fig. 1A). The immunohistochemical demonstration of ACTH was higher in post-menopausal cancers as compared to pre-menopausal cases. The difference was close to the level of statistical significance ($P=0.056$, border-line significant). In post-menopausal group, 18 tumors (18/82, 22.0%) revealed immunoreactivity for ACTH, whereas 7 tumors (7/68, 10.3%) were positive for ACTH among pre-menopausal women (Table 1). In this study, metastatic involvement of the lymph node was present in 85 patients (lymph node positive cases, 56.7%). Amongst these metastases cases, 17 tumors (17/85, 20.0%) showed the positive immunostaining of ACTH. On the other hand, out of 65 cases who were free from involvement of lymph nodes (lymph node negative cases), 8 tumors (8/65, 12.3%) revealed immunoreactivity for ACTH (Table 1). Like lymph node involvement, ER status is considered as an important prognostic parameter in breast cancer. In the present study, 41 (27.3%) cases were positive for ER (Fig. 1B). Interestingly, a significant association was observed between the presence of ACTH in tumors and their ER status ($P<0.001$). Out of 41 ER positive cases, 18 cases (18/41, 43.9%) were positive for ACTH and out of 109 ER negative cases, 7 cases (7/109, 6.4%) showed positive immunostaining of ACTH. Alternatively, 72% (18/25) of ACTH producing tumors revealed ER positivity and 93.6% (102/109) of ER negative cancers were also negative for ACTH immunoreactivity (Table 1).

**Discussion**

Immunohistochemical technique has created an enormous opportunity to visualize different neuroendocrine cells in a number of carcinomas originating from exocrine glandular tissues. It has been thought that endocrine characters of breast cancer do not originate from pre-existing endocrine cells, but rather indicate the expression of a potential differentiation pathway in neoplastic cells. In general, there is a direct relationship between number of neuroendocrine cells of breast tumour and patients' age. Higher frequency of ACTH positive cases among post-menopausal patients in our study probably denoted the same phenomenon. Interestingly, the prognostic importance of

<table>
<thead>
<tr>
<th>ACTH peptides</th>
<th>Positive (n=25)</th>
<th>Negative (n=125)</th>
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<tbody>
<tr>
<td>Menopausal status</td>
<td>Post-menopausal (n=82)</td>
<td>18 (72.0%)</td>
</tr>
<tr>
<td>Pre-menopausal (n=68)</td>
<td>7 (28.0%)</td>
<td>61 (48.8%)</td>
</tr>
<tr>
<td>$\chi^2 = 3.64$, $P = 0.056$, OR = 2.45, CI = 0.89-7.01</td>
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<tr>
<td>Lymph node status</td>
<td>Positive (n=85)</td>
<td>17 (68.0%)</td>
</tr>
<tr>
<td>Negative (n=65)</td>
<td>8 (32.0%)</td>
<td>57 (45.6%)</td>
</tr>
<tr>
<td>$\chi^2 = 1.57$, $P = 0.21$, OR = 1.78, CI = 0.66-4.90</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ER status</td>
<td>Positive (n=41)</td>
<td>18 (72.0%)</td>
</tr>
<tr>
<td>Negative (n=109)</td>
<td>7 (28.0%)</td>
<td>102 (81.6%)</td>
</tr>
<tr>
<td>$\chi^2 = 30.13$, $P&lt;0.001^*$, OR = 11.40, CI = 3.90-34.51</td>
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$^*$Significant at $P<0.05$
neuroendocrine differentiation has been widely studied in carcinomas of prostate, lung and colon. On the contrary, little is known about prognosis in neuroendocrine differentiated tumors of the breast. Only few studies have been conducted in this direction and the investigators\textsuperscript{19-22} did not find any significant association between the presence of neuroendocrine cells and tumour size, grade, stage, lymph node involvement, vascular invasion, or c-erbB-2 oncprotein status of the tumors. Also, in the present study, no significant association was observed between ACTH immunoexpression and metastatic involvement of lymph nodes. Nevertheless, investigations are necessary to understand ACTH expression in breast cancer and its relationship with hormonal receptors\textsuperscript{2}. Ray et al.\textsuperscript{20} have noticed a significant association between ACTH expression and ER status of breast tumors; however, progesterone receptor (PR) did not show any such association. In that study\textsuperscript{20}, only 15.4\% PR positive breast cancer cases revealed ACTH expression, whereas 23\% PR negative cases were positive for ACTH.

Primary source of estrogens in post-menopausal women is from the conversion of androsterone to estrone in peripheral tissues including adipose tissue, thus post-menopausal obesity increases the risk of breast cancer through increased production of estrogens. Interestingly, several studies have demonstrated that women with breast cancer have higher blood levels of androgens\textsuperscript{23-25}. The source of excess androgens among breast cancer patients may be adrenal gland. Disturbances in extra-adrenal regulators like ACTH or ectopic source of ACTH may lead to excessive adrenal sex-steroid production\textsuperscript{20}24. It has been mentioned earlier that in the present study, immunohistochemical expression of ACTH peptide was much higher among post-menopausal breast cancers compared to pre-menopausal group. Similar findings have also been documented by one of the previous studies\textsuperscript{19}. Already, it has been explained that in post-menopausal women, the ovarian contribution of estrogen is either abolished or markedly diminished, and estrogen is synthesized from adrenal androgens. In the present study, no patient showed the manifestation of ACTH hyperactivity. Although, the amount of ACTH secreted by the tumors was not sufficient to cause clinical manifestation of hormonal hyperactivity, however, this ectopic source of ACTH can stimulate adrenal cortex continuously and in a sub-clinical manner to produce excess androgens, and may modify the pathological course of the disease. Also, there are reports that recorded similar observation\textsuperscript{26}.

Unlike all previous studies\textsuperscript{4,19,20,22-29}, the present study analyzed tissue specimens from a large number (n=150) of primary breast cancer cases. Interestingly, this study noticed a significant association between immunopositivity of ACTH and ER. The present finding confirmed the earlier observations\textsuperscript{19,20}. ACTH expression in breast cancer tissues and its significant association with ER perhaps indicated a perplexing steroid environment. Further, ACTH expression may have a relation with immune mechanisms within tumour microenvironment\textsuperscript{29}. How tumors acquire the capacity to produce ACTH and the precise role of this hormone are unknown. These are important questions for future research to understand the complicated pathophysiology of breast cancer.

References


