Clinical Study

A comparative study of the int-2 gene product in primary and secondary parathyroid lesions

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Abstract

Objective: The family of fibroblast growth factors stimulates proliferation of cells of mesenchymal, epithelial and neuroectodermal origin. One of the members of this family, the product of proto-oncogene int-2, fibroblast growth factor-3, has been found to stimulate mitosis of parathyroid cells in culture. Primary and secondary hyperparathyroidism have no clear differences with regard to the histopathological features of the diseased parathyroid glands.

Design: This study was undertaken in order to determine whether int-2 protein is immunohistochemically expressed in normal and abnormal parathyroid glands and to investigate whether there is a differential expression of the int-2 gene product between primary and secondary parathyroid disease.

Methods: A sheep anti-human int-2 antibody was applied to tissue sections from 37 samples of primary parathyroid disease (12 sporadic adenomas, 25 hyperplastic glands), from 30 samples of renal hyperparathyroidism, and from seven normal controls. Int-2 immunostaining was evaluated semi-quantitatively.

Results: None of the normal parathyroid glands stained positively. Int-2 immunopositive expression was more frequently detected in specimens of uraemic patients than in those of patients with primary parathyroid growth processes ($P = 0.029$). The reason for this differential expression appears to be the higher proportion of oxyphilic cells growing in hyperplastic glands of patients with secondary hyperparathyroidism; the latter cells were specifically found to be int-2 immunoreactive.

Conclusion: The int-2 gene product is likely to participate in the proliferation of this parathyroid cell subpopulation.

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Introduction

Fibroblast growth factors (FGFs) comprise a family of at least eight members involved in the control of proliferation of glandular tissues; these polypeptides interact with a complex class of cell surface receptors. Normal rodent parathyroid cell lines contain multiple classes of FGF receptors with potentially variable specificity for FGF family members (1). FGF3 is encoded by the proto-oncogene int-2; it has been reported to stimulate mitosis of parathyroid cells in culture and can be oncogenically activated by insertional mutagenesis. The int-2 proto-oncogene is located on chromosome 11q13.3, a region already identified as being susceptible to rearrangement/mutation in parathyroid disease. Alteration in FGF3 regulation is a possible mechanism by which growth abnormalities could be induced in this region of the human genome (2).

Little is known about the factors that control cell proliferation within the parathyroid. In hyperparathyroidism (HPT), the parathyroid glands are enlarged owing to an increase in the size and number of parathyroid cells. The predominance of focal lesions in adenomatous growth of primary HPT, in contrast to the generalized hyperplasia of renal patients (secondary HPT), suggests distinct pathways of growth processes in these conditions.

RT-PCR has already been used to evaluate FGF3 expression in normal, adenomatous and hyperplastic parathyroid tissues. It has been argued that heterogeneous FGF3 expression observed in these various parathyroid lesions should be re-evaluated using immunohistochemistry (2), since the latter procedure preserves tissue architecture and thus allows the localization of FGF3 expression in specific cell subclones; in this way, correlations between histology and the int-2 gene product can be made and this may help clarify the significance of FGF3 expression in diseased parathyroid tissue. So, the aim of this study was to determine whether int-2 protein is detectable in tissues of normal

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and abnormal parathyroid glands and to assess any changes in the int-2 immunoreactivity status with regard to the aetiology of various growth abnormalities (i.e. primary and secondary HPT).

**Materials and methods**

Human parathyroid tissues were obtained from parathyroid glands removed at neck exploration from 37 patients with sporadic primary HPT (12 ordinary adenomas, 25 hyperplastic glands) and from 30 patients with secondary HPT due to renal failure. Adenomas were distinguished from primary chief-cell hyperplasias on morphological grounds and often on the presence of a microscopically normal second gland. Tissue blocks were cut at 3 μm and mounted on poly-L-lysine-coated glass slides. The samples were stained immunohistochemically according to the avidin–biotin–peroxidase complex procedure (ABComplex, HRP; Dako, Glostrup, Denmark). A sheep anti-human int-2 antibody (TCS Biologicals Ltd, Buckingham, Bucks, UK) was diluted 100 times and incubated overnight. Control staining was performed employing non-immunized sheep serum instead of the primary antibody. As controls, seven normal parathyroid glands extirpated by mistake during thyroid operations were also examined. Immunostaining was evaluated semi-quantitatively: samples with int-2 immunopositivity in 0–5% of parathyroid cells were considered to be int-2 immunonegative while those with more than 5% of immunopositive cells were considered int-2 immunopositive cases. High int-2 immunopositivity was defined as the presence of staining in more than 50% of parathyroid cells. The results were statistically analysed by the $\chi^2(x)$ test.

**Results**

None of the normal parathyroid glands stained positively. Among primary lesions, int-2 protein was positively expressed (i.e. in >5% of parathyroid cells) in one adenoma and in six cases of chief-cell hyperplasia. The proportion of immunoreactive specimens was 18.9% (7/37) in patients with primary lesions and 43.3% (13/30) in the uraemic patients; the difference between the two groups was significant ($P = 0.029$). Int-2 positive staining was independent of the lesion’s size as well as of the presence of regression changes (i.e. fibrosis). Immunoreactivity for int-2 was mainly seen both in the cytoplasm and on the cell surface; so this marker appears to exist in both the cytoplasm and the cell membrane. As regards int-2 quantitative expression, int-2 immunoreaction was only occasionally detected at high levels (in more than half of the examined parathyroid cells); this increased immunoreactivity also seemed to be irrespective of the gland’s size. Int-2 immunostaining mainly demonstrated a focal and distinctly granular pattern, being specifically expressed on oxyphilic cells (Fig. 1), often in the form of neighbouring hyperplastic nodules as well as in transitional oxyphilic cells with anisokaryosis.

**Discussion**

Histopathological distinction between primary chief-cell hyperplasia and primary adenoma of the parathyroid is
not easy; therefore, a new classification of primary parathyroid enlargements in one gland disease, and multiple gland disease, has been proposed. Parathyroid tissues from patients with primary or secondary HPT do not differ significantly on histological grounds either.

In cases of ordinary primary adenoma, as well as in those of classic primary hyperplasia, chief cells predominate as a rule. Cases with a clear presence of a mixed cell population were characterized as hyperplastic. So, 11 of the 12 adenomatous tumours of this study were all practically negative for the presence of oxyphilic cells and this is probably associated with the almost complete absence of int-2 immunostaining in the examined adenomas. In hyperplastic lesions, the incidence of int-2 immunopositivity status appeared to depend on the different amount of oxyphilic cells dispersed in the different hyperplastic glands. In nodular, primary chief-cell hyperplasia, a high proportion of oxyphilic cells is reported to be noticeable (3). It is noteworthy that in the six out of seven int-2 immunopositive primary lesions of the present study, a nodular pattern of growth with obvious participation of oxyphilic cells was observed. In secondary HPT, hyperplastic nodular collections of transitional oxyphilic cells and pure oxyphilic cells within nodules are more common (3). The pathogenesis of nodule formation is based on two concepts: either the hyperplastic nodular tissue is considered to result from chronic intense stimulation by a trophic hormone, eventually causing the growth of polyclonal nodules (a concept known as non-neoplastic endocrine hyperplasia (NNEH)), or else the nodules are thought to represent true clonal tumours (4). As best shown in hyperplastic thyroid glands, there are some characteristics that do not comply with the simple concept of NNEH, i.e. the inevitable nodular transformation, the frequent autonomy and irreversibility of nodular growth, the loss of function in many cells and nodules, and the loss of coordination between cellular growth and function (4).

It has been claimed that nodules within parathyroid lesions contain subpopulations of cells with a consistently higher proliferative rate than non-nodular areas and are thus more likely to develop genetic abnormalities (5). Transforming growth factor-alpha has already been reported to interact with its receptor to promote parathyroid cell proliferation, perhaps by an autocrine mechanism (6). In the present study, immunoreactivity for int-2 was observed both in the cytoplasm and on the cell surface of parathyroid oxyphilic cells; this finding suggests an autocrine and paracrine action of FGF3 in parathyroid lesions. Particularly in renal HPT, parathyroid glands initially grow diffusely and polyclonally. At a later stage, nodules are formed; cells in these nodules are transformed monoclonally and proliferate aggressively. Nodular hyperplasia is thus likely to represent monoclonal parathyroid neoplasia (4, 7). Nodular hyperplastic glands characterizedly have been found to possess higher percentages of oxyphilic cells (8). The number of such cells happened to be extremely higher in the 13 immunopositive specimens of secondary hyperplasia in which these specific cells were often found to be int-2 immunoreactive; therefore, int-2 gene product expression appears to be involved in some step of the pathogenesis of parathyroid hyperplasia in chronic renal failure. Our findings imply that oxyphilic parathyroid cells are potentially responsive to FGF3, which is likely to participate in the proliferation of this cell subpopulation in diseased parathyroid glands. It is of interest that the increase of oxyphilic cells has already been correlated with the expression of another member of the FGF family, FGF2 (9); actually, nodules consisting of oxyphilic cells have been observed as assemblies of cells with marked emergence of FGF2. In general, the pathogenesis of oncocyctic transformation and thus of mitochondrial proliferation which characterizes oxyphilic cells is still unresolved (10) and the role of cytokines and growth factors is still unclear. Therefore, in addition to FGF3, the concerned activity of other growth factors, such as FGF2, that also control glandular growth, should be comparatively investigated.

We should point out that our normal samples were characterized by int-2 immunonegativity: this lack of immunopositivity is in parallel with the study of Lambert et al. (2) in which FGF3-amplified RT-PCR products were barely detectable or absent from normal parathyroid gland samples. More than one-half of the diseased tissues studied were found to be immunonegative for FGF3, like normal parathyroid tissue. This finding may imply that FGF3 plays a pathogenetic role only in a minority of parathyroid lesions. Nevertheless, the int-2 proto-oncogene product, FGF3, does demonstrate some differential expression between primary and secondary parathyroid disease states; this may indicate changes in capacity for response and growth requirements between normal, primary and secondary hyperplastic parathyroid tissues. There is, however, a possibility that the differential expression of FGF3 between primary and secondary parathyroid disease might be an epi-phenomenon since only 43.3% of patients with secondary HPT demonstrated FGF3 positivity; so, the significance of our observation needs further investigation in larger series and verification by other techniques.

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