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Familial multiple endocrine neoplasia type 1 (FMEN 1) is an autosomal dominant disorder characterized by tumors of the parathyroid glands, pancreatic islets, and anterior pituitary. Hyperplasia appears to be the typical histopathological lesion in FMEN 1 endocrine tumors. A circulating mitogen related to basic fibroblast growth factor was active on proliferation of clonal bovine and human parathyroid endothelial cells. Moreover, the FMEN 1 mitogen modulated differentiation of human parathyroid endothelial cell in vitro. All these facts suggested that an extrinsic factor was active on parathyroid endothelial cell growth and differentiation. The FMEN 1 gene maps to chromosome 11q13, and allelic loss in this region has been shown in FMEN 1 parathyroid and pancreatic islet tumors and rarely in anterior pituitary tumors. Together these results support the theory that FMEN 1 parathyroid clonal lesions can develop in the context of generalized hyperplasia. Similarly, in uremic hyperparathyroidism, where parathyroid hyperplasia is thought to be the primary lesion, loss of constitutional heterozygosity for chromosome 11 markers coexists in parathyroid tissue with a polyclonal pattern. Future efforts of scientists working on this genetic disorder will focus on the cloning of the FMEN 1 gene and the development of a suitable bioassay system to study its function. (Henry Ford Hosp Med J 1992;40:181-5)

During the past seven years, we have been working on the cellular biology of parathyroid tissue, with a particular interest in "hyperplastic" parathyroid tumors of patients affected by familial multiple endocrine neoplasia type 1 (FMEN 1), a disorder in which subjects develop not only hyperparathyroidism but also tumors of the pituitary glands and the endocrine pancreas (1).

This model became important for understanding the significant events in parathyroid tumor development. We review the progress of our laboratory and that of others in defining the molecular abnormalities of FMEN 1 endocrine tumors.

Mechanisms Underlying Tumor Development in FMEN 1 Syndrome

DNA restriction fragment length polymorphism studies of lymphocyte DNA have linked the germline genetic abnormality for FMEN 1 to the long arm of chromosome 11 region q13 (2). It was initially proposed (2) that the mechanism of the tumorigenesis via the chromosome 11 in FMEN 1 tumors might be similar to that of retinoblastoma (3) and of several other heritable cancers (4-6), where loss of a recessive tumor suppressor function is thought to play the initial role in tumor development. This theory is based on the "two hit" hypothesis of carcinogenesis developed by Knudson (3) for formation of retinoblastoma. In the familial form of retinoblastoma, the first "hit" is a germline genetic defect which is assumed to be phenotypically silent as long as the normal allele is functionally intact. Alternatively, the first hit in target tissues might not be silent but may

contribute to the generalized hyperplasia that precedes tumor development in familial cancers. The stimulus for this polyclonal hyperplastic growth may reflect an intrinsic abnormality of the target cell or else an extrinsic stimulus for the entire cell population. The second "hit" becomes manifest in occasional cells via loss of the remaining normal gene allele. Subsequent clonal tumor growth ensues from the cells that have suffered these two hits. This process is usually multifocal and each tumor comes from a different cell clone (7). This multiplicity reflects the initial genetic abnormality of the hyperplastic cell population.

Pathology of FMEN 1 Tumors

The tumors in FMEN 1 syndrome tend to be multiple in the involved organ. Moreover, even though FMEN 1 syndrome has a generalized hyperplastic phase (8), a complete spectrum of hyperplasia, microadenomas, adenomas, carcinomas, and carcinoid changes have been found on pathological examination. In FMEN 1 patients the major histologic abnormality is parathyroid hyperplasia. Gland size is markedly irregular in parathyroid hyperplasia and sometimes one or even two of the glands can be of normal weight. The nature of parathyroid lesions is usually
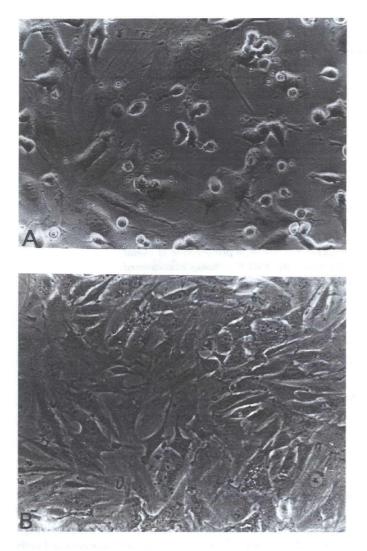
Fig 1—Primary cultures of FMEN 1 glucagonoma tissue. Cells were cultured for seven (A) and 30 (B) days in Coon's modified Ham's F12 medium containing 10% FCS. Original magnification X250.

that of chief cell hyperplasia. Nevertheless, a number of FMEN 1 patients have been reported with one or more parathyroid adenomas. However, proof of long-term cure of hyperparathyroidism following removal of a single parathyroid adenoma is rarely found in FMEN 1. These observations are suggestive of the coexistence of both forms of parathyroid disease, adenoma and hyperplasia, in the same genetic setting.

Similar to the lesions in the parathyroid glands, the abnormalities in the islet cells in the FMEN 1 syndrome consist of diffuse hyperplasia, affecting either a single type of cell or two or more different types. The endocrine pancreas in FMEN 1 syndrome displays single or multiple benign or malignant islet cell neoplasms.

In patients with the FMEN 1 syndrome, the anterior pituitary lesion is commonly an adenoma, generally solitary. However, multiple pituitary tumors with increased plasma prolactin and growth hormone concentrations have been reported. Histologi-

cally, pituitary lesions can consist of chromophobe, eosinophil, basophil, or mixed cell types.

Adrenal abnormalities in FMEN 1 syndrome generally consist of diffuse or nodular hyperplasia. Occasionally there may be a cortical adenoma or even carcinoma. In only a few cases does the clinical history indicate that the tumors have functioned.

The pattern of thyroid involvement is inconsistent. Thyroid adenoma, colloid goiter, thyroid carcinoma, and thyrotoxicosis have been reported.

Primary carcinoids of the bronchus and thymus do not differ from sporadic tumors, while carcinoids of the gastric mucosa are characterized by multicentricity. Indeed, hyperplasia of argyrophil endocrine cells is regularly noted in the hypertrophic oxyntic mucosa of Zollinger-Ellison patients, a finding generally attributed to the trophic action of gastrin on the main type of endocrine cell located in this mucosa, the so-called enterochromaffin-like (ECL) cell (9). In addition to ECL hyperplasia, tumors of this cell type having the morphological appearance of carcinoids arise frequently in patients with gastrinoma in the setting of FMEN 1 syndrome (10). These findings have been interpreted as an effect of gastrin upon genetically altered ECL cells to promote tumor growth.

Tumors of FMEN 1 patients have been extensively characterized by morphologists; however, only limited data are available on preneoplastic lesions developing in man. Transgenic mice bearing tumors of the anterior pituitary and pancreas have been proposed as useful tools to improve our understanding of heritable endocrine tumor disease in human pathology (11). The morphological steps of tumor development in these animals were analyzed and preneoplastic features were described. Hyperplasia together with dysplasia and dysplasia without hyperplasia were the lesions preceding tumor formation in these transgenic animals (11).

Cell Biology of FMEN 1 Tumors

Studies with cell cultures have begun to clarify some of the mechanisms which underlie growth dysfunction in the parathyroid tissue. This progress has been mainly due to the development of rat epithelial (12) and bovine endothelial (13) clonal cell lines from parathyroid tissue. The use of such in vitro models made it possible to demonstrate that a mitogen closely related to basic fibroblast growth factor (bFGF) is circulating at high concentrations in FMEN 1 syndrome (14,15). Bovine parathyroid endothelial cells represented a target for this growth factor, suggesting the possibility that a bFGF-like factor may act systemically on parathyroid angiogenesis and, therefore, indirectly on proliferation of the epithelial component of parathyroid tissue. We have also shown that bovine parathyroid endothelial cells release factor(s) able to stimulate proliferation of rat parathyroid epithelial cells and vice versa (16). These observations pointed to the importance of considering the stromal cells of the endocrine tissue as active components of the metabolic activities of the glands. Moreover, these findings suggested the possibility that a soluble endothelial cell growth factor may play a critical role in the growth of the parathyroid tissue in FMEN 1, supporting the hypothesis of a generalized polyclonal proliferation of

the glands. However, the main limitation of our studies was the lack of human cell models to evaluate the mechanisms which underlie cell proliferation and cell to cell interactions in the endocrine organs involved in the FMEN 1 syndrome. We have, therefore, developed both primary cultures as well as clonal cell lines from FMEN 1 endocrine tumors.

Primary cultures are an ideal system for in vitro studies on cell differentiation, reflecting well the in vivo tissue heterogeneity. However, this cellular heterogeneity is also the main cause of cell culture variability observed in multiple experiments. In fact, depending on the composition of the tissue of origin (ratio of stromal/epithelial components, etc.) and also on the tissue manipulation (enzyme digestion, adhesion properties, growth capability, etc.), great heterogeneity exists between one tissue preparation and the other. Moreover, primary cultures are destined to be overgrown by fibroblast-like cells (Fig 1 [A and B]) and therefore the epithelial component has a limited life span. Even though all of these limitations must be considered, preliminary studies using primary cultures of FMEN 1 endocrine tissue appear promising. Parathyroid cells obtained from FMEN 1 parathyroid tissue and cultured for seven days in the presence of 10% fetal calf serum (FCS) exhibited an epithelial morphology with a tendency to not proliferate (Fig 2A). The replacement of FCS with 10% MEN 1 plasma induced cell proliferation and spreading (Fig 2B). We have also developed primary cultures from pancreatic islet tumors of patients with FMEN 1 syndrome. In our experience pancreatic endocrine cells adhere to the plastic dishes (Fig 1A) or could be maintained as suspension cultures (Fig 3). Primary cultures of FMEN 1 pituitary tissue formed clusters reminiscent of the pituitary follicular cells (Fig 4).

Recently, we have cloned an endothelial (HPE) cell line from FMEN 1 parathyroid tissue. The fact that the bovine parathyroid endothelium was the target of the mitogenic effect of the bFGF-like mitogen (14,15) prompted further tests on the newly developed human cell line. Preliminary results showed clearly not only a potent proliferative effect of FMEN 1 plasma on HPE cell proliferation, but also an induction of endothelial differentiative features required in angiogenesis. The growth of new capillaries, whether in development, wound healing, tumor angiogenesis, arthritis, or diabetes, requires lumen formation and migration of endothelial cells. Confluent HPE cells cultured in the presence of 10% FMEN 1 plasma exhibited a peculiar "ring" shape (Fig 5). This morphological correlate may indicate a role of the FMEN 1 plasma in the lumen formation via vacuolization of the endothelial cells or else by cylindrical curvature of the cell membrane. When HPE cells were incubated in medium containing FCS they either remained quiescent or moved slowly. In response to different concentrations of FMEN 1 plasma, the rate of cell migration, measured as the surface of the tracks made by the cells as they migrated across gold-coated coverslips, increased proportionately.

Genetics of FMEN 1 Tumors

The mapping of the FMEN 1 gene to chromosome 11 and the implication of the inactivation of negative modulation in the
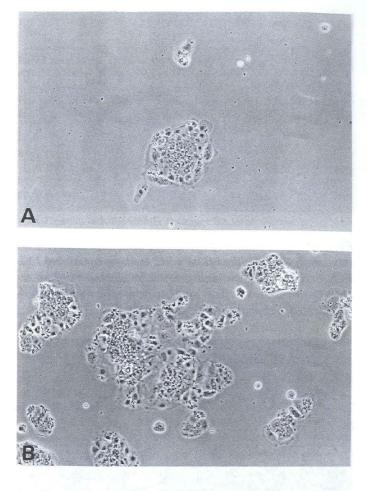

Fig 2—Primary cultures of FMEN 1 parathyroid tissue. Cells were cultured for seven days in Coon's modified Ham's F12 medium containing 10% FCS (A) and 10% FMEN 1 plasma (B). Original magnification X250.

pancreatic growth disturbance in FMEN 1 (2) prompted further studies on loss of constitutional heterozygosity in FMEN 1 endocrine tumors.

Loss of DNA alleles from one copy of the long arm of chromosome 11 region q13 has been found in both sporadic and FMEN 1 associated tumors (17-20). These results are in agreement with the two-mutational model. In sporadic tumors the loss of constitutional heterozygosity is indirect evidence of monoclonality. However, in familial hyperplasia such losses do not necessarily reflect a monoclonal composition of the tumor. Since these losses serve to eliminate the wild type allele at the disease locus, polyclonal and monoclonal tumors may give similar patterns of allele losses for chromosome 11 markers. Interestingly, allelic losses specific to chromosome 11 are typical of the larger FMEN 1-associated parathyroid lesions (17), suggesting that clonal outgrowth might arise in the context of generalized hyperplasia in FMEN 1 parathyroid tissue. In order to better understand the relationships between clonal lesions and hyperplasia in FMEN 1 parathyroid tumors, we have chosen the model of uremic hyperparathyroidism, believed to be hyperplastic in origin. We found loss of constitutional heterozygosity
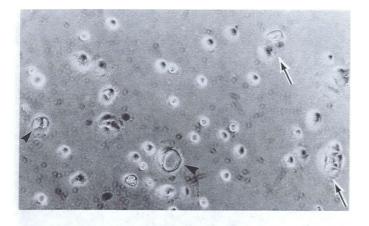
Fig 3—Primary cultures of FMEN 1 insulinoma. Cells were cultured in Coon's modified Ham's F12 medium containing 10% FCS. Several cells grew in suspension. Small clusters of adherent cells (arrows) as well as "ring" structures (arrowheads) are represented. Original magnification X250.
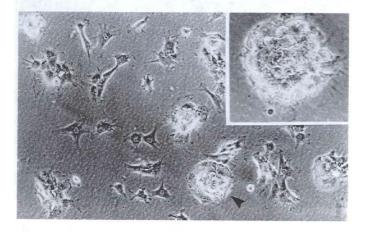

Fig 4—Primary cultures of FMEN 1 prolactinoma. Cells were cultured in Coon's modified Ham's F12 medium containing 10% FCS. Cells formed thick clusters (arrowhead and inset) resembling the follicle-like structures described in primary cultures of bovine pituitary cells. Original magnification X250 and X400 (inset).

for chromosome 11 markers in two of nine glands from five uremic patients (21). Interestingly, the two glands with allelic losses were significantly greater in mass than those without loss, supporting the theory that parathyroid adenoma can develop through overgrowth by a monoclonal tumor in the context of generalized hyperplasia. Parathyroid lesions have represented a rich opportunity to study molecular changes that occur during tumor development. However, the lack of correlation between standard histopathology (hyperplasia versus adenoma) and molecular biology (polyclonal versus monoclonal lesions) studies makes it desirable to prepare DNA from microscopic sections of tumors, where contiguous sections would be analyzed by histopathology.

Loss of constitutional heterozygosity has been found also in FMEN 1 pancreatic islet cell hyperplasia and in insulinomas

Fig 5—Clonal HPE cells maintained in culture for 20 days in Coon's modified Ham's F12 containing 10% FMEN 1 plasma. Cells exhibited a typical "ring" shape. Original magnification X400.

(2,22). In contrast to parathyroid tumors, many sporadic pancreatic islet neoplasms, even when malignant, do not develop through homozygous inactivation of the FMEN 1 gene (22).

The FMEN 1 pituitary lesions are most commonly prolactinomas, a tumor which is only rarely managed by surgical intervention. Therefore, studies of loss of heterozygosity in pituitary tumors have been comprised mainly of sporadic pituitary tumors (19). The observation of relatively few allelic losses on chromosome 11 in FMEN 1 or sporadic pituitary adenomas suggested that the recessive nature of the germline defect proposed for FMEN 1 parathyroid and pancreatic tissues might not be operative in pituitary tumors.

Finally, FMEN 1 bronchial carcinoid (22), FMEN 1 benign adrenal enlargements, and sporadic adrenocortical adenomas showed retained genotypes for markers flanking the FMEN 1 region.

An alternative explanation for the low incidence for reduction of homozygosity in a number of FMEN 1 endocrine tumors should be given serious consideration. The two allelic hit theory of Knudson (3) might not be operative in these tumors. However, it is also conceivable that failure to detect reduction of homozygosity might be due to a mechanism of inactivation of the normal allele function that does not involve the loss of a large segment of chromosome 11 (i.e., point mutations). Alternatively, subsequent genetic events necessary for frank neoplasm formation may involve not the opposite normal allele but other genetic loci, as suggested in Wilm's tumor (23,24).

Cytogenetics of FMEN 1 Tumors

One important line of evidence in support of a genetic origin for cancer is the frequent finding of chromosomal abnormalities in patients with specific types of tumor. Perhaps the best characterized of these are deletions involving the chromosome region 13q14, which are found in tumor tissue of 5% of cases of retinoblastoma with normal constitutional karyotypes. Initially, karyotype analysis in FMEN 1 patients resulted in an inconsis-

tent pattern of chromosomal instability in family members with the FMEN 1 syndrome (25). Recently, analysis of cultured lymphocytes and fibroblasts of FMEN 1 patients showed a number of chromosome abnormalities with no rearrangements involving chromosome 11 (26). Similarly, primary cultures of FMEN 1 pancreatic tumors revealed a number of chromosome abnormalities with several double minute chromosomes of variable size and no specific involvement of chromosome 11 (27).

Prospects

Clearly, elucidation of the genetic events that underlie the development of tumors in FMEN 1 will teach much about the range of molecular changes that can contribute to the early stages of human cancer. Cytogenetic, cellular, and molecular biology studies of FMEN 1 tumors will contribute to this understanding. The immediate goal of several laboratories is the cloning of the FMEN 1 gene. However, a biologic assay for the FMEN 1 gene function would be the ultimate proof of its suppressor nature, similarly to what developed for the retinoblastoma gene (28). This will allow introduction of the gene into cultured tumor cells that contain inactivated endogenous FMEN 1 genes. Therefore, the development of an assay system for the FMEN 1 gene function remains an important goal.

Acknowledgments

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