Genetic Mechanisms of Neoplasia in MEN 2

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Several possible mechanisms for the initiation and progression of tumors in multiple endocrine neoplasia type 2 (MEN 2) merit consideration. Localization of MEN2A to the pericentromeric area of chromosome 10 indicates the site of the initial mutagenic event but does not explain the tissue specificity observed. The consistency of tissue involvement within families, despite the variability between families, suggests that the tumors result from separate but contiguous tissue-specific genes arranged in a particular linear order. Linkage studies in MEN 2A and 2B families are compatible with this contiguous gene theory. Data suggest that Knudson's two-mutational-event theory is applicable in MEN 2, with cellular hyperplasia resulting from the initial heritable mutation. The second event could be a homozygous allelic mutation, but the lack of consistent loss of heterozygosity of chromosome 10 markers in tumors suggests other mechanisms. Observations in MEN 2 may be explained by the heritable chromosome 10 mutation causing hyperplasia, with the hyperplastic cells being converted to cancer cells by second mutations at any of many possible sites. Tumor progression probably involves subsequent events at other loci. These hypotheses may have important clinical implications. (Henry Ford Hosp Med J 1989;37:116-9)

Effects of the Inherited Mutation

In 1973, Wolfe and others (2) reported the new pathologic entity of C-cell hyperplasia in young members of MEN 2 families. At this time we suggested (3) that this C-cell hyperplasia represented the manifestation of the first or inherited mutational event preceding medullary thyroid cancer (MTC) in Knudson's (4) two-mutational-event theory for the initiation of neoplasia. Much later we realized that if C-cell hyperplasia were the result of the heritable mutational event, it should be present in every hereditary MTC case but in none of the sporadic cases. C-cell hyperplasia distal to the cancers has thus served as a differentiating pathologic feature between sporadic and hereditary MTC (5,6).

In studying the ages of onset for sporadic and hereditary palpable MTCs, we found that these age-of-onset curves fit Knudson's two-mutational model for the initiation of cancer (5). Using G6PD studies, Baylin and his group (7,8) found MTC and pheochromocytomas to be the result of final clonal mutations in hereditarily susceptible cells in MEN 2 patients, which also fits with Knudson's theory (4). The finding of adrenal medullary hyperplasia in MEN 2 patients by the Mayo Clinic (9) and the New England Medical Center (10) groups fits into this theory as well. Like C-cell hyperplasia (11), adrenal medullary hyperplasia is a visible manifestation of the heritable mutational event.

Tissue-Specific Effects of the Initial Mutation

Our collaborative studies with Tashjian (3,5,6) have provided the opportunity to observe the spectrum of manifestations in many MEN 2A families and a few MEN 2B (mucosal neuroma) families. We observed that the tissues involved are generally consistent within families, even though a variety of tumor involvement is noted between families. We have suggested that this consistency of tissue involvement within a family could be clinically useful in predicting those organs likely to be affected in an individual (12). In our largest MEN 2 family with 37 affected members, we have observed MTC and parathyroid tumors but no pheochromocytomas or adrenal medullary hyperplasia even in six affected members aged 51 to 67 or in three patients at autopsy. In this family we can operate on the thyroid or parathyroid glands without great concern for adrenal tumors. However, in families with the full spectrum of involvement, many members with calcitonin elevations had to have their pheochromocytomas removed before neck surgery could be performed.

Because the pattern of tumor involvement does breed true within the 12 MEN 2A and four MEN 2B families studied longest and most thoroughly, we have postulated that the different components of MEN 2 are the result of separate mutations that may be tightly linked or adjacent and which have tissue-specific functions (13). In 1986, Schmickel (14) coined the term contiguous gene syndrome, citing as examples the Prader-Willi and...
Table 1
Tumor Involvement in MEN 2 Kindreds and Linkage Results with Chromosome 10 Markers from the Literature (16-25)

<table>
<thead>
<tr>
<th>Linkage to Chromosome 10 Markers*</th>
<th>Max Lod Scores</th>
</tr>
</thead>
<tbody>
<tr>
<td>Parathyroid</td>
<td>MTC</td>
</tr>
</tbody>
</table>

**MEN 2A**
- + + + 23.76
- + + 2.5
- + 3.27
- + 3.88

**MEN 2B**
- + + + 4.33

* Nakamura et al (22) report evidence for tight linkage between MEN 2A and chromosome 10 markers in 27 MEN 2A families from 14 countries and no evidence for genetic heterogeneity. Yamamoto et al (25) found tight linkage to RBP3 in five Japanese families.

Table 2
MEN 2B Versus Chromosome 10 Markers

<table>
<thead>
<tr>
<th>Marker</th>
<th>0</th>
<th>0.001</th>
<th>0.05</th>
<th>0.1</th>
<th>0.2</th>
<th>0.3</th>
<th>Peak Lods</th>
<th>θ at Peak</th>
</tr>
</thead>
<tbody>
<tr>
<td>D10Z1</td>
<td>4.33</td>
<td>4.32</td>
<td>3.87</td>
<td>3.41</td>
<td>2.45</td>
<td>1.47</td>
<td>4.33</td>
<td>0</td>
</tr>
<tr>
<td>RBP3</td>
<td>-1.35</td>
<td>0.24</td>
<td>1.58</td>
<td>1.52</td>
<td>1.09</td>
<td>0.57</td>
<td>1.59</td>
<td>0.062</td>
</tr>
<tr>
<td>D10S15</td>
<td>(MCK2)</td>
<td>-0.72</td>
<td>1.11</td>
<td>2.45</td>
<td>2.38</td>
<td>1.91</td>
<td>1.29</td>
<td>2.46</td>
</tr>
<tr>
<td>D10S5</td>
<td>(-1.93</td>
<td>-0.32</td>
<td>1.09</td>
<td>1.10</td>
<td>0.82</td>
<td>0.45</td>
<td>1.12</td>
<td>0.075</td>
</tr>
</tbody>
</table>

DiGeorge syndromes, the aniridia-Wilms tumor association, and various beta-delta thalassemias. The pattern of overlap involvement in these 16 families is consistent with a particular linear order of the specific adjacent or contiguous genes—parathyroid, thyroid C-cells, adrenal medulla, and mucosal neuromas (13) (Fig 1). Experience with the retinoblastoma model suggests that the same genes involved in initiation and progression of the various MEN 2 tumors will be implicated in the events causing various sporadic endocrine tumors (15).

A prediction of this contiguous gene theory in MEN 2 is that studies should show linkage to the same general chromosomal area in the two-component (MTC, parathyroid) families as in the three-component (MTC, parathyroid, pheochromocytoma) families. The linkage studies of Simpson et al (16), which included our two-component S family and three-component W family, provided evidence for linkage to the same chromosome 10 markers. No definite linkage evidence has yet been reported to indicate differences in gene location in other two- or three-component MEN 2 families (Table 1) (16-25).

Another prediction of this theory is that the genes for MEN 2A and MEN 2B should be linked to markers on chromosome 10. Most MEN 2B cases are the result of new mutations. With much assistance, we have been able to study ten MEN 2B families in whom data and DNA are available for two or three generations (Table 2). These families have provided strong evidence for linkage of the MEN 2B gene to the chromosome 10 centromere marker (D10Z1) (26) with a lod score of 4.33 at a zero recombination level (24). The peak lod score for MEN 2B versus D10S15 (MCK2) was 2.46 at θ = 0.06, and the peak lod score for MEN 2B versus RBP3 was 1.59 at θ = 0.062. The lack of crossovers with D10Z1 and the crossover with RBP3 in the one family suggest that D10Z1 is either closer than RBP3 to MEN 2B or that D10Z1 and RBP3 are on opposite sides of the MEN 2B gene. Multipoint analysis in MEN 2B versus various chromosome 10 markers provides curves similar to those observed with MEN 2A and the same markers (22) (Fig 2).

A possible suggestion from the contiguous gene theory is that the tumors in MEN 2 are initiated by deletions of tumor-suppressor genes as in the retinoblastoma model. The action of these genes seems to be specific to certain tissues. However, no chromosome 10 deletion was visible in 23 MEN 2 families whose chromosomes we have studied by high-resolution chromosome techniques (27). Additionally, no consistent loss of heterozygosity of chromosome 10 markers has been found in MEN 2 tumors (28-32) as has been found with chromosome 13 markers in retinoblastomas (15).
DNA Markers in Tumors

Landsvater et al (29) found losses of chromosome 10 markers in only one of 42 tumors studied. Nelkin and others (30,31) have described losses of all informative chromosome 10 markers in two of 16 MTCs studied, with no losses in the pheochromocytoma of one of these patients or in the pheochromocytoma of that patient's sister. Loss of heterozygosity of chromosome 1 markers was reported by Mathew et al (28) in 1987, and alterations in a small area of the short arm of chromosome 1 was recently reported by Samaan et al (33). C. D. James (personal communication, 1989) from Cavenee's laboratory and Henry Ford Hospital has found loss of heterozygosity of a chromosome 17 restriction fragment length polymorphism (RFLP) in a pheochromocytoma of a patient whose MTC did not show the same loss. He has also found losses of heterozygosity of several RFLPs on chromosome 13 in one case of far advanced thyroid cancer and losses of a chromosome 22 RFLP and two chromosome 20 RFLPs in another far advanced case. Although this lack of consistent loss of heterozygosity of chromosome 10 markers suggests that the second event is not a loss of a large segment homologous to the chromosome bearing the inherited defect, a small deletion or point mutation in the normal allele has not been excluded. If the second event is a homologous allelic deletion, the losses of heterozygosity elsewhere are most likely changes associated with tumor progression.

Effects of Further Mutations

Nelkin et al (31) have suggested that oncogenesis in MEN 2 may not occur by the same recessive mechanism as outlined by Cavenee et al (15) for retinoblastoma. They (31) suggest a mechanism similar to that proposed for familial polyposis coli and colorectal tumors in which the mutation in the hereditary gene on chromosome 5 (34) results in hyperplastic cell growth (as do the genes in MEN 2) and subsequent events involving genes at other loci result in further progressive neoplastic changes. In colorectal tumor progression to more malignant stages, an increasing frequency of genetic alterations has been reported (35-38), especially in 5q, chromosomes 17 and 18, and in the ras gene (36). Multiple allelic losses have been found to be dispersed throughout the genome in many of the 56 colorectal tumors studied by the Vogelstein group (36-38). An explanation proposed (37) was that the heritable mutation included or was linked to a gene whose product affected neoplastic growth, and that inactivation of many different suppressor genes could have incremental effects on cell growth. In this theory only one of the parental alleles would need to be mutated to promote neoplasia, and individual changes would represent single components in a complex process of tumor evolution. This is similar to the mechanisms reported by James et al (39) of loss of alleles on one of the two number 10 chromosomes in the progression of glial tumors. Studies of MEN 2 tumors using markers from many chromosomal sites will undoubtedly provide evidence for mutations at various loci associated with tumor progression.

In suggesting possible mechanisms for MEN 2 tumors other than a homozygous allelic deletion, Nelkin et al (30,31) postulated a dominant second event occurring in hyperplastic tissue at another locus on either the same or a different chromosome, or recessive events at other loci. In 1973 we emphasized that, if C-cell hyperplasia were the manifestation of the initial heritable mutation, the agents causing the second event would need to be ubiquitous because of the high penetrance of the MEN 2 gene (3). Perhaps this level of penetrance could best be explained by the inherited chromosome 10 mutation causing both hyperplasia and an increased susceptibility to mutation and by the hyperplastic cells being converted to cancer cells by second mutations at any of many possible sites.

Clinical Implications

What we have suggested are some hypotheses as to mechanisms of neoplasia in the MEN 2 syndromes. Evidence favors the view that Knudson's two-mutational-event theory is applicable in the initiation of the various tumors in MEN 2. Knudson's theory has much supporting evidence; when it should cease to be known as theory and become Knudson's law should be only a matter of time. What are the possible clinical implications of Knudson's theory in MEN 2? Since the first mutational events are heritable mutations, the ages of onset and sizes of the various tumors should be quite variable depending on the time of the chance occurrence of the second mutational event. It is even possible that in some individuals the second event would, by chance, never occur in any of the hereditarily susceptible cells or that the additionally mutated cells might not survive, suggesting nonpenetration in those gene carriers. Easton et al (40) have emphasized the variability in age of penetrance of the gene based on clinical findings. However, it seems unlikely that true nonpenetration of the gene occurs very often, if at all, in an individual subjected early in life to periodic stimulated calcitonin screening procedures, since the C-cell hyperplasia, the manifestation of the first mutational event, should result at some time in elevated calcitonin levels even before the second event has occurred.

One clinical implication of this theory is that if it were possible to prevent the second mutational event from occurring in any of the predisposed cells, only the hyperplasia would occur. It seems impossible to maintain known carriers in a mutagen-free environment, although some epidemiologists have suggested a “Seven Day Adventist life-style” to decrease cancer risks: no alcohol or tobacco exposure, no meat, and many green-yellow vegetables daily (41). One factor physicians can control is exposure to known mutagenic chemicals as well as ionizing radiation from roentgenographic or isotopic studies except when risk-benefit ratios seem very low; perhaps the neck organs and adrenals should be protected during roentgenographic studies of these patients as is done with the gonads in individuals of childbearing age. Since MTCs (and other tumors of MEN 2A) are generally slow-growing and nonaggressive (42), perhaps the greatest benefit of protecting gene carriers from mutagenic exposure can be in limiting occurrence of the additional mutagenic events which are associated with tumor progression. Samaan et al (33) have provided evidence that radiation therapy is associated with decreased survival in MTC patients and that chemotherapy does not appear to increase survival.

These hypotheses or views on tumor mechanisms in MEN 2 are subject to testing and possible eventual verification or rejec-
tion. In his conclusion to *The Descent of Man*, Darwin (43) stated: "... false views, if supported by some evidence, do little harm, for everyone takes a salutary pleasure in proving their falseness; and when this is done, one path toward error is closed and the road to truth is often at the same time opened."

**Acknowledgment**

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**References**

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