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Nicholas Schulz,* Friedrich Propst,‡ Michael M. Rosenberg,§ R. Ilona Linnoila,§ Richard S. Paules,* Douglas Schulte,* and George F. Vande Woude*

We have previously described a neurological phenotype for transgenic mice carrying the c-Mos proto-oncogene. Pheochromocytomas and C-cell thyroid neoplasms occur in these transgenic lines in patterns that are similar to those seen in multiple endocrine neoplasia type 2 (MEN 2). Characterization of the pathological lesions via immunohistochemistry underscores similarities between MEN 2 and these transgenic mice. When transgenic mice that do not display the MEN 2 phenotype are crossed to a different background, the progeny display the MEN 2 phenotype. Thus the interaction of the background with the transgene is such that it can suppress tumor information. This observation bears special relevance to the human syndrome in that this model system may be used to study the question of penetrance of phenotype. (Henry Ford Hosp Med J 1992;40:307-11)

The role of oncogenes in the mechanism of carcinogenesis has been explored through the use of transgenic animals (1). In these systems the site of tumor formation is usually directed by the promoter sequence used to express the oncogene. For example, when a mouse mammary tumor virus promoter is linked to the c-myc oncogene, an increased incidence of mammary tumors is observed in stochastic fashion (2). In contrast, when an IgG enhancer is used with c-myc, lymphoid neoplasms develop (3). An insulin promoter linked to the SV40 large T antigen produces pancreatic β cell tumors (4). Thus far, however, these model systems have not yielded a pattern of tumors resembling that seen in familial neoplasia syndromes such as multiple endocrine neoplasia type 2 (MEN 2).

Transgenic mice carrying a constitutively activated mos proto-oncogene display neurological phenotypes manifested by behavioral abnormalities, including circling, ataxia, head tilt, and bobbing (5,6). Histopathology reveals neuronal and axonal degeneration and gliosis (6). In three of four Mos transgenic lines displaying the severe neurological defect, greater than 60% of the animals develop multicentric pheochromocytomas and/or medullary (C-cell) thyroid neoplasms after long latent periods. Moreover, the tumor histologies and patterns of presentation within these lines bear remarkable resemblance to those observed in human MEN 2 (7-9). The tumor presentation pattern varies in a line-dependent manner and phenotype penetrance is background dependent.

Materials and Methods

Generation of transgenic lines
Transgenic mice were generated as previously described (5,6). Murine Mos cDNA clones linked to the Moloney virus long terminal repeat (LTR) were microinjected into fertilized eggs from either FVB/N or B6C3F2 mice (10).

Histopathology
Formalin fixed tissues were embedded in paraffin, cut in five micron sections, and stained with hematoxylin and eosin. An improved immunoglobulin-bridge technique was used for the immunohistochemical staining (11).

RNA expression analysis
Total cellular RNA was prepared as described (12). In situ hybridization analysis was carried out using S35 CTP on paraformaldehyde-fixed frozen sections. S1 hybridization was carried out as previously described (5,6).

Results

Histopathology
Four of our Mos transgenic lines display aberrant lens-fiber differentiation as well as brain lesions consisting of neuronal and axonal degeneration and gliosis (5,6). Despite this, these animals live for periods of longer than one year. Examination of tissues at 8 months of age reveals pheochromocytomas and/or medullary (C-cell) thyroid carcinomas in three of four of these Mos transgenic lines. Thus, we find that 58% of line 1 transgenic Mos mice develop bilateral adrenal gland pheochromocytomas (Table 1). Multifocal pheochromocytomas are ob- Submitted for publication: November 18, 1991. Accepted for publication: January 27, 1992.

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Table 1
Tumor Incidence in Transgenic and Control Mice

<table>
<thead>
<tr>
<th>Transgenic Mouse Line</th>
<th>Background</th>
<th>Number of Animals</th>
<th>Pheochromocytomas Only</th>
<th>Medullary Thyroid Neoplasia Only</th>
<th>Both</th>
<th>Total</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>FVB/N</td>
<td>154 (79/75*)</td>
<td>58% (39/51)</td>
<td>0% (0/0)</td>
<td>4% (1/5)</td>
<td>62% (40/56)</td>
</tr>
<tr>
<td>2</td>
<td>FVB/N</td>
<td>83 (39/44)</td>
<td>0% (0/0)</td>
<td>63% (19/33)</td>
<td>4% (0/3)</td>
<td>66% (19/36)</td>
</tr>
<tr>
<td>3</td>
<td>FVB/N</td>
<td>89 (48/41)</td>
<td>2% (1/1)</td>
<td>0% (0/0)</td>
<td>0% (0/0)</td>
<td>2% (1/1)</td>
</tr>
<tr>
<td>4</td>
<td>B6C3</td>
<td>62 (29/33)</td>
<td>23% (7/7)</td>
<td>13% (4/4)</td>
<td>32% (8/12)</td>
<td>68% (19/23)</td>
</tr>
<tr>
<td>pLSM-1.2</td>
<td>B6C3</td>
<td>37 (17/20)</td>
<td>0% (0/0)</td>
<td>0% (0/0)</td>
<td>0% (0/0)</td>
<td>0% (0/0)</td>
</tr>
<tr>
<td>Control</td>
<td>FVB/N</td>
<td>46 (21/25)</td>
<td>2% (1/0)</td>
<td>0% (0/0)</td>
<td>0% (0/0)</td>
<td>2% (1/0)</td>
</tr>
</tbody>
</table>

*Actual number of males/females.

Fig 1—Multiple pheochromocytomas within one adrenal (hematoxylin-eosin stain).

In human pheochromocytomas, one observes high level expression of markers of neuroendocrine differentiation (11). The tumors in the transgenic Mos animals express some of the neuroendocrine markers that are detected in human tumors, such as neuron-specific enolase (NSE) (11). Pheochromocytomas from a line 1 mouse reveal a variable NSE nodular staining pattern, with lesions staining from intense to not detectable (Fig 4). The spontaneous pheochromocytoma from a normal FVB/N mouse as well as unaffected adrenal glands from line 2 and 3 mice show diffuse staining for NSE (data not shown). Calcitonin (CT) staining of thyroid tumors shows multiple nests of CT-positive cells with a staining pattern indistinguishable from that observed in human tumors (13).

Immunohistochemistry
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It is most common for MEN 2 patients to develop C-cell thyroid neoplasms as well as pheochromocytomas, the same pattern of tumor development observed in the line 4 transgenic mice. Some kindreds, however, develop MTCs without develop-
oping pheochromocytomas. This is a pattern observed in line 2 mice. Since the clinical presentation of pheochromocytomas without MTC is rare in MEN 2, we examined the thyroids of line 1 mice for the presence of C-cell hyperplasia, via CT staining. This indeed reveals the precursor lesion of MTC (13).

**Transgenic mice carrying a kinase deficient Mos**

In order to investigate the effect that the LTR promoter had on tissue specificity and on tumorigenesis, we generated two lines of transgenic mice (pLSM-1, pLSM-2 [Table 1]) that contained the identical transgene with the exception of a 350 bp deletion in the 5' region. This deletes the ATP binding domain and renders the protein produced kinase deficient. As can be seen in Table 1, no tumor formation was found. Additionally, when we examined tissues in these mouse lines for expression of the transgene via Northern hybridization analysis, we found high level expression in muscle and kidney and undetectable level in brain, adrenal, and thyroid (data not shown).

**Effect of background on phenotype**

The tumor presentation pattern was line-dependent (Table 1) suggesting that the transgene integration site or the background of the animal played an important role. To evaluate this, the three Mos transgenic FVB/N lines were crossed with BALB/c mice (Table 2). The same disease phenotype was seen in the F1 animals of the first two lines. The F1 animals of the line 3 X BALB/c cross, however, display a high frequency of both pheochromocytomas and medullary thyroid C-cell carcinomas even though neither neoplasm is found in the parental line. Thus, it is the integration site and/or background which affects the penetrance of the transgene on phenotype.

Of interest are preliminary results involving line 3 crosses to yet another background. When C3H mice are crossed to line 3 transgenics, the phenotype observed is similar to that seen in hu-
m Associated with Human MEN 2

The differences in tumor presentation patterns in the various lines may be attributable to variations in the level of transgene expression or may reflect earlier activation of expression in the target organ of the tumor-bearing lines. This could be due to the transgene integration site (15-19) or the genetic background used. As illustrated in Table 2, the interaction of transgene with genetic background can influence the penetrance of the phenotype. The tumor phenotypes of the F1 progeny of the FVB/N Mos transgenic lines crossed with BALB/c may change because of alterations in transgene expression. Alternatively, it may be that a second genetic event (as suggested by the long latent period before disease is observed in these animals) may be suppressed in line 3 or predisposed to in the F1 progeny. These transgenic lines provide a valuable opportunity to study the genetic and molecular basis of tumor induction and may help in elucidating the mechanism of tissue targeting in human syndromes.

MEN 2 has been assigned to chromosome 10 by linkage studies (20-22), while Mos is located on human chromosome 8 (23). However, the marked similarity between the pathologies of MEN 2 and the Mos transgenic mice suggests that the Mos-LTR transgene may function in the same pathway that gives rise to MEN 2. In that regard, we have examined nine tumors from MEN 2 patients and find only one positive for Mos expression (data not shown). C-mos transcripts were reported as being non-detectable in a larger number of MEN 2 tumors by Moley et al (24).

The c-Mos product has been identified as an essential component of cytostatic factor (CSF) (25), an activity believed to be responsible for arresting mature oocytes at metaphase II and for stabilizing maturation-promoting factor (MPF). MPF is considered a universal regulator of meiosis and mitosis in eukaryotes (26-28). We have postulated that the transformed phenotype induced by the Mos product may be due to the expression of M-phase activities during interphase. Due to its downstream M-phase function, Mos may be a proximal effector of the transformed phenotype by comprising earlier signal transduction controls. Mos is expressed in adult (29-31) and embryonal brain (data not shown), and its constitutive expression in the transgenic animals in cells of neural crest origin may be responsible for tumor formation in the adrenal medulla and thyroid (unpublished data, not shown). The long latent period for tumor formation in these mice suggests that there are multiple steps in neoplastic progression, perhaps some of which are related to the chromosomal loci associated with human MEN 2.

Acknowledgments

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*Actual number of males/females.
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References