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Patterns of Neoplasia in c-Mos Transgenic Mice and Their Relevance to Multiple Endocrine Neoplasia

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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 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 S^{35} 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-

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Table 1
Tumor Incidence in Transgenic and Control Mice

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

*Actual number of males/females.

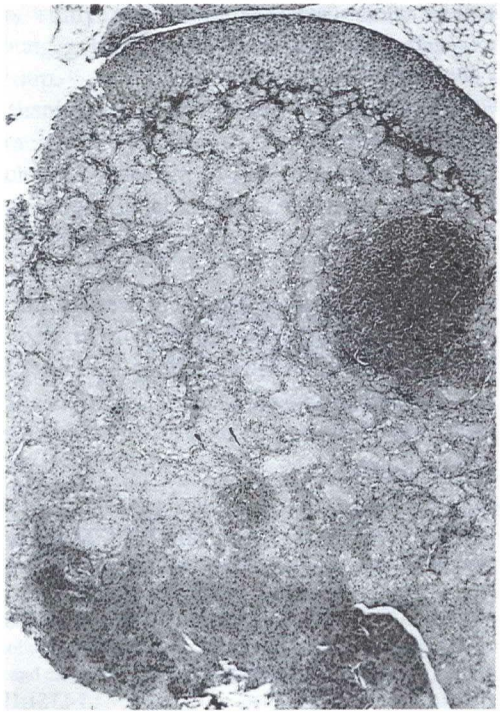

Fig 1—Multiple pheochromocytomas within one adrenal (hematoxylin-eosin stain).

served within one adrenal gland, or the entire gland can be replaced by tumor (Fig 1). Line 2 Mos transgenic mice display a 62% incidence of multifocal C-cell hyperplasia of the thyroid and frank medullary thyroid carcinoma (MTC) (Table 1). Like the pheochromocytomas of the line 1 animals, the thyroid lesions of the line 2 animals are multifocal and the tumors are bilateral, indicating that they also arise in a polyclonal fashion (Fig 2). Line 4 Mos transgenic animals develop multifocal pheochromocytomas as well as medullary thyroid neoplasms, with animals quite often having both types of tumors (Table 1).

In contrast to lines 1, 2, and 4 mice, the line 3 Mos transgenic mice fail to develop pheochromocytomas or C-cell thyroid neoplasms above the sporadic background level observed in the nontransgenic control animals (Table 1), even though their lens-fiber development defect and neuropathology are indistinguishable from the other lines (5,6).

RNA expression studies

To demonstrate that the tumors observed in these animals were related to Mos expression, we examined the adrenal and thyroid tumors from line 4 Mos transgenic animals for Mos RNA expression by Northern analysis. We found that the adrenal and thyroid tumors, like the affected brains, express high levels of Mos RNA (13). The unaffected organs, like liver and kidney, however, are negative by Northern analysis, and the tissues from nontransgenic control litter mates show little or no expression of Mos RNA (13). When the thyroid tumors are examined via in situ hybridization for mos expression, one sees high level expression of the transgene specifically over areas of tumor involvement (Fig 3).

To investigate whether c-mos is expressed in tissue derived from human MEN 2 tumors, we carried out S1 nuclease protection assays on both pheochromocytomas and MTCs. Of nine tumors tested, only one showed low level mos expression. Furthermore, no detectable expression of c-mos was found in the MZCRC, TT, and 6-23 cell lines, all of which were initially derived from human C-cell thyroid cancers (data not shown).

Immunohistochemistry

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).

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 devel-

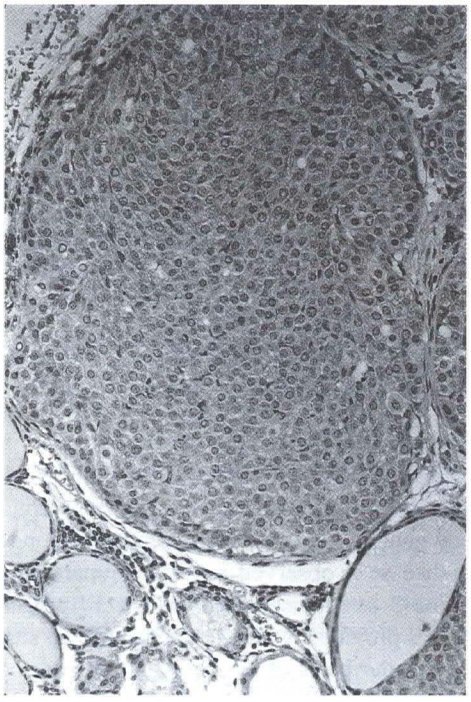

Fig 2—Medullary thryoid carcinoma (hematoxylin-eosin stain).

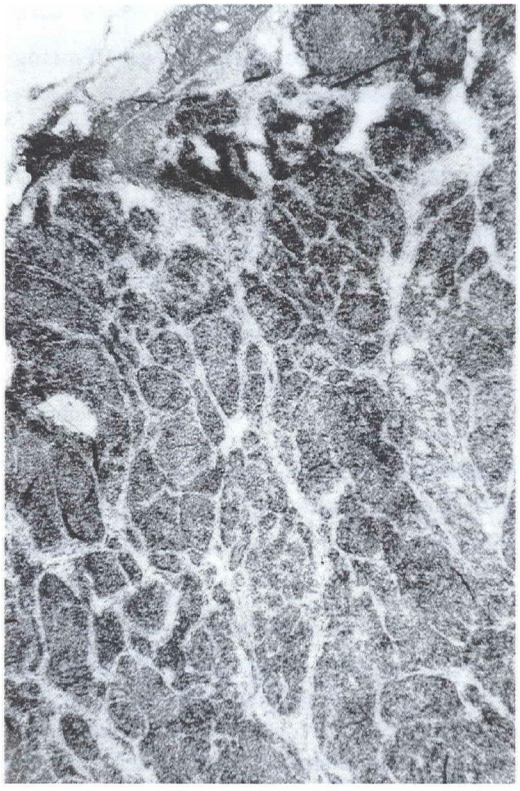

Fig 3—In situ hybridization of medullary thyroid carcinoma. Dark-black grains represent signal from S^{35}-labeled mos-probe.

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 F_1 animals of the first two lines. The F_1 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.

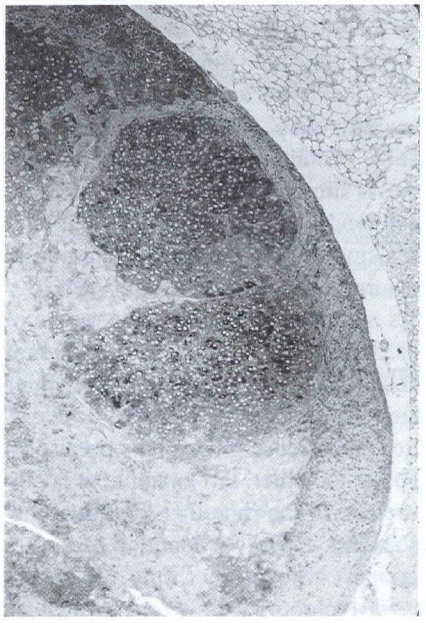

Fig 4—Immunohistochemical staining of multiple pheochromocytomas exhibiting variable staining with neuron-specific enolase.

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-

Table 2
Tumor Incidence in Offspring of Transgenic Mice Crossed to BALB/c Mice

Cross	Number of Animals	Percentage of Mice With Tumors: Pheochromocytomas Only	Medullary Thyroid Neoplasia Only	Both	Total
1 × BALB/c	49 (21/29*)	51% (12/13)	2% (0/1)	12% (1/5)	65% (13/19)
2 × BALB/c	31 (16/15)	0% (0/0)	52% (7/9)	16% (1/4)	68% (8/13)
3 × BALB/c	15 (7/8)	7% (1/0)	33% (3/2)	20% (1/2)	60% (5/4)

*Actual number of males/females.

mans with the MEN 1 syndrome with a high incidence of pituitary adenomas and pancreatic islet cell hyperplasia being seen (unpublished data).

Discussion

We have previously shown that extensive neuropathological changes such as gliosis and axonal degeneration are observed in mouse transgenic lines on two backgrounds harboring the LTR-Mos allele (5,6). Here we show a high incidence of pheochromocytomas and/or medullary thyroid neoplasms (62% to 68%) in three of the four Mos transgenic lines after approximately 8 months of age. Although all lines display similar lens cell and neurological defects, the frequency and type of tumor vary (Table 1). Thus, animals from lines 1 and 2 display either pheochromocytomas or medullary thyroid neoplasms, respectively, with few animals developing both tumors. In contrast, the line 4 mice present either tumor type and often develop both pheochromocytomas and thyroid C-cell carcinomas.

The variation of tumor presentation pattern between the transgenic lines is similar to that observed in humans with MEN 2. In the human syndrome, the tumor presentation pattern is consistent within a kindred (11). The most commonly observed pattern of tumor formation in humans with MEN 2 is the presence of both MTCs and pheochromocytomas, as we observe in the line 4 animals. Staining the thyroids of line 1 animals for CT revealed evidence for C-cell hyperplasia, the precursor lesion of MTC. The study of these two lines could reveal mechanisms whereby there is preference given to the predisposition towards one tumor type or the other. For example, it is possible that the differences are due to the time that the transgene is expressed during development. A far less common form of MEN 2 is exhibited by patients who present only MTCs. These patients never develop pheochromocytomas (14), which is similar to the presentation observed in the line 2 Mos transgenic animals.

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 F_1 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 F_1 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 nondetectable 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.

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