Targeted Expression of Human MYCN Selectively Causes Pancreatic Neuroendocrine Tumors in Transgenic Zebrafish

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ABSTRACT

The zebrafish model organism has been used extensively for studies of genetic pathways in development, indicating its potential applicability to cancer. Here we show that targeted expression of MYCN in cells of the pancreatic islet induces neuroendocrine carcinoma. Four transgenic fish developed abdominal tumors between 4 and 6 months of age, and histologic analysis revealed lobulated arrangements of neoplastic cells with expression of the MYCN transgene. The tumors also expressed insulin mRNA, and pancreatic exocrine cells and ducts were identified within the neoplasms, indicating a pancreatic origin for the tumor. Transmission electron microscopy revealed cytoplasmic, endocrine-dense core granules, analogous to those found in human neuroendocrine tumors. Our studies establish a zebrafish transgenic model of pancreatic neuroendocrine carcinoma, setting the stage to evaluate molecular pathways downstream of MYCN in this vertebrate forward genetic model system.

INTRODUCTION

The zebrafish system has been broadly used for elucidating genetic pathways in development (1–4). The zebrafish has also been shown to be a relevant vertebrate system to model human cancer, displaying many similarities in tumorigenic pathways, despite the evolutionary divergence of mammals and fish more than 300 million years ago (5, 6). The zebrafish (whose genome contains cell-cycle genes, tumor suppressor genes, and oncogenes found in humans and other mammals) has enormous potential as a vertebrate system in which to identify novel molecular pathways of oncogenesis, especially because zebrafish are prone to develop tumors (5, 7–11). Zebrafish have also played an important role as a vertebrate model system in carcinogenesis to determine environmental effects, genetic susceptibility, and environmental-genomic interactions (5, 12–14).

Recently, our laboratory has developed the first transgenic model of leukemia in the zebrafish, by expressing the murine Myc gene in developing thyocytes, under the control of the Rag-2 promoter (6). Given the proven utility of the zebrafish system for forward genetic screens to dissect developmental pathways (1–4), it should be possible to find modifier genes that either accelerate or reduce the rate of onset of leukemia in these transgenic lines. The zebrafish system is also conducive to high-throughput target validation and drug screening, providing a crucial link between high-throughput in vitro assays and in vivo disease models (15).

MYC, MYCN, and MYCL are the three members of the Myc oncoprotein family with well-established roles in the pathogenesis of many human neoplastic diseases (16). MYCN, for example, is amplified and misexpressed in a variety of different tumors including ~25% of childhood neuroblastomas, where it signifies an adverse prognosis (17, 18), as well as in tumors of neuroectodermal origin, including medulloblastoma, retinoblastoma, astrocytoma, glioblastoma, rhabdomyosarcoma, and small-cell lung cancer (19–23).

In this study, we generated transgenic zebrafish by targeted expression of the human MYCN transgene under the control of a promoter that targets gene expression in pancreatic neuroendocrine β cells as well as in muscle cells and neurons. These transgenic fish selectively developed pancreatic β-cell tumors that express insulin mRNA and histologically resemble human pancreatic neuroendocrine carcinomas.

MATERIALS AND METHODS

Cloning of Zebrafish myod Gene (z-myod) Genomic DNA, Construction of Plasmids, and Microinjection of DNA into Zebrafish Embryos. A z-myod genomic PAC clone was isolated by screening a zebrafish PAC library with a z-myod cDNA probe. A 6-kb promoter fragment was then isolated from the z-myod PAC with ApaI and EcoR V and cloned into a pSK+ vector. The z-myod-EGFP construct was made by inserting the EGFP in frame with the ATG codon of the z-myod gene. The 258-bp core enhancer of the human MYOD gene (24) was cloned by PCR and then inserted at the 5’ end of the z-myod promoter construct (zmyod-EGFP, Fig. 1A) to make the core-zmyod–EGFP construct (Fig. 2A). The z-myod–MYCN and core-zmyod–MYCN constructs were generated by replacing the EGFP with the MYCN cDNA in the zmyod-EGFP and core-zmyod–EGFP constructs. The DNA for injection was released from vector by Pronase digestion and then purified from the gel with a QIAEX II Gel Purification Kit (Qiagen Inc., Valencia, CA). The F0 mosaic fish were generated by injecting the linearized transgenes (100 ng/μl) into the cytoplasm of fertilized embryos at the one-cell stage.

Fish Maintenance and Embryonic Staging. Zebrafish were maintained and developmentally staged according to Westerfield et al. (25). Briefly, wild-type fish were mated, and the embryos were collected and grown in E3 medium (5 mmol/L NaCl, 0.17 mmol/L KCl, 0.4 mmol/L CaCl₂, and 0.16 mmol/L MgSO₄) at 28.5°C. Between 18 and 24 hours post-fertilization (hpf), embryos were transferred to E3 medium containing 0.003% 1-phenyl-2-thiourea to inhibit pigment formation and to prolong their optical transparency. Embryos were fixed at various stages of development in 4% paraformaldehyde at 4°C overnight. Fixed embryos were washed in PBS with 0.1% Tween 20 and then stained in methanol at −20°C.

Whole-Mount In situ Hybridization. Digoxigenin- and fluorescein-labeled RNA probes were transcribed from linear cDNA constructs according to the manufacturer’s instructions (Roche Molecular Biochemicals, Indianapolis, IN). Probes for zebrafish insulin, glucagon, and somatostatin were kindly provided by Dr. F. Argenton (Department of Biology, University of Padova, Padova, Italy). Probes were isolated by reverse transcription-PCR with primers based on the sequences in the National Center for Biotechnology Information database for chromogranin A (NM025152; primers: forward, 5’ AACACCCAG-CACGCCCTAATG; and reverse, 5’ AGTCCTTTAGGGAGGCTGGTC) and synaptophysin (NM958377; primers: forward, 5’ CCAGGTTAGCAGCTGTTTTG; and reverse, 5’ GCTGTTAGCCCAAGTACAGG). Whole-mount in situ hybridization assays were done as described previously (26).

Paraffin Embedding and Sectioning. Euthanized fish were placed in 4% paraformaldehyde at 4°C for 4 days and then transferred to 0.25 mol/L EDTA (pH 8.0) for no less than 2 days. The fish were then dehydrated in alcohol, cleared in xylene, and infiltrated with paraffin. Tissue sections (4-μm thick) from paraffin-embedded tissue blocks were placed on charged slides, depar-
affinized in xylene, rehydrated through graded alcohol solutions, and stained with hematoxylin/eosin (H&E).

Immunohistochemistry. Immunohistochemical studies for green fluorescent protein (GFP) were done as described previously (6). Immunofluorescence for insulin was done on 10-μm frozen sections with anti-insulin K36aC10 (1:1,000; Sigma, St. Louis, MO), antiglucagon (1:100; Chemicon, Temecula, CA), and Cy3-conjugated antimouse IgG was used as secondary antibody. Confocal images were taken on a Zeiss LSM 510 microscope.

Electron Microscopy. Tumor tissue was fixed in a glutaraldehyde/paraformaldehyde mixture (2.5% glutaraldehyde; 2% paraformaldehyde) in 0.1 mol/L sodium cacodylate buffer (pH 7.4). After a brief rinse in 5% sucrose in 0.1 mol/L sodium cacodylate buffer (pH 7.4), tissues were post-fixed in osmium-S-collidine solution [1.33% osmium tetroxide in 0.066 mol/L S-collidine buffer (pH 7.4)] for 2 hours. The tissue was then dehydrated in graded EtOH solutions, cleared in propylene oxide, and infiltrated first in a 1:1 mixture of propylene oxide and Poly/Bed mixture for 60 minutes, followed by infiltration in undiluted Poly/Bed for 30 minutes. Semithin sections (1.0 μm) were stained with alkaline toluidine blue, and ultrathin sections were cut on an ultramicrotome, picked up on 200 mesh copper grids, and stained on the grid with saturated uranyl acetate solution for 15 minutes and 0.1 to 0.4% of lead citrate for 45 seconds. The grids were examined in a Jeol JEM 1010 electron microscope at 80 kV acceleration voltage. Images were recorded with an AMT digital camera.

RESULTS

Analysis of Zebrafish z-myod Promoter Sequences In vivo. In hopes of generating transgenic lines that express transgenes in muscle cell progenitors, we cloned 6.0 kb of genomic sequence upstream of the initiator codon of z-myod and fused these sequences to the gene encoding enhanced green fluorescence protein (EGFP; Fig. 1A). This transgene was microinjected into the cytoplasm of fifty fertilized zebrafish embryos (one-cell stage) to generate F0 mosaic fish. The F1 progeny of one of these fish expressed EGFP during embryogenesis, indicating germline transmission of the transgene. In this stable line, EGFP was expressed primarily in the interneurons of the hindbrain and spinal cord and lacked expression in the skeletal musculature (Fig. 1, B–E), indicating that the genomic sequences tested had promoter activity but lacked sequences required for expression in developing muscle cells. EGFP was also ectopically expressed in islet cells of the pancreas (Fig. 1, D–K).

![Figure 1](https://example.com/fig1.png)

Fig. 1. EGFP expression in the z-myod-EGFP transgenic line. A. The z-myod-EGFP transgenic construct was made by fusing the EGFP coding sequence to a 6-kb promoter fragment of the z-myod gene. Zebrafish embryos microinjected at the one-cell stage began expressing EGFP as early as 6 hpf (data not shown). B. At 30 hpf, EGFP is expressed in the hindbrain (arrowhead) and notochord (arrow). C. At 48 hpf, EGFP is strongly expressed in the hindbrain (arrowhead), notochord (arrow), and neurons in the spinal cord (open arrowhead). D and E. EGFP is expressed in pancreatic islet cells (arrow) at 5 days post-fertilization (dpf), as shown in dorsolateral and dorsal views, respectively. Expression in the hindbrain and developing pectoral fins is indicated (open arrowhead). F–H, sagittal frozen section through the pancreatic islet of 5 dpf zebrafish (400×). F, bright field. G, EGFP fluorescence. H, merged view [exocrine cells (e) and islet cells (i) are indicated]. I–K, paraffin-embedded section through the pancreatic islet of the adult zebrafish. I, H&E stain. J, mRNA in situ hybridization with an insulin antisense riboprobe. K, immunohistochemical staining with an anti-GFP antibody (dark brown cells, arrows). Pancreatic islet cells (arrows) and exocrine cells (e) are indicated. (hb, hindbrain).

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for expression in muscle cells (24, 27, 28). Rather than search for an
analogous element in the zebrafish gene, we cloned the human MYOD
258-bp core enhancer upstream of the 6.0-kb zebrafish promoter
sequence generating a construct, core-zmyod–EGFP (Fig. 2A). Eighty
zebrafish embryos were injected with this construct, grown to adult-
hood, and the offspring of two of these fish were found to express
EGFP, indicating germline transmission of the transgene. These lines
showed EGFP expression in the muscle cells of the somites at 24 hpf
and in the jaw muscles beginning at 72 hpf. The pattern of transgene
expression in muscle cells with this construct was nearly identical to
the pattern of expression of the endogenous z-myod gene, as detected
by whole-mount in situ hybridization assay with an antisense z-myod
riboprobe (Fig. 2, B–H). However, expression levels of the transgene
in the hindbrain, spinal cord, and pancreatic islet were similar to those
previously obtained with the 6.0-kb zebrafish genomic fragment,
indicating that although the human MyoD core enhancer sequences
were able to activate transgene expression by muscle cells, the ectopic
expression in neural cells, and the pancreas was not suppressed. (Fig.
2, D and I–L). Although EGFP expression is most prominent in the
exocrine pancreas, expression was also evident in a subset of the
dorsal views of the zebrafish in panels B and C, respectively. F, ventral view of the head at 5 dpf, showing high levels of expression of EGFP in the
jaw muscles. G, ventral view at 5 dpf, showing endogenous z-myod expression, by mRNA in situ hybridization. H, schematic diagram showing the
jaw muscles evident in F and G. I, lateral view of EGFP expression in the head and upper abdomen of a 5 dpf zebrafish (arrow, pancreatic islet). J–L,
frozen cross section through the pancreas at 5 dpf. J, bright-field view (e, exocrine cells; i, islet are indicated; R, right side). K, EGFP fluorescence
(superficial slow muscle, arrow); and L, merged view of sections cut along the plane shown by the dashed lines in panel I. M–O, colocalization of
insulin and EGFP in islet cells. M, immunofluorescence of insulin in an islet cell (red cells). N, EGFP fluorescence. O, merged view, arrows indicate a
cell expressing insulin and EGFP. P–R, colocalization of glucagon and EGFP in islet cells. P, immunofluorescence analysis of glucagon expression
(red cells). Q, EGFP fluorescence. R, merged view: arrows indicate a cell expressing glucagon and EGFP. (ima, intermandibularis anterior; imp, inter-
mandibularis posterior; am, adductor mandibulae; hh, hypohyoidus; sh, sternothoideus)
The remaining four transgenic fish developed abdominal tumors between 4 and 6 months of age (Fig. 4, A–D). The tumors were anatomically distinct from the liver, and histologic analysis of paraffin sections revealed an encapsulated, lobulated, or nested arrangement of neoplastic-appearing cells. These cells were polygonal in shape and possessed a high nuclear/cytoplasmic ratio, distinct nucleoli and small to moderate amounts of eosinophilic cytoplasm. Delicate fibrovascular stromal tissue gave some tumors a clearly nested or nodular appearance. The mitotic rate was low, and single cell necrosis was minimal; however, the expansile tumors were locally invasive into adjacent nonpancreatic tissue (Fig. 4H). Small residual aggregates of normal-appearing pancreatic exocrine cells and ducts were identified within the neoplasm, which together with the histologic features, suggested a pancreatic origin for the tumor (Fig. 4, E–G). To aid in confirming this histologic evaluation, transmission electron microscopy was done on tissues from two tumors. In one of the two tumors, well-granulated tumor cells were identified with solid, round to

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### Table 1  Characteristics of tumors in MYCN mosaic transgenic zebrafish

<table>
<thead>
<tr>
<th>Case</th>
<th>Promoter</th>
<th>Oncogene</th>
<th>Age</th>
<th>Site</th>
<th>MYCN chr</th>
<th>syn</th>
<th>ins</th>
<th>glu</th>
<th>soma</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>z-myodD</td>
<td>MYCN</td>
<td>5 months</td>
<td>abd</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>2</td>
<td>z-myodD</td>
<td>MYCN</td>
<td>6 months</td>
<td>abd</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
<td>–</td>
</tr>
<tr>
<td>3</td>
<td>core-z-myodD</td>
<td>MYCN</td>
<td>3 months</td>
<td>head</td>
<td>+</td>
<td>–</td>
<td>+</td>
<td>–</td>
<td>–</td>
</tr>
<tr>
<td>4</td>
<td>core-z-myodD</td>
<td>MYCN</td>
<td>5 months</td>
<td>abd</td>
<td>+</td>
<td>–</td>
<td>–</td>
<td>+</td>
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<tr>
<td>5</td>
<td>core-z-myodD</td>
<td>MYCN</td>
<td>6 months</td>
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**Abbreviations:** abd, abdomen; syn, synaptophysin; ins, insulin; glu, glucagon; soma, somatostatin.
slightly irregular haloed cytoplasmic neurosecretory granules of low to intermediate density, suggestive of those seen in human neuroendocrine tumors (Fig. 4J). In addition, intermembrane tight junctions of epithelial cells were evident (Fig. 4J).

To additionally establish the endocrine nature of these tumors, in situ hybridization assays were done with mRNA probes encoding hormones, the expression levels of which are used as markers for neuroendocrine tumors (29). Synaptophysin is an integral membrane protein of small synaptic vesicles in brain and endocrine cells (30). Insulin is synthesized by the β cells of pancreatic islets (31). Glucagon is a 29-amino acid pancreatic hormone that counteracts the glucose-lowering action of insulin by stimulating glycogenolysis and gluconeogenesis (32). Chromogranin A is secreted by a great variety of peptide-producing endocrine neoplasms; pheochromocytoma, parathyroid adenoma, medullary thyroid carcinoma, carcinoid, oat-cell lung cancer, pancreatic islet-cell tumors, and aortic-body tumor (33). Somatostatin is widely distributed throughout the body and is an important regulator of endocrine and nervous system function (34). The tumor cells of Case 3 expressed zebrafish synaptophysin (z-syn) RNA but not zebrafish insulin (z-ins), glucagon (z-glu), chromogranin (z-chr), or somatostatin (z-soma; Table 1; data not shown). The tumor cells of Cases 1, 2, 4, and 5 all expressed both high levels of human MYCN mRNA (Fig. 5, A and B) as well as zebrafish insulin mRNA (Fig. 5, C–F). One fish (Table 1, Case 4) also revealed slightly positive staining of glucagon (z-glu; data not shown). We did not detect expression of zebrafish chromogranin (z-chr) or somatostatin (z-soma; Table 1; data not shown). Taken together, our studies indicate that the z-myod promoter-driving MYCN expression in the zebrafish causes the development of neuroendocrine epithelial tumors that are classified as neuroendocrine carcinomas, with anatomic and gene expression findings in four of the five tumors, suggesting origins in insulin-producing islet cells of the pancreas.

At one year of age, all of the F0 MYCN transgene-injected fish were sacrificed, and serial transverse sections were stained with H&E and analyzed histologically for the presence of tumors. We did not detect any additional neuroendocrine tumors or any other type of tumor in this analysis, indicating that although the zmyod promoters drive transgene expression in other organs, the misexpression of MYCN does not initiate cancer in other tissue types. It is possible that in tissues other than the endocrine pancreas, the zmyod promoters drive the expression of MYCN in cells that have differentiated further, which lack self-renewal capacity and thus cannot form malignant tumors.

**DISCUSSION**

We have developed a zebrafish transgenic model system for pancreatic neuroendocrine carcinoma. In humans, pancreatic neuroendocrine tumors include both benign and malignant epithelial neoplasms that show evidence of endocrine cell differentiation (35). These tumors often secrete active hormones, such as insulin or glucagon, and patients may suffer from pronounced paraneoplastic syndromes because of the overproduction of these hormones. Insulin-secreting β-islet cell tumors are the most common type of pancreatic neuroendocrine neoplasms in man. The locally invasive growth pattern identified in our zebrafish tumors favors a malignant rather than a benign lesion.

Relatively little is known about the genetic events that occur during the initiation and progression of pancreatic β-cell neoplasms in humans. However, several observations suggest that activation of the proto-oncogenes, MYC and RAS and overexpression of transforming growth factor α (TGF-α) often occur with malignant progression and that mutations inactivating the p53 tumor suppressor protein are common (36, 37). Pavelic et al. (36, 37) have proposed that MYC activation appears to be an early event in human β-cell tumor progression, because it is expressed at higher levels in β-cell hyperplastic islets than in normal islet cells and at even higher levels in benign and malignant tumors.

We used an upstream fragment of the zebrafish z-myod gene as the promoter to generate transgenic animal expressing the human MYCN oncogene. Mammalian MyoD promoters have a very complicated structure. A 258-bp enhancer located 20 kb upstream of the transcriptional start site is necessary to drive transgene expression in muscle cell progenitors in the mouse, because a minimal promoter fragment, lacking this upstream motif, primarily mediates neuronal expression (24, 27, 28). Our results in zebrafish were quite similar, in that a 6-kb minimal promoter fragment of z-myod resulted in EGFP expression that was most pronounced in the hindbrain, spinal cord, and pancreatic islets (Fig. 1, A and B). These results suggest that the 6-kb promoter of z-myod has activity but lacks the elements necessary to initiate muscle expression, as had been previously observed for the mouse minimal promoter of MyoD in transgenic mice (24, 27). In an attempt to obtain expression in zebrafish muscle cells, we tested the 258-bp core enhancer of the human MYOD gene upstream of our z-myod promoter fragment, forming a human-zebrafish chimeric promoter. Interestingly, this chimeric promoter functioned well to drive high levels of transgene expression in muscle cells of the zebrafish, both when transiently injected at the one-cell stage and also in the progeny of two different EGFP-expressing stable lines. Muscle cells in both the trunk and the craniofacial regions expressed high levels of EGFP. However, the 258-bp core enhancer of the human MYOD gene did not prevent the ectopic expression of EGFP in neuronal and pancreatic islet cells in our transgenic lines, suggesting that our chimeric construct still lacks repressor sequence motifs that commonly prevent expression of the endogenous z-myod gene in these tissues. Our
success in using human enhancer elements to drive muscle expression in zebrafish suggests evolutionary conservation of regulatory enhancer sequence elements and trans-acting transcription factors between these two species and raises the possibility that the zebrafish system could be used to help identify and analyze the sequence motifs and transacting factors, the homologues of which perform similar roles during mammalian development.

The c-Myc proto-oncogene is implicated in pancreatic β-cell proliferation in tumorigenesis and may contribute to apoptosis of the same cells in diabetes (38). Although the vast majority of functional and transgenic studies of islet cell tumors have focused on the c-Myc protein, N-Myc functions in a very similar manner in a number of cell culture assays (39, 40). Indeed, when the coding region of the mouse c-Myc gene was replaced with the N-Myc coding sequence by homologous recombination, the resulting homozygous knock-in mice, in which the c-Myc promoter drives the synthesis of N-Myc mRNA instead of c-Myc mRNA, were viable and appeared normal, indicating that the N-Myc protein can functionally replace c-Myc if it is appropriately regulated (41). β-cell tumors that express insulin mRNA and histologically resemble human pancreatic endocrine carcinomas have been produced by transgenic expression of c-Myc, indicating the importance of the Myc pathway in the molecular pathogenesis of pancreatic neuroendocrine carcinoma (42). Studies in transgenic mice have shown that Myc activation initially promotes both proliferation and apoptosis in pancreatic β cells and that tumors arise when the apoptosis pathway is suppressed, indicating the need for at least two mutational events before tumors arise (42). Studies with a conditional c-Myc allele also showed that Myc expression is required not only for initiation for the tumor but also to maintain the neoplastic phenotype of established tumors (42). More recently, Lewis et al. (43) have generated a mouse model for pancreatic cancer through the somatic delivery of oncogene-bearing avian retroviruses to mice that express TVA, the receptor for avian leukemia sarcoma virus subgroup A (ALSV-A), under the control of the elastase promoter. Infection of elastase-tv-a transgenic mice, either wild-type or null for the Ink4a/Arf locus, with viruses encoding mouse polyoma virus middle T antigen induced highly penetrant acinar and ductal tumors. In contrast, infection of elastase-tv-a, Ink4a/Arf null mice with viruses encoding the c-Myc oncogene led to the formation of pancreatic endocrine tumors exclusively, indicating the importance of Myc in the tumorigenesis of pancreatic endocrine tumors (43).

We were surprised that we did not recover rhabdomyosarcoma in transgenic fish expressing MYCN driven by the core-zmyod promoter, because we observed high levels of expression of GFP in embryonic muscle cells with this promoter, and MYCN is amplified and overexpressed in most human rhabdomyosarcomas (44). There are several possibilities to explain this observation. Although expressed at high levels in muscle cells, core-zmyod may not drive expression in muscle stem cells with the capacity for self-renewal, thus preventing the acquisition of additional mutations needed for the formation of a malignant tumor. Alternatively, high levels of MYCN expression driven by this promoter may lead to apoptosis of muscle stem cells that integrate the transgene, preventing transformation. Lastly, it remains possible that MYCN overexpression is not a rate-limiting step in rhabdomyosarcoma and that other mutations are needed before developing myoblasts are susceptible to MYCN-induced transformation.

One advantage of the zebrafish model lies in its ability to accommodate large-scale “forward-genetic” screens to identify modifier genes. For such screens we will need to establish a stable transgenic line expressing MYCN, which we have not thus far recovered, although we were able to generate stable lines expressing EGFP alone from both promoters. A likely possibility is that the promoter we are using drives MYCN expression in germ cells that integrate the transgene, which is toxic to them. We are now injecting a Cre-Lox conditional transgene to establish stable lines that only express EGFP until MYCN is induced through the controlled expression of Cre. This system has worked with other promoters, so we feel confident that it will ultimately be successful.

Once forward-genetic screens become possible in our system, germline mutations induced by either ethyl-nitrosourea or retroviral integration into large numbers of mutagenized transgenic zebrafish will be analyzed for mutations that accelerate or retard the rate of development of Myc-induced tumors. For example, mutations that inactivate tumor suppressor genes and accelerate the onset of clonal tumors should be identified, because the mutant fish would carry inactivating mutations of single tumor suppressor alleles throughout development, increasing the likelihood of acquiring inactivating mutations or deletions affecting both alleles of the same gene. In addition, mutations identified in unbiased screens that delay or prevent tumorigenesis may provide insight into human homologues that could serve as targets for the development of small molecule inhibitors.

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