Zebrafish with Mutations in Mismatch Repair Genes Develop Neurofibromas and Other Tumors

Harma Feitsma,1 Raoul V. Kuiper,2 Jeroen Korving,1 Isaac J. Nijman,1 and Edwin Cuppen1

1Hubrecht Institute for Developmental Biology and Stem Cell Research, Cancer Genomics Center and 2Faculty of Veterinary Medicine, Department of Pathobiology, Utrecht University, Utrecht, the Netherlands

Abstract
Defective mismatch repair (MMR) in humans causes hereditary nonpolyposis colorectal cancer. This genetic predisposition to colon cancer is linked to heterozygous familial mutations, and loss-of-heterozygosity is necessary for tumor development. In contrast, the rare cases with biallelic MMR mutations are juvenile patients with brain tumors, skin neurofibromas, and café-au-lait spots, resembling the neurofibromatosis syndrome. Many of them also display lymphomas and leukemias, which phenotypically resembles the frequent lymphoma development in mouse MMR knockouts. Here, we describe the identification and characterization of novel knockout mutants of the three major MMR genes, mlh1, msh2, and msh6, in zebrafish and show that they develop tumors at low frequencies. Predominantly, neurofibromas/malignant peripheral nerve sheath tumors were observed; however, a range of other tumor types was also observed. Our findings indicate that zebrafish mimic distinct features of the human disease and are complementary to mouse models.

Introduction
Mismatch repair (MMR) corrects small replication errors that are caused by the DNA polymerase and skipped by its proofreading capacity (1). In humans, defective MMR is associated with early-onset colorectal cancer in the hereditary nonpolyposis colorectal cancer (HNPCC) syndrome (2). According to the second-hit hypothesis (3), only one extra mutation, one that inactivates the wild-type allele of the MMR gene, is necessary to create a genomic instable environment that strongly enhances cancer development. Perhaps due to the high cell turnover in the colon, this organ is most prone to the loss-of-heterozygosity followed by tumorigenesis, but several other types of tumors have also been found in HNPCC patients (4).

In the years following the discovery of HNPCC, continuous monitoring of HNPCC families has revealed several cases of humans with innate inactivating mutations in both alleles of a MMR gene, resulting from inbreeding or coincidence (5–19). These patients lack the MMR protein from the beginning and therefore develop cancer at extremely early ages, generally before the age of 10 years. From a summary of the tumors that occurred in this group of patients (Table 1), it becomes clear that the tumor spectrum is markedly different from HNPCC patients. Most striking is the high frequency of brain tumors, such as glioblastoma, medulloblastoma, oligodendroglioma, primitive neuroectodermal tumor (PNET), and astrocytoma. In combination with a high incidence of neurofibromas in the skin and café-au-lait spots, this resembles the neurofibromatosis type I syndrome (20). Second most frequent are lymphomas and leukemias (Table 1). Gastrointestinal tumors occur (Table 1) but at much lower rate than in HNPCC patients and at a later age than the other tumor types. Apparently, constant genomic instability in all cells causes different cancers, which prompted some authors to call this the MMR deficiency syndrome (13) or childhood cancer syndrome (19), whereas others consider it a variant of Turcot’s syndrome (14, 15, 21). A number of cases were found for the four most important MMR genes, MSH2, MLH1, MSH6, and PMS2. Striking is that biallelic inactivation of PMS2 leads to juvenile cancer, whereas heterozygous mutated PMS2 has not frequently been detected in cases of HNPCC (2).

To obtain animal models for HNPCC, mouse knockouts for all MMR genes have been generated (22–33). Homozygous mutants predominantly die of lymphomas (Table 1). Intestinal tumors occur at much lower frequencies than in HNPCC, which led some researchers to conclude that these mouse mutants were not properly modeling HNPCC. However, when comparing the tumor spectra of these homozygous mouse models to the spectrum of humans with biallelic mutations (Table 1), it becomes clear that they are similar in terms of frequent lymphoma development and relatively low abundance of gastrointestinal tumors. A difference is that brain tumors are very rare and neurofibromas and café-au-lait spots are absent in mouse MMR knockouts (Table 1).

Here, we describe the isolation of zebrafish mutants for the MMR genes mlh1, msh2, and msh6 and show that homozygous mutants of all three lines develop neoplasms, with a frequency of 6% to 45%. Most frequently, neurofibromas/malignant peripheral nerve sheath tumors (MPNST) in the eye and abdomen were observed; however, other tumor types also occurred, such as PNET in the brain and hemangiosarcoma on the head.

Materials and Methods
PCR. The sequences of all oligonucleotides that were used are given in Supplementary Table S1.

Generation of mutant zebrafish lines. All animal experiments were approved by the Animal Care Committee of the Royal Dutch Academy of Science according to the Dutch legal ethical guidelines. For obtaining msh6 and msh2 mutant fish ampicils that covered most of the large exon 4 of the zebrafish msh6 gene and exon 5 and 6 of the zebrafish msh2 gene, respectively, were used in an ethynitrosourea (ENU)–driven target-selected mutagenesis screen (34). The obtained mutant fish were outcrossed with wild-type fish, and heterozygous offspring was subsequently incrossed. Genotyping was done either by PCR amplification and resequencing or by using KASPAR genotyping technology (Kbioscience). The mlh1 (hu1919) mutants were generated as described previously (35).
Reverse transcription-PCR and quantitative PCR. RNA was isolated from tail tissue of adult zebrafish and wild-type embryos using FastRNA Pro Green kit (Q-biogene) and reverse transcribed by using the RETROscript kit (Ambion). For msh2 reverse transcription-PCR (RT-PCR), cDNA-specific primers on the boundary of exons 3 and 4 and within exon 6 were used, resulting in a 534-bp fragment in the wild-type. For msh6 quantitative PCR, cDNA-specific primers within exon 8 were used. As a control, the msh2 primers mentioned above were used. The reactions contained SYBR Green Supermix (Bio-Rad) and were run and analyzed with the MyIQ single color real-time PCR detection system and software (Bio-Rad). A dilution series of wild-type cDNA was used as a standard. All genotypes were run in 5-fold. The cDNA concentrations were calculated in arbitrary units compared with the wild-type average and expressed as the ratio of msh6 to msh2.

Microsatellite instability. Two mononucleotide (A22 and A25) and two dinucleotide repeat loci in the germ line were targeted in the msh2 mutant. Primers were designed within the repeat loci. PCR products were separated by gel electrophoresis. Each of the products was sequenced on an ABI 3730XL capillary DNA analyzer (Applied Biosystems). A dilution series of wild-type cDNA was used as a standard. All genotypes were run in 5-fold. The cDNA concentrations were calculated in arbitrary units compared with the wild-type average and expressed as the ratio of msh6 to msh2.

Cancer development. Homozygous mutant fish and heterozygous siblings of all three mutant lines were monitored weekly. Animals with signs of disease or apparent cell masses were sacrificed. Fish were fixed in 4% paraformaldehyde at 4°C for 12 h, embedded in paraffin, sectioned at 4 μm, and stained with hematoxylin and eosin (H&E) for histologic characterization. The genomic organization of the msh2 gene is highly homologous from human to fish, including complete conservation of the exon-intron boundaries of exon 5 (Supplementary Fig. S1). Interestingly, in humans, in-frame deletion of exon 5 of MSH2 is the most common mutation in HNPCC families, accounting for 11% of all known pathogenic MSH2 mutations (36). It results in an incomplete protein (37), and carriers have a high risk of developing cancers (38), which generally are microsatellite instable (39). This suggests that the splice site mutation of msh2 in zebrafish results in loss of function of the gene and provides a translational animal model for MSH2-driven disease in humans.

Results

Generation of MMR mutants in zebrafish. Using ENU-driven target-selected mutagenesis, we isolated three zebrafish mutants with putative loss-of-function mutations in the major MMR genes msh6, msh2, and mll1.

The msh6 mutant (hu1811) carries a point mutation causing a premature stop codon in the large fourth exon (Fig. 1A). This exon is well conserved in comparison with the similarly large fourth exon of the human MSH6 gene. According to the database of the International Society for Gastrointestinal Tumors, among HNPCC families, there are several alleles known of point mutations causing premature translation termination in this exon, increasing the likelihood that our zebrafish mutation will be pathogenic. We could not confirm the knockout phenotype at the protein level in the zebrafish mutant, as we were not able to find an anti-MSH6 antibody that recognizes the zebrafish protein. However, we could show by quantitative PCR that the levels of msh6 transcript are decreased to ~44% of the wild-type level in heterozygous and ~15% in homozygous mutants, presumably due to nonsense-mediated decay of the premature stop codon-containing transcript (Fig. 1B).

The mutation in msh2 (hu1886) changes the G of the splice donor site of exon 5 to an A (Fig. 1A). Using RT-PCR, we could show that it results in a 150 bp shorter mRNA (Fig. 1C). Sequencing of this product showed that this is the result of an in-frame deletion of exon 5 (Fig. 1C). The genomic organization of the msh2 gene is highly homologous from human to fish, including complete conservation of the exon-intron boundaries of exon 5 (Supplementary Fig. S1). Interestingly, in humans, in-frame deletion of exon 5 of MSH2 is the most common mutation in HNPCC families, accounting for 11% of all known pathogenic MSH2 mutations (36). It results in an incomplete protein (37), and carriers have a high risk of developing cancers (38), which generally are microsatellite instable (39). This suggests that the splice site mutation of msh2 in zebrafish results in loss of function of the gene and provides a translational animal model for MSH2-driven disease in humans.

The third MMR mutant that is used in this study contains a premature stop mutation in mll1 (hu1919; Fig. 1A and D). We recently showed that this mutation results in full loss-of-function of MLH1 and causes a strong meiotic phenotype (35).

MMR-deficient zebrafish show microsatellite instability. One of the hallmarks of defective MMR is instability of microsatellite sequences (2). We studied microsatellite instability (MSI) within two mononucleotide and two dinucleotide repeat loci in the germ

Table 1. Tumors in human patients with biallelic inactivation of MMR genes

<table>
<thead>
<tr>
<th>Gene</th>
<th>Cancer (%)</th>
<th>Brain tumor*</th>
<th>Lymphoma</th>
<th>Leukemia</th>
<th>Gl tumor</th>
<th>Skin tumor</th>
<th>Other</th>
<th>CALS</th>
<th>References</th>
</tr>
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<tr>
<td>Human</td>
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<tr>
<td>MSH2</td>
<td>3</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>1</td>
<td>(5, 6)</td>
<td></td>
</tr>
<tr>
<td>MLH1</td>
<td>11</td>
<td>4</td>
<td>2</td>
<td>2</td>
<td>2</td>
<td>4 †</td>
<td>2</td>
<td>10</td>
<td>(7–11)</td>
</tr>
<tr>
<td>MSH6</td>
<td>10</td>
<td>8</td>
<td>4</td>
<td>1</td>
<td>4</td>
<td>9</td>
<td></td>
<td></td>
<td>(10–14)</td>
</tr>
<tr>
<td>PMS2</td>
<td>27</td>
<td>17</td>
<td>5</td>
<td>4</td>
<td>10</td>
<td>3</td>
<td>2</td>
<td>20</td>
<td>(11, 15–19)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>51</td>
<td>30 (59)</td>
<td>12 (24)</td>
<td>8 (16)</td>
<td>16 (31)</td>
<td>4 (8)</td>
<td>5 (10)</td>
<td>40 (78)</td>
<td></td>
</tr>
<tr>
<td>Mouse</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Msh2</td>
<td>207</td>
<td>204 (99)</td>
<td>4</td>
<td>161</td>
<td>10</td>
<td>60</td>
<td>12</td>
<td>18</td>
<td>(22–26)</td>
</tr>
<tr>
<td>Mlh1</td>
<td>76</td>
<td>69 (91)</td>
<td>41</td>
<td>40</td>
<td>8</td>
<td>4</td>
<td></td>
<td></td>
<td>(27–29)</td>
</tr>
<tr>
<td>Msh6</td>
<td>35</td>
<td>35 (100)</td>
<td>20</td>
<td>2</td>
<td>8</td>
<td>7</td>
<td>8</td>
<td></td>
<td>(30–32)</td>
</tr>
<tr>
<td>Pms2</td>
<td>45</td>
<td>34 (76)</td>
<td>31</td>
<td>4</td>
<td>16</td>
<td>8</td>
<td>3</td>
<td></td>
<td>(28, 29, 33)</td>
</tr>
<tr>
<td>Total (%)</td>
<td>363</td>
<td>342 (94)</td>
<td>4 (1) ‡</td>
<td>253 (74)</td>
<td>12 (4) ‡</td>
<td>108 (32) ‡</td>
<td>27 (8) ‡</td>
<td>32 (9) ‡</td>
<td></td>
</tr>
</tbody>
</table>

Abbreviations: GI, gastrointestinal; CALS, cafe-au-lait spots.
*In humans: glioma, glioblastoma, medulloblastoma, oligodendroglioma, PNET, supratentorial PNET, and astrocytoma; in mouse: not further specified.
† Neurofibromas.
‡ Percentage of mice with this type of tumor of the total number of mice that developed cancer.
line of msh2 and msh6 mutants—mlh1 mutant males are sterile (35) and could therefore not be included in this assay. Homozygous mutant males were crossed with wild-type females and microsatellites were typed in the progeny. As the latter are heterozygous and thus MMR proficient, any observed clonal instability should have occurred in the germ line of the male founder (Fig. 2). Three percent of offspring of msh2+/−/− males have altered microsatellite lengths, both in mononucleotide and dinucleotide repeat sequences. Msh6+/−/− progeny predominantly shows instability of mononucleotide markers in 4% of the cases. No MSI was detected in progeny from wild-type or heterozygous males (Table 2).

MMR-deficient zebrafish develop tumors. From age 6 months, MMR mutant zebrafish started developing cancer. Tumors were only found in homozygous mutants and mostly developed in the 2nd year of life (Table 3). The cancer incidence was relatively low but was much higher than generally found in wild-types, 33% on average for all three lines together. In total, 25 tumors were detected, of which 13 in mlh1+/−/−, 11 in msh6+/−/−, and 1 in msh2+/−/− fish (Table 3).

The tumor frequency in homozygous mutants was significantly higher than in heterozygous siblings for the mlh1 (n = 29 for homozygotes, n = 30 for heterozygotes; P = 2 × 10−5) and msh6 (n = 31 for homozygotes, n = 12 for heterozygotes; P = 0.01) mutants. This is not the case for the msh2 mutant (n = 16 for homozygotes, n = 15 for heterozygotes; P = 0.5), as the number of animals and tumors is low. This is most likely caused by the reduced general fitness of the line. For all three mutants together, the difference in tumor frequency is highly significant (n = 76 for homozygotes, n = 57 for heterozygotes; P = 1 × 10−7).

Tumors are characterized as neurofibromas/MPNST and other types. Frequently, tumors were poorly differentiated; however, a high proportion (76%) showed features consistent with piscine neural crest origin tumors, such as unencapsulated growth of spindle-shaped neoplastic cells in interconnected fascicles and scattered presence of pigmentary cells (iridophores and melanocytes). The neoplastic growth invaded surrounding structures, including bone and cartilage of the skull, connective tissue and sclera, and striated muscle. As clearly distinct Antoni A and B patterns were not recognized, pulsading was generally absent, and whorls of tumor cells resembling Pacinian-like corpuscles were more frequently observed, the diagnosis neurofibrosarcoma was favored to malignant Schwannoma for these tumors. However, due to the lack of reliable immunohistochemical markers for these lesions in zebrafish, the distinction between these two types of MPNSTs cannot be made definitely, so that we use both characterizations together. Neurofibromas/MPNSTs were most frequent in the eye (Fig. 3A) and in the abdomen (not shown). Most tumors in the trunk and one extraocular cranial tumor were located dorsally, extending into the renal tissue and skeletal muscle surrounding the skull and vertebral column, or invading bones and

![Figure 1](https://example.com/figure1.png)
cartilage of the skull. Therefore, a meningotheelial component cannot be excluded, which could explain the histologic similarity between intraocular, cranial, and abdominally located neoplasms.

Furthermore, two distinct cases of cranial neural crest tumors were observed. In one case, a mass protruding from the rostral aspect of the cranium and invasively growing between the eyes histologically consisted of clusters and nests of small polygonal to cuboid cells. These cells frequently had apical cilia and were forming rosettes and tubules, separated by areas where cells were more stellate with abundant myxoid intercellular material (Fig. 3B). Based on the histologic appearance and localization, this tumor was diagnosed as olfactory neuroblastoma. A third type of neural

<table>
<thead>
<tr>
<th>Genotype male</th>
<th>Mononucleotide</th>
<th>Dinucleotide</th>
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<tbody>
<tr>
<td></td>
<td>A22</td>
<td>A25</td>
</tr>
<tr>
<td>msh2+/--</td>
<td>0/95</td>
<td>0/95</td>
</tr>
<tr>
<td>msh2+/--</td>
<td>4/244</td>
<td>9/273</td>
</tr>
<tr>
<td>msh6+/--</td>
<td>0/42</td>
<td>0/42</td>
</tr>
<tr>
<td>msh6+/--</td>
<td>5/219</td>
<td>14/222</td>
</tr>
<tr>
<td>Wild-type</td>
<td>0/156</td>
<td>0/151</td>
</tr>
</tbody>
</table>

*Average percentage of embryos that is instable for this type of markers.

Figure 2. MSI in MMR mutant zebrafish. Examples of instability in a mononucleotide (left) and a dinucleotide (right) marker are shown. Top row, allele lengths of a msh2+/-- male; second row, allele lengths of a wild-type female. Embryos of a cross of those two show changes in the paternal allele length, as shown in the two bottom rows.
crest tumor was found inside the cranium of one fish, extending from the brain through cartilage and bones of the skull into the surrounding tissues, including the eye. Histologically, the neoplasm consisted of poorly differentiated small polymorphic cells with limited amounts of basophilic cytoplasm and distinct cellular borders, growing in solid sheets (Fig. 3C). This morphology was consistent with PNET diagnosis.

Tumors other than those of neuroectodermal origin included a cavernous hemangioma (not shown) and a hemangiosarcoma (Fig. 3D), both in the head. Histologically, these lesions were characterized by plump fusiform cells growing in a cribiform pattern, with intercellular spaces frequently containing erythrocytes. Hyperplastic epithelial lesions with formation of irregular dermal islets of dysplastic epithelial cells without connection to the epidermis were observed in the head of one fish, consistent with squamous cell carcinoma (not shown). One animal showed a poorly differentiated densely cellular neoplasm located ventrally between the branchial arches, invading the tissues surrounding the ventral aorta, consisting of small cuboidal to polygonal cells with indistinct borders growing in solid sheets, with occasional palisading of tumor cells and rare formation of rosettes or follicles containing small amounts of brightly eosinophilic material. Consistent with the location of the teleost thyroid, this tumor was diagnosed as a solid thyroid carcinoma (not shown).

Although the above neoplasms showed marked invasion of the surrounding tissue, mitotic figures were infrequent in all tumors and no indications for metastasis were observed. The tumor classification was done solely on morphologic characteristics, because the necessary immunohistochemical stainings (for glial fibrillary acidic protein, synaptophysin, epithelial membrane antigen, neuronal nuclei, and s100) to confirm these tumor types was not done on zebrafish. Some exemplary tumors (antigen, neuronal nuclei, and s100) to confirm these tumor types were fibrillary acidic protein, synaptophysin, epithelial membrane antigen, neuronal nuclei, and s100.

<table>
<thead>
<tr>
<th>Genotype</th>
<th>n</th>
<th>Cancer (%)</th>
<th>Av. age cancer (mo)</th>
<th>Ocular nf.</th>
<th>Abdominal nf.</th>
<th>Other nf.</th>
<th>Other neural*</th>
<th>Cranial nonneural 1</th>
</tr>
</thead>
<tbody>
<tr>
<td>mlh1−/−</td>
<td>29</td>
<td>13 (45)</td>
<td>14</td>
<td>1</td>
<td>8</td>
<td>1</td>
<td>2</td>
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<td>30</td>
<td>0 (0)</td>
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<td></td>
<td></td>
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<td></td>
<td></td>
</tr>
<tr>
<td>msh2−/−</td>
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<td>1 (6)</td>
<td>22</td>
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<tr>
<td>msh2+/−</td>
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<tr>
<td>msh6−/−</td>
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<td>11 (35)</td>
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</tr>
<tr>
<td>msh6+/−</td>
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<tr>
<td>Total−/−</td>
<td>76</td>
<td>25 (33)</td>
<td>17</td>
<td>4 (16) 1</td>
<td>13 (52) 1</td>
<td>2 (8) 1</td>
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Abbreviation: nf., neurofibroma.
1Primary neuroectodermal tumor and olfactory neuroblastoma.
*Hemangioma, hemangiosarcoma, thyroid carcinoma, and squamous cell carcinoma.

Discussion

We generated the first MMR-deficient models in zebrafish by knocking out the major MMR genes mlh1, msh2, and msh6, respectively. Homozygous mutants for msh2 and msh6 show MSI, which is a hallmark of defective MMR. The frequency at which we find instability (3–4%) is lower than generally observed in mice (15–40%; refs. 22, 29, 33, 40); however, this can be explained by the fact that we studied markers in the germ line, where mutation rates are usually lower than in somatic cells that were used in the mouse studies. The per generation mutation rate for a large set of repeat markers in wild-type zebrafish was found to be 1.5 × 10−4 (41), which means that it is increased around 200-fold in our mutants. In mouse somatic cells, the microsatellite mutation frequency was on average 50-fold higher in the mutant than in wild types (40), which is a comparable increase. The observed MSI in both mononucleotide and dinucleotide repeats in msh2 mutants and in mononucleotide repeats only in msh6 mutants is consistent with the respective functions of MSH2 and MSH6 in repair of different types of mutations (1), although dinucleotide repeat instability has been observed in tumors of mouse msh6 knockouts (32). Homozygous mutants of all three fish lines are prone to tumor development at low but significant incidence and primarily develop neurofibromas/MPNSTs in the eye and abdomen, but also a PNET, an olfactory neuroblastoma, a hemangioma and hemangiosarcoma, a squamous cell carcinoma, and a thyroid carcinoma were observed. Although the number of tumors is low, these data suggest that the zebrafish model resembles an important part of the phenotype of human patients, where biallelic MMR inactivation causes a neurofibromatosis type I–like phenotype, a phenotype that is not seen in mouse MMR knockouts. Similar to mouse mutants, tumors were only found in homozygous mutant fish.

Because lack of MMR results in a specific form of genomic instability in repeat sequences, we tested the hypothesis that the observed differences in tumor types in different species may be caused by the species-specific presence of repeat sequences in the coding sequence of selected tumor suppressor genes. We performed an in silico analysis of human genes involved in neural, gastrointestinal, or hematologic cancers or generally in many types of cancers and their mouse and zebrafish homologues, but found no significant differences in numbers of repeats between the three species (Supplementary Text, Supplementary Table S2, and Supplementary Figs. S2 and S3). Even more, targeted analysis of neurofibromatosis I, a strong candidate gene for neural tumors, did not work on zebrafish. Some exemplary tumors (antigen, neuronal nuclei, and s100) to confirm these tumor types were fibrillary acidic protein, synaptophysin, epithelial membrane antigen, neuronal nuclei, and s100.
not show a correlation between the number of repeat stretches and the development of neural tumors in human, mouse, and zebrafish. Nonetheless, it could still be interesting to sequence the genes containing large repeats in tumor samples of different species, to obtain information about their inactivation rates, although the presence of frameshift mutations does not necessarily mean that inactivation of the gene was essential for tumor development. Importantly, types of mutations are known to depend on selection pressure in tumor development and are thus hard to predict. A good example of this was given by Smits and colleagues, who showed that, in msh2-deficient background, apc mutations in intestinal tumors were mostly located in small dinucleotide repeats in mice wild-type in apc, where both alleles needed inactivation. In contrast, they were point mutations more upstream in the gene in apc heterozygous mice, where loss of heterozygosity had to take place (25). Here, it is therefore more likely that the organism-specific tumor spectra are the result of physiologic and anatomic differences, such as relative organ sizes and life span.

Only very recently, zebrafish has become a model for studying genetics and molecular biology of cancer. Three tumor suppressor mutants in zebrafish have been generated by reverse genetics: tp53, pten, and apc (42–44), all of which spontaneously develop tumors. Homozygous tp53 mutants develop MPNSTs in the eye and abdomen (42), similar to the MMR mutant fish. This type of tumors was also frequent in tumor suppressor mutants from forward genetic screens, e.g., ribosomal protein mutants from a retroviral insertion screen and mutants from a screen for genome instability (45, 46). MPNSTs have been reported in several fish species, but these are certainly not the only type of neoplasm in fish. For example, tp53 knockout medaka develop a broader range of tumors (47). Also, the ocular tumors that were found in pten knockout fish seem of a different type, although they have not been characterized in detail (43), and apc mutant fish develop intestinal and liver neoplasms (44). This, as well as the occurrence of other tumors besides neurofibromas in the MMR mutants, indicates that different genetic predispositions lead to different types of cancer, similar to the situation in humans.

Loss of MMR results in high and intermediate frequencies of hematologic defects in mice and humans, respectively. Lymphomas and leukemias generally arise due to genomic rearrangements that
result in misexpression of certain oncogenes (48). The number of known tumor suppressor genes is low, decreasing the chance that MSI will be causal to lymphoma development. The high occurrence of misrecombination in immune cells is likely the result of the large number of immunoglobulin gene rearrangements that have to be made during VDJ junction and class switch recombination to create a functional immune system. Because the MMR genes Mlh1, msh2, and msh6 are known to play a role in class switch recombination (49–52), it is tempting to speculate that their deficiency can directly lead to immune malignancies, without the need for creating a genomic instable situation that mutates other tumor suppressors. The fact that some patients with biallelic MMR inactivation were reported to be IgA-deficient supports this idea (11). Also, MMR-deficient mice show a reduction in isotype switching (49, 50, 52). Zebrafish have three classes of immunoglobulin heavy chains, which are genomically organized by VDJ junction or alternative splicing. Class switch recombination does not occur (53), which may explain why MMR deficiency does not lead to lymphoma development in zebrafish, although lymphomas and leukemias have been observed in zebrafish (e.g., ref. 54).

The question why mice are more prone to lymphoma development than humans remains unanswered.

From our msh2 line, we did not observe many fish dying from cancer. This is partly because homozygous mutants generally had a weak appearance and died of cancer-unrelated causes (results not shown). In addition, nearly all homozygous mutants were males and had significantly lower body weights than siblings. Although we cannot exclude that these phenotypes result from background mutations, it is striking that in humans, very few homozygous msh2 cases were encountered whereas heterozygous patients are common, which could suggest that homozygous msh2 inactivation reduces viability in general. It should be mentioned that such mechanism or phenotype has not been reported for msh2 knockout mice.

Taken together, we have shown that mutants for MMR genes in zebrafish display MSI and are at increased risk for tumor development. This is similar to mice and humans, although the tumor spectra are different. From this and other recent publications, it can be concluded that zebrafish proves to be a useful cancer model that provides new insights and experimental possibilities, and complements studies in mouse and human.

Disclosure of Potential Conflicts of Interest

No potential conflicts of interest were disclosed.

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References

and aneuploid as well as triploid progeny in females. 


Zebrafish with Mutations in Mismatch Repair Genes Develop Neurofibromas and Other Tumors

Harma Feitsma, Raoul V. Kuiper, Jeroen Korving, et al.


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