Hikui disease in nine koi carp (Cyprinus carpio): first description of a cutaneous perivascular wall tumour

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Background – Hikui disease is a well known disfiguring disease of koi carp (Cyprinus carpio) primarily affecting fish with red pigmentation. It causes light orange to golden yellow, multifocal to coalescing raised patches, starting from the red cutaneous areas. Some cases respond to surgery or topical treatment, but recurrence is common.

Objectives – To describe the clinical and pathological presentation of Hikui disease and its cause.

Animals – Nine affected koi carp belonging to private hobbyists.

Methods – Eight fish underwent surgery or biopsy; one was euthanized. Tissues were submitted for histology, immunohistochemistry and transmission electron microscopy.

Results – Five fish showed typical lesions of Hikui disease, whereas four fish showed an atypical presentation characterized by focal or multifocal, oedematous, dark red cutaneous plaques or nodules. Histology showed unencapsulated, infiltrating and densely cellular neoplasms composed of spindle cells arranged in bundles, rows and whorls frequently centred on capillaries. Immunohistochemistry for smooth muscle actin labelled neoplastic cells in all cases. Ultrastructure showed neoplastic cells with slender cytoplasmic processes encircling the capillaries, a thin basal membrane and occasional plasmalemmal vesicles.

Conclusions and clinical importance – All of the data supported a neoplastic process producing perivascular wall tumours. Immunoreactivity to smooth muscle actin and the ultrastructural features were indicative of a pericyte origin (haemangiopericytoma). This is the first report dealing with Hikui disease that has achieved a conclusive diagnosis. The neoplastic nature of this condition suggests the potential usefulness of a surgical approach in the clinical management of less severe cases.

Introduction

Hikui disease is a disfiguring skin disease of koi carp (Cyprinus carpio). It presents clinically as raised patches that can progress and become eroded or ulcerated. Although this condition is well recognized by Japanese breeders, koi carp hobbyists and veterinarians, and widely discussed on web forums, little has been published on the prevalence, aetiology or pathogenesis, and neither a definitive cause nor a diagnosis have been achieved. The disease is widespread. According to one anecdotal survey of koi carp owners in Belgium and the Netherlands, 41% of koi carp owners have had at least one or more fish displaying Hikui disease in their pond. It is supposedly more common in Go-Sanke (Kohaku, Sanke and Showa), Goshiki and Goromo colour varieties, all of which have a large amount of red (“Hi” in Japanese) pigmentation and can spread to other coloured areas on affected fish. It does not appear to occur in koi carp without red pigment, even if they are kept in the same pond. Both males and females can be affected. Although fish rarely die from the primary lesions, the clinical progression is unpredictable. Lesions most commonly occur on the dorsum and head, rarely on the ventrum or lateral aspects of the fish. Lesions start as areas of epithelial thickening but often erode and ulcerate. Lesions can become haemorrhagic. Healed lesions are often discoloured, scarred and without normal pigmentation.

Topical and surgical treatments have been used but it is difficult to evaluate their efficacy because lesions can resolve spontaneously. If lesions are extensive, fish may be euthanized. Little is known about the cause and infections, although protozoal, nutritional deficiency, poor water quality, actinic disease (i.e. sunburn), high organic load in the water, neoplasia and heritable causes have been hypothesized.1–3 Ultrastructural studies on these skin lesions failed to clearly confirm the presence of viral particles.3

The goal of this case report is to describe the histological, immunohistochemical and ultrastructural findings in nine fish with cutaneous lesions compatible with Hikui disease.
Case report

All koi carp belonged to private hobbyists and were held in ponds of 10,000–50,000 L, which were either shaded or in full sun. The colour varieties included Kohaku, Showa Sanshoku, Taisho Sanshoku, Benigoi and Hi Utsuri (Table 1). All fish were from Japanese breeders except for one which came from Israel. The overall condition of the fish was indicative of good health, and they ate and swam normally; they had been treated topically with a formalin-malachite green mixture for ectoparasites prior to examination. Affected fish presented with multifocal raised cutaneous thickenings on the dorsum, head and on the lateral body. Erosions, ulcers, hyperaemia and haemorrhage were present in some fish. The disease caused the red pigmented skin to become oedematous, separating and lifting the scales. Some

<table>
<thead>
<tr>
<th>Case</th>
<th>Gross appearance</th>
<th>Body length (cm)</th>
<th>Age (years)</th>
<th>Sex</th>
<th>Pond volume (L)</th>
<th>Number of koi affected</th>
<th>Sun</th>
<th>Follow-up</th>
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<td>60</td>
<td>10</td>
<td>N/A</td>
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<td>Several</td>
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<td>50</td>
<td>10</td>
<td>Male</td>
<td>10,000</td>
<td>More than one</td>
<td>Mostly sun</td>
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<td>60</td>
<td>10–15</td>
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<td>Completely in the sun</td>
<td>Healed completely after surgery</td>
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<td>30</td>
<td>15</td>
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<td>16,000</td>
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<td>15–20</td>
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<td>5–6</td>
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<td>Lesions still present</td>
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<td>More than one</td>
<td>50/50 sun/shadow</td>
<td>Lesions still present: one animal euthanized during sampling</td>
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</table>

Table 1. Data about anamnesis and 18 month follow-up of the nine koi carp examined based on a survey given to owners

Figure 1. Skin, koi carp. Gross appearance of Hikui. (a,b) Typical focal or multifocal to coalescing, light orange to golden yellow, thickened cutaneous patches are present. In some cases the affected skin was shed, exposing the skull bones. (c,d) Atypical presentation of Hikui showing dark red, soft or gelatinous, irregular thickened patches also present in white areas.
affected scales were covered by green algae present in the pond.

Clinical examination revealed two presentations. The first was the typical clinical presentation represented by multifocal to coalescing, raised, light orange to golden yellow cutaneous patches, that involved the red skin areas and spread into white areas (5 of 9 fish) (Figure 1a,b). Affected skin was often friable and multifocally ulcerated, and in one case parts of the skull bones were exposed (Figure 1). The second or atypical presentation consisted of focal or multifocal, oedematous, dark red, raised cutaneous plaques or nodules, involving the red skin areas and the white areas (4 of 9 fish) (Figure 1c,d).

Fish were sedated with tricaine methanesulfonate (MS-222, Sigma-Aldrich Co.; St. Louis, MO, USA). Skin samples were collected from all nine fish, either with a punch tool or by surgical excision in the more severe cases. Affected fish were treated with cryotherapy and/or topical treatment with propolis tincture. At follow-up 18 months after sampling, two fish still had lesions but they had not progressed, four fish had a worsening of lesions or recurrence and one had died of unrelated causes. Two fish healed completely after surgery.

Table 1. Although the disease is reported to have a seasonality, this was not observed.

**Diagnostic investigations**

Tissue samples were processed routinely and stained with H&E. Immunohistochemistry was performed with a panel of antibodies used to characterize perivascular wall tumours (PWT) and peripheral nerve sheath tumours (PNST) in domestic animals and fish. Details are described in Appendix S1.

Transmission electron microscopy (TEM) was performed from a selection of formalin-fixed samples from four fish. Samples were placed in 2.5% glutaraldehyde in 0.1M cacodylate buffer, post-fixed with 1% osmium tetroxide, dehydrated and embedded in Durcupan ACM resin (Sigma-Aldrich). From toluidine blue stained semithin sections (0.7 μm), selected ultrathin sections (60 nm) were obtained and stained with uranyl acetate and lead citrate. The ultrastructural observations were made with a Philips EM 208 transmission electron microscope (Philips; Eindhoven, Holland) operating at 80 kV.

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**Figure 2.** Skin; koi carp. Histological features of Hikui. (a,b) Neoplastic cells are elongated and arranged in bundles and rows showing a storiform pattern or in whorls centred on capillaries (Haematoxylin and eosin, H&E, ×20). (c,d) The cell borders are indistinct, whereas the cells have a tiny amount of light eosinophilic cytoplasm, a high nuclear/cytoplasmic ratio, and central, oval plumped nuclei with finely stippled chromatin and single or multiple nucleoli well evident. Anisocytosis and anisokaryosis are moderate. Atypical mitoses are frequent (arrow) (H&E, ×40).
Nested PCR was employed from fresh tissue from one fish that was euthanized, for detection of Cyprinid herpesvirus 1 (CyHV-1) according to published procedures. Histological findings in all cases revealed a dermal nonencapsulated densely cellular and infiltrating neoplasm. It was composed of mesenchymal cells arranged in bundles, rows and whorls, frequently centred on capillaries, supported by a minimal amount of fibrovascular stroma. The neoplastic cells were elongated with indistinct cell borders, a very small amount of light eosinophilic cytoplasm, a high nuclear/cytoplasmic ratio, oval central plumped nuclei with finely stippled chromatin, and single or multiple prominent magenta nucleoli. Anisocytosis and anisokaryosis were moderate. The mitotic rate was 1 per HPF with frequent atypical mitoses (Figure 2). A few inflammatory cells, mainly lymphocytes and melanomacrophages, were present and occasional erythrophores were also detected. The dermal scales were effaced by the neoplastic tissue and totally disappeared. The epidermis showed hydropic cellular degeneration and spongiosis. In two cases there was a focal ulcerative dermatitis. The histological diagnosis was consistent with PWT and a concurrent focal–multifocal ulcerative dermatitis.

Immunohistochemistry showed positive staining for actin either as small groups of cells or mild diffuse staining of cells (Figure 3a). S100 showed specific immunolabelling of rare neoplastic cells (2 of 9 cases), while GFAP and desmin were negative. Positive controls of canine and cyprinid tissues were positive for all of the markers, except for desmin in cyprinid heart.

Electron microscopy showed spindle-shaped neoplastic cells arranged in parallel bundles and occasionally forming a whorling arrangement. Cytoplasmic processes of neoplastic cells were long and slender with well-developed branching, encircling the capillaries or radiating from them (Figure 3b,c). Intercellular junctions were not observed. A thin basal membrane frequently was observed with occasional plasmalemmal vesicles (Figure 3d). The cytoplasm of neoplastic cells contained electron-dense lipid droplets or myelin figures. Nuclei were oval, slightly to moderately indented, with chromatin either dispersed or condensed. Morphologically these cells were consistent with pericytes. Interspersed within the neoplastic cells, occasional chromatophores containing melanosomes or pterinosomes were detected. Viral particles were not found in the analysed samples. Cutaneous lesions tested negative for CyHV-1 by nested PCR.
Discussion

It has been suggested that Hikui disease is caused by a fibrosarcoma or a dermatofibroma protuberances.1 In the cases presenting herein, the histological features, immunohistochemistry and ultrastructure led to a diagnosis of PWT. Perivascular wall tumours are neoplasms deriving from the vascular wall cells, excluding the endothelial lining. Haemangiopericytoma, included in PWT, derives from pericytes that are capillary subendothelial lining cells. In the dog, haemangiopericytoma is diagnosed on the basis of a pericapillary whorling growth. Peripheral nerve sheath tumours and PWT may have overlapping microscopic features; occasional whorls can be found in PNST but are less extensive. Furthermore, pericytes in the dog express vimentin and, variably, pan actin and α-smooth muscle actin. The latter in fish has been used to characterize swimbladder leiomyosarcoma associated with retrovirus.15

The combination of histology and immunohistochemistry is essential for the diagnosis of canine PWT and to differentiate them from PNST.5,9,11 The literature reports for dogs that actin immunolabelling has never been observed in PNST and is rarely found in human cases, while it is frequently expressed in canine PWT.5,10,11 GFAP and S100, which are markers for PNST in the dog and fish, were negative or only rarely expressed in our cases.5–7

In fish species, haemangiopericytomas are rare.12,13 In the cases described here, neoplastic cells showed ultrastructural features typical of PWT and especially of haemangiopericytoma: a whorling arrangement and long, branched cytoplasmic processes that encircled the capillaries, which are as reported for dog and fish.10,12,13

Neoplastic cells lacked pterinosomes – spherical cytoplasmic particles containing pigments – which are abundant in pigmented cell tumours in fish, thus ruling out a pigmented cell origin for this disease.4 “Carp pox”, a typical cutaneous proliferation of viral aetiology (CyHV-1), elicits a severe epithelial hyperplasia. Other virally induced neoplasia of fish caused by a retrovirus are the walleye dermal sarcoma, the hookednose cutaneous fibroma/fibrosarcoma and bicolour damselfish neurofibromatosis.14,15 In fish, detection of retrovirus-like particles by TEM may be due to coincidental expression or selective replicative advantage of mitotically active cells.15 One fish described here tested negative for CyHV-1. Ultrastructure did not detect viral particles. Further studies are necessary to rule out a viral aetiology.

This is the first report dealing with Hikui disease that has provided a conclusive diagnosis of PVT.

Acknowledgments

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References

5. Suzuki S, Uchida K, Nakayama H. The effects of tumor location on diagnostic criteria for canine malignant peripheral nerve sheath tumours (MPNSTs) and the markers for distinction between canine MPNSTs and canine perivascular wall tumours. Vet Pathol 2014; 51: 722–736.

Supporting Information

Additional Supporting Information may be found in the online version of this article.

Appendix S1. Immunohistochemical methods.

Résumé

Contexte – La maladie d’Hikui est une atteinte déformante bien connue de la carpe koi (Cyprinus carpio) affectant primairement les poissons à pigmentation rouge. Elle cause des patchs orange clair à jaune, multifocaux à coalescents, partant des zones cutanées rouges. Certains cas répondent à la chirurgie ou au traitement topique mais les récidives sont fréquentes.

Objectifs – Décrire la présentation clinique et pathologique de la maladie d’Hikui et de ses causes.

Sujets – Neufs carpes koi atteintes appartenant à un propriétaire privé.

Méthodes – Huit poissons ont subi une chirurgie ou une biopsie; un a été euthanasié. Les tissus ont été soumis pour histologie, immunohistochimie et microscopie électronique à transmission.
Conclusions et importance clinique – Toutes les données supportent un processus néoplasique produisant des tumeurs des parois vasculaires. L’immunoréactivité à l’actine des cellules musculaires lisses et les critères ultrastructuraux étaient évocateurs d’une origine pérycytaire (hémangiopericytome). Ceci est la première description de la maladie d’Hikui qui aboutit à une conclusion diagnostique. La nature néoplasique de cette atteinte suggère l’utilité potentielle d’une approche chirurgicale pour la gestion clinique des cas les moins sévères.

Resumen

Introducción – La enfermedad de Hikui es una enfermedad bien conocida de los peces koi (Cyprinus carpio) que afecta principalmente a los peces con pigmentación roja. Produce una descoloración anaranjada a amarillenta multifocal confluyente en placas elevadas empezando en las zonas de color rojo. Algunos casos responden a cirugía o tratamiento trópico, pero es frecuente observar recidiva.

Objetivos – describir la presentación clínica y patológica de la enfermedad de Hikui y su causa

Animales – nueve carpas koi de propietarios privados afectadas por la enfermedad

Métodos – ocho de los peces sufrieron cirugía o biopsia; uno fue eutanasiado. Los tejidos fueron remitidos para estudio histopatológico, inmunohistocquímica y microscopía electrónica de transmisión.

Resultados – cinco peces mostraron signos típicos de la enfermedad de Hikui y 4 peces mostraron una presentación atípica caracterizada por nódulos o placas focales o multifocales, edematosas, de color rojo oscuro. La histopatología demostró la presencia de neoplasias no encapsuladas, infiltrativas y densamente celulares de células fusiformes organizadas en haces, filas y espirales frecuentemente centradas alrededor de capilares. La inmunohistoquímica para actina de músculo liso marcó las células neoplásicas en todos los casos. El análisis ultraestructural mostró células neoplásicas con procesos citoplasmáticos delicados alrededor de las capilares y una fina membrana basal y ocasionalmente vesículas en el plasmasoma.

Conclusión e importancia clínica – toda la información indica que la enfermedad de Hikui es un proceso neoplásico de células perivasculares. La reactividad para actina de músculo liso y las características ultraestructurales son indicativas de un origen en pericitos (hemangiopericitoma). Este es la primera publicación tratando la enfermedad de Hikui que llega a un diagnóstico conclusivo. La naturaleza neoplásica del proceso sugiere el uso potencial de la cirugía en el manejo clínico en los casos menos severos.

Zusammenfassung

Hintergrund – Die Hikui Erkrankung ist eine gut bekannte verunstaltende Erkrankung des Koi Karpfen (Cyprinus carpio), die in erster Linie Fische mit Rotfärbung betrifft. Sie verursacht hell orange bis goldgelbe verfärbte, multifokale bis koaleszierende erhabene Flecken, die von den roten Hautgebieten ausgehen. Einige Fälle werden durch chirurgische Intervention oder durch topische Behandlung besser, aber ein Wiederauftreten kommt häufig vor.

Ziele – Eine Beschreibung der klinischen und pathologischen Präsentation der Hikui Erkrankung und ihre Ursache.

Tiere – Neun betroffene Koi Karpfen, die privaten Hobbyhaltern gehörten.

Methoden – Acht Fische wurden chirurgisch behandelt oder es wurden Biopsien genommen; ein Fisch wurde euthanasiert. Die Gewebeproben wurden zur histologischen und zur immunhistochemischen Untersuchung sowie zur Untersuchung mittels Transmissionselektronenmikroskop eingesendet.

Ergebnisse – Fünf Fische zeigten typische Veränderungen der Hikui Erkrankung, während vier Fische eine atypische Präsenz aufwiesen, die durch fokale oder multifokale, ödömatöse, dunkelrote kutane Plaques oder Knoten charakterisiert war. Histologisch zeigte sich das Bild von nicht eingekapselten, infiltrierenden und dichten zellulären Neoplasmen, die aus Spindelzellen zusammengesetzt waren, die in Reihen und Wirbeln angeordnet waren, die sich häufig um die Kapillaren drängten. Immunhistochemisch wurde mittels markiertem Aktin der glatten Muskulatur in allen Fällen neoplastische Zellen nachgewiesen. Die Ultrastruktur zeigte neoplastische Zellen mit zarten zytoplastischen Fortsätzen in der Umgebung der Kapillaren, eine dünne Basalmembran und gelegentliche Membranbläschen.

Schlussfolgerungen und klinische Bedeutung – Alle Untersuchungsergebnisse bekräftigten das Vorkommen eines neoplastischen Prozesses, durch den perivaskuläre Wandtumoren entstanden. Die Immunreaktion auf Aktin der glatten Muskulatur und die ultrastrukturellen Merkmale waren ein Hinweis auf perizytären Ursprung (Hämagangiopericytum). Es handelt sich hierbei um den ersten Bericht, welcher sich mit der Hikui Erkrankung befasst, und eine eindeutige Schlussfolgerung zulässt. Die Tatsache, dass es sich
Hikui disease koi carp: vascular wall tumour

要約
背景 — 紅疹い症は、主に赤い色素をもつ魚に罹患する、鯉 (Cyprinus carpio)の外見を損なう知られる炎症である。皮膚の赤い部位から始まる、淡いオレンジ色から黄色の多病巣および病巣の形成性病変を生じる。外科手術や外用療法に反応する症例もあるが、一般的には再発する。
目的 — 紅疹い症の臨床および病理学的な所見とその原因を解説することである。
供与動物 — 個人が愛好家が所有する9匹の罹患した鯉
方法 — 5匹の鯉は外科手術あるいは生検を行い、1匹は安楽死され、組織は組織学、免疫組織化学、および透過電子顕微鏡法のために提出された。
結果 — 5匹の鯉は紅疹い症の典型的な変状を示していた一方、4匹は局所性あるいは多病巣性で、浮腫性の暗赤色の皮膚損傷あるいは結節を特徴とする多病巣性の外見を示していた。組織学的には、続発性および局所性、一例に並び、しばしば毛細血管が中心に存在する螺旋状に配列する偽組織細胞により構成される細胞が密に詰まった腫瘍を特徴としていた。超微細構造法では、毛細血管を取り囲む多核の細胞質を持った腫瘍細胞、薄い基底膜、および時折みられる細胞質内小胞が認められた。
結論および臨床的な重要性 — すべてのデータは血管周囲壁腫瘍を形成している腫瘍パーキーであることを支持している。
平滑筋アクチンに対する免疫反応性および超微細構造の特徴は、血管周囲壁腫瘍の特徴であることを示していた。この報告は診断が得られた鯉の症例における、この疾患の臨床的および病理学的な診断における、外科的なアプローチの潜在的な有用性を示唆している。

摘要
背景 — 众所周知、Hikui病是一种影响锦鲤美观的疾病，主要感染鱼的红色皮肤区域。该病从红色区域开始，皮肤变为橙色或金黄色，引起更多病灶或斑块融合。有些病例需要手术或局部治疗，但经常复发。
目的 — 描述Hikui病临床症状和病理学表现以及病因。
動物 — 9条属于私人繁殖的Hikui患病锦鲤。
方法 — 8条鱼进行外科手术或活组织取样; 其中一条进行安乐死，将其组织组织进行组织学、免疫组化和透射电子显微镜检查。
結果 — 5条鱼表现出Hikui病的特征病变，4条鱼表现为单独病变或多灶性病变，水肿、深红色皮肤斑块或结节等非典型特征病变。组织学表现为无明显界限的、浸润性、密集的肿瘤细胞，由纤维性、或常见螺旋状围绕毛细血管周圈的纤维和细胞组成。所有病例免疫组化用平滑肌肌动蛋白标记肿瘤细胞。超微结构显示肿瘤细胞长的细胞质包围毛细血管、薄基底膜，偶尔围绕基底膜。
総合と臨床意义 — 所有的数据显示，该病为血管壁肿瘤。平滑肌肌动蛋白的免疫反应和超微结构特征。提示为外膜细胞源性（血管外皮细胞瘤）。这是第一篇有关Hikui病确诊方面的报道，对于临床少数需要手术治疗的严重病例，肿瘤的性质对于手术通路的选择具有提示意义。