An unusual cuticular tumor-like growth on the abdomen of a lobster, *Homarus americanus*

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**A R T I C L E   I N F O**

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**A B S T R A C T**

Tumors are rare in crustaceans, and whereas a few have been reported from the lobster *Homarus americanus* none have been adequately described. A lobster with an unusual, large, blue-colored tumor-like growth projecting laterally outward from the first abdominal somite was caught off Stonington, Maine, USA. The growth was rugose and covered by a relatively normal appearing cuticle with dispersed focal melanization. The underlying stroma consisted of an internal area of rescaffolded fibrous connective tissue, restructured muscle fibers, few arterioles, and an epidermal area comprised of columnar, highly vacuolated epithelial cells. No infectious pathogens or unusual inclusions were observed with microscopy and no eukaryotic pathogens were detected via molecular sequencing. Given the nature of the histology and the appearance of the growth, we identify the mass as a benign papilliform hamartoma that likely originated as a result of abnormal wound repair possibly initiated around ecdysis. This represents the first tumor-like hamartoma reported from a lobster, and the second hamartoma reported from a crustacean.

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1. Introduction

Reports of tumors are rare in crustaceans. A hamartoma has been reported from postlarval shrimp found at heavily polluted sites (Overstreet and Van Devender, 1978) and four cases of neoplasms have been reported, with three from shrimp and one from a crab (see review by Brock and Lightner, 1990). The hamartomas from shrimp were observed in a number of postlarvae collected from sites with heavy pollution. They presented as muscular overgrowths protruding through the abdominal somites (Overstreet and Van Devender, 1978). For the neoplasms, three have been reported in soft tissues: a lymphosarcoma of the hematopoietic tissue, restructured muscle fibers, and an epidermal area comprised of columnar, highly vacuolated epithelial cells. No infectious pathogens or unusual inclusions were observed with microscopy and no eukaryotic pathogens were detected via molecular sequencing. Given the nature of the histology and the appearance of the growth, we identify the mass as a benign papilliform hamartoma that likely originated as a result of abnormal wound repair possibly initiated around ecdysis. This represents the first tumor-like hamartoma reported from a lobster, and the second hamartoma reported from a crustacean.

2. Material and methods

The lobster was packed in seaweed and blue ice and shipped to the Virginia Institute of Marine Science by overnight express where it arrived alive and in good condition. It was assigned identification number ME040 as part of a larger series of animals from Maine...

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(the 100 Lobster project: Shields et al., 2012). The animal was grossly examined and measured, and hemolymph was aseptically drawn from the juncture of the basis and the ischium of the right fifth walking leg after swabbing with 95% ethanol. A few drops of hemolymph were placed onto marine agar (Difco 2216) and TCBS agar (thiosulfate citrate bile salts, Difco) to assess possible bacterial infection. After examining a hemolymph smear with a compound microscope, and saving hemolymph into 95% ethanol, the animal was killed by injection of ice-cold saturated KCl into the ventral nerve ganglia (Battison et al., 2000). Immediately after death, the carapace was removed and tissue samples from several organs were dissected and placed in Z-Fix (Fisher Scientific) fixative for 48 h. The tumor-like mass was carefully cut free from the abdomen, bisected along a frontal-lateral plane and pieces were placed into fixative. Tissues medial to the mass were also dissected from the host and placed into Z-Fix. All of the tissues were processed through routine histological procedures, cut at 5–6 μm, and stained with Mayer’s hematoxylin and eosin. Hard tissues including pieces of gill, chitinous portions of the mass and adjacent cuticle were decalcified in an EDTA-citric acid solution as in Luna (1968) prior to processing through paraffin histology.

To confirm that no protistan or metazoan, such as the intestine of an aberrant rhizophoran, were present in the mass, a degenerate primer set targeting a portion of the SSU gene was applied to a genomic DNA sample extracted directly from the tumor tissue. Briefly, a 1 mm² sub-sample (obtained roughly 3–4 mm inwards from the outer edge of the tumor) was excised from a section of the mass preserved in 95% ethanol. The tissue sub-sample was placed in molecular grade water for 30 min to allow for removal of residual ethanol prior to homogenization in 180 μl of ATL lysis buffer and 20 μl proteinase K (Qiagen, Valencia, CA). Total genomic DNA was subsequently extracted using a DNeasy Tissue Kit (Qiagen) following the manufacturer’s protocol and stored at −20°C. A generalized euakaryotic PCR primer set, nSSU A (5’-AACCTGRTGTGATCTCGTCGCAGT-3’) and nSSU B (5’-GATCCTTCGGCCAGTTACCTAC-3’) (modified from Medlin et al., 1988), was used to amplify part of the SSU rRNA gene. Each 20 μl reaction contained 10 mM Tris–HCl, pH 8.3, 50 mM KCl, 1.5 mM MgCl₂, 0.2 mM of each dNTP, 0.2 μM of each primer, 0.5 unit of Taq polymerase (Applied Biosystems, Foster City, CA, USA), and 1 μl genomic DNA. Amplifications were performed with an initial denaturation temperature of 94°C for 4 min, followed by 35 cycles at 94°C for 60 s, 50°C for 60 s, 68°C for 90 s, with a final elongation step at 72°C for 2 min. The amplified products were treated with shrimp alkaline phosphatase (SAP) and exonuclease I (Exo I) (Amersham Biosciences, Piscataway, NJ, USA) prior to sequencing. PCR products were bi-directionally sequenced using the Big Dye Terminator kit (Applied Biosystems) with M13 primers included and one eighth the recommended concentration of Big Dye. The sequencing reaction products were precipitated using ethanol/sodium acetate and re-suspended in 18 μl of Hi-Di formamide (Applied Biosystems) and 10 μl of each were electrophoresed on an ABI 3130 Prism genetic analyzer (Applied Biosystems). Eight clones from a sample of the tumor-like mass were prepared and sequenced bi-directionally. Resulting SSU sequences were analyzed using CodonCode Aligner (version 3.7.1.1) and compared to those deposited in GenBank using basic local alignment search tool (BLAST) searches (Altschul et al., 1990).

3. Results

The lobster was a mature, non-ovigerous female, 83.1 mm carapace length. A large, rughose, bright blue tumor-like mass projected laterally from the left side of the first abdominal somite (Fig. 1A and B). The tumor was 25.2 mm in breadth, 16.3 mm high, and appeared to have a basal attachment diameter of approximately 6.0 mm. The mass arose directly from the abdomen, with a slight deformation of the left, posterior cephalothorax adjacent to the affected somite. There was no apparent disruption to the cuticle around the abdomen other than the area affected. The mass was firm, not flexible, and had a cauliflower-like appearance (Fig. 1C and D). Three small bivalve larvae (spat) were located within the folds of the mass, and small white flecks on the surface were diatoms adhering to the cuticle.

Gross examination of cross sections of the mass (Fig. 2A) indicated that it was comprised primarily of fibrous connective tissue. While convoluted, the cuticle appeared relatively normal except some regions were thickened. Some apical regions had little epicuticle present, and dispersed focal melanization was evident on the dorsal surfaces (Fig. 2B). Histologically, there was no indication of hyperchromatosis in the epidermis. The epidermis and subepidermis within the tumor were continuous with the adjacent unaffected regions. The underlying restructured epidermis was comprised primarily of columnar epithelial cells, with goblet vacuoles at their apices, overlying a stroma of fibrous connective tissue with scattered muscle fibers, and few arterioles. No tegmental glands, setal inserts, or pigment cells were present in the epidermis (Fig. 2B–D). Hemocyte infiltrates composed primarily of granulocytes were observed within the stroma (Fig. 3A–C). Mediially, within the affected somite, the epithelial cells had a normal non-vacuolated, cuboidal appearance, but the muscle and connective tissues showed signs of rescaffolding as if from wound repair. The fibrous connective tissue cells appeared hypertrophied, and in some cases they possessed apoptotic nuclei (Fig. 3D). No mitotic figures were observed in the affected tissues. Deeper within the somite, the histology of the muscle was normal. Histological examination of the main internal organs (hepatopancreas, heart, muscle, ovary, midgut, gill, eyes, cuticle, hemapoietic tissues) did not present any unusual findings. The hepatopancreas was normal, without large numbers of reserve inclusion cells, and the fixed phagocytes surrounding the hepatopancreatic arterioles were not activated. Several gregarines (Porospora gigantea) were present within the midgut and an acanthocephalan cystacanth (Polymerus botulis) was present in the fibrous connective tissues adjacent to the submucosa. A single, distinct granulomatous lesion was present in the submucosa of the midgut. The eyes and eyestalks were normal, and there was no indication of idiopathic blindness as in Maniscalco and Shields (2006). The lobster was in the intermolt stage as determined by microscopic examination of the cuticle around the eyestalks and from an unaffected portion of the abdominal somite.

There were no microscopic signs of pathogens present within the tumor or the adjacent tissues nor did bacterial colonies grow on the agar plates. The molecular sequencing did not find evidence for the presence of other eukaryotic organisms in the tumor. A single amplification product was produced using the general eukaryotic primer set and genomic DNA sample from the mass. Consensus sequences could not be generated from the forward and reverse sequencing reactions as there was no overlap in the sequences.
However, all sixteen partial SSU sequences generated in this study were 100% identical to the 5' and 3' ends of *H. americanus* SSU sequences in GenBank (AF235971 and FJ174917, respectively). Sequences representing the 5' and 3' ends of *H. americanus* SSU
gene were deposited in GenBank under accession numbers KF578382-KF578397.

4. Discussion

We identify the mass as a benign, papilliform harmatoma, in the broad sense, as a benign, focal mass of tissue arising from an abnormal growth of cells. However, it is not clear if this is a harmatoma or a true neoplasm. No mitotic figures were observed within the affected tissues, but mitosis is controlled by molting hormones in Crustacea (see Sparks and Lightner, 1973) and the animal was in intermolt. Moreover invertebrate neoplasms can have low mitotic indices (Odintsova et al., 2011). The extensive rescaffolding of fibrous connective tissue and muscle fibers, the lack of tegmental glands and setal inserts, and the highly vacuolated epithelia indicate that the harmatoma was likely induced by injury. The lobster case is similar to the papilliform neoplasm reported from F. aztecus by Sparks and Lightner (1973). In the shrimp, the tumor was an uncolored cauliflower-like growth on the sixth abdominal somite. The histology of the neoplasm in shrimp is strikingly similar to that observed here with the exception that tegmental glands and setal inserts were not observed in the neoplasm from the lobster.

The hamartoma was not caused by an active infection or aberrant infection by a eukaryotic organism, nor were inclusions present within the tissues that might indicate a viral etiology. We speculate that it arose as a response to internal bleeding or to wound repair to the abdominal somite, possibly occurring around ecdisis. If the hamartoma was initiated around ecdisis, then the rugose, cauliflower-like nature of it may have formed as a herniation of new cuticle and epidermis due to the turgor of internal pressure from imbibition during molting as has been observed in shrimp (Lightner et al., 1987). However, the lack of tegmental glands within the stroma is unusual and suggests that the epidermis did not simply unfold improperly during ecdisis. The intensely blue coloration is also noteworthy and may be due abnormal levels of free astaxanthins in the cuticle that would decrease the red coloration (Tlusty and Hyland, 2005), but that remains to be determined.

It is not clear if lobsters with similar tumor-like growths would survive molting because the hamartoma lacked tegmental glands, which provide lubricants during molting (Talbot and Demers, 1993), and a large growth such as this would likely adhere to the new instar during molting. However, this animal may have survived molting, because the lesion was adjacent to the less sclerotized arthrodial membrane separating the abdomen from the cephalothorax, close to the ecdisial suture at the juncture of the cephalothorax and abdomen. If additional animals with this condition are found alive, we suggest that they be held through a molt cycle to determine if they can survive molting.

Hamartomas are extremely rare in lobsters. We know of three anecdotal reports of these intensely blue growths, including the present case, and these cases were all reported through the email listserver for Crustacea (crust-l@vims.edu) or through electronic media to groups focused on lobster health issues. Additional specimens would no doubt shed more light on the etiology and rarity of this condition.

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Fig. 3. Histological features of the stroma within the tumor from the lobster. (A) Stroma comprised of loosely arranged fibrous connective tissue (c), sparse muscle fibers (m) attached to the cuticle (c), and cellular infiltrates (arrows). Bar = 500 μm. (B) Hemocyte infiltrates in the stroma. Bar = 100 μm. (C) Hemocyte infiltrates comprised primarily of granulocytes in the stroma. Note the apoptotic nuclei (arrows) within the hypertrophied fibrous connective tissues. No mitotic figures were present in the stroma. Bar = 50 μm. (D) Tissues in the abdominal somite subjacent to the tumor with muscle fibers and fibrous connective tissue showing rescaffolding. Bar = 100 μm.
References


