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DISEASE IN WILDLIFE OR EXOTIC SPECIES

Putative Rodlet Cell Neoplasms in the Livers of Two White Suckers (*Catostomus commersonii*)

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Summary

Although discovered more than a century ago, piscine rodlet cells (RCs) remain somewhat of a mystery to scientists in terms of their origin and function. Initially described as parasites, and later as potential secretory cells, the prevailing theory is that RCs are leucocyte-like cells that possess pathogen defence capabilities. The current case report involves a novel type of neoplasm discovered in the livers of two adult female white suckers (*Catostomus commersonii*) that were collected as part of a survey of fish from the St. Mary's River Area of Concern, in which sediment contaminated by polyaromatic hydrocarbons has been associated historically with a high prevalence of liver neoplasms in white suckers. The two tumours in this study were investigated by light microscopy, histochemical staining, immunohistochemical labelling for S100 protein and transmission electron microscopy. The evidence from these investigations suggests that these neoplasms may be derived from de-differentiated RCs or RC precursors. The unanticipated existence of these solid mesenchymal-like tumours may prompt a reassessment of the current dogma regarding the physiological function of RCs.

Keywords: Catostomus commersonii; liver neoplasia; rodlet cell; white sucker

Introduction

Rodlet cells (RCs) are universally characterized as 'enigmatic' because the precise ontogeny, physiological function and even essential nature (i.e. host or non-host) of these fish-specific cells have not been established definitively, notwithstanding the many decades of scientific scrutiny that followed their original discovery in the late 19th century (Manera and Dezfuli, 2004). Initially described as unicellular parasites *Rhabdospora thelohani* (Thélohan, 1892), and later postulated to have secretory or sensory functions (Leino, 1974; Morrison and Odense, 1978; Imagawa *et al.*, 1998; Mendonca *et al.*, 2005; DePasquale, 2014a,b), the current consensus suggests that RCs are endogenous rather than parasitic and possibly represent a unique component of the piscine innate pathogen defence system (Imagawa *et al.*, 1990; Reite and Evensen, 2006; Dezfuli *et al.*, 2007a,b,c, 2016; Matisz *et al.*, 2010). Work conducted at several institutions has demonstrated associations between the abundance and microanatomical location of RCs (alone or in combination with eosinophilic granule cells, macrophages, lymphocytes and other leucocytes) and the presence of parasitic helminth, myxozoan or microsporidian organisms (Leino, 1996; Dezfuli *et al.*, 2000, 2003, 2007a,b,c; Mazon *et al.*, 2007). Increased numbers of RCs have also been observed in certain tissues from fish that were exposed to various chemical or physical contaminants, endogenous hormones or other stressors (Iger and Abraham, 1997; Dezfuli *et al.*, 2006; Giari *et al.*, 2006, 2007; Jordanova *et al.*, 2007; Poltronieri *et al.*, 2009; Jovanovic *et al.*, 2014; Schultz *et al.*, 2014). RCs have been observed in fish as early as 5 days post-fertilization (Mazon *et al.*, 2007) and descriptions of RC developmental stages have been published (Leino, 1974; Kramer

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and Potter, 2002; Siderits and Bielek, 2009; Vigliano *et al.*, 2009; Dezfuli *et al.*, 2010a,b; Matisz *et al.*, 2010; Laura *et al.*, 2012; Abd-Elhafeez and Soliman, 2016). Tissue migration of RCs concomitant with maturation has been observed ultrastructurally (Leino, 1974; Laura *et al.*, 2012), although RCs in culture did not demonstrate motility (DePasquale, 2014a,b). Morphological studies have reported that RCs are capable of discharging their cytoplasmic contents (Leino, 1974; Smith *et al.*, 1995a,b,c; Dezfuli *et al.*, 2000; Kramer and Potter, 2002; Laura *et al.*, 2012; DePasquale, 2014a,b) and several chemical substances that possess anti-pathogen or cytotoxic properties (e.g. tumour necrosis factor [TNF]-α, piscicidin, peroxidase activity, protective glycoproteins, inducible nitric oxide synthase, lysozyme and matrix metalloproteinase-9) have been demonstrated immunohistochemically or by other means within RCs (Silphaduang *et al.*, 2006; Srivastava *et al.*, 2012; Yashpal *et al.*, 2014; Abd-Elhafeez and Soliman, 2016; Bosi *et al.*, 2017) or as components of rodlet granules (Iger and Abraham, 1997).

However, despite some categorical claims that the RC is a unique form of leucocyte-like immune cell, the supporting evidence for this is still largely circumstantial. High concentrations of RCs are frequently found in tissues where no pathogens or signs of inflammation are apparent (Smith *et al.*, 1995a,b,c; Arellano *et al.*, 2001; Mendonca *et al.*, 2005; Abd-Elhafeez and Soliman, 2016) and conversely, RC responses in certain pathogen/host combinations can be variable or non-existent, even when an extensive inflammatory response or infectious challenge is present (Mazon *et al.*, 2007; Dezfuli *et al.*, 2010a,b). Likewise, expulsion of RC cytoplasmic contents, which is a proposed pathogen defence mechanism, is often described to occur in the apparent absence of provocation by pathogens or other stressors (Bielek, 2002; Kramer and Potter, 2002; Laura *et al.*, 2012). No phagocytic capability has been reported for RCs, and unlike the granules of fish leucocytes, once expelled, the rodlet structures themselves seem to be inert in terms of interactions with surrounding tissues (Barber *et al.*, 1979; Bielek, 2002). To date, no molecular investigations have been performed to assess the leucocyte status of RCs, although several studies have compared the DNA content of RC nuclei to that of other fish cell nuclei (Barber and Westermann, 1983, 1985, 1986), the results of which seem to support the contention that RCs are of host origin. Most significantly, there are no conclusive study results that demonstrate that fish pathogens have been killed, damaged or deterred by encounters with RCs.

The light microscopical and ultrastructural appearance of RCs has been described in numerous publications, as reviewed by Manera and Dezfuli (2004). RCs are present ubiquitously in a wide variety of freshwater, marine and euryhaline fishes (Morrison and Odense, 1978) and have been observed in numerous external and internal tissue types, most frequently associated with epithelial, endothelial or mesothelial cell layers and their respective basement membranes. They can be found individually when present in small numbers, while at the other extreme, they may be observed palisading along the endothelial linings of blood vessels or the luminal or serosal surfaces of viscera; in the latter case, RCs may almost entirely supplant the native epithelial or mesothelial cells. Although oriented most frequently with their apical poles directed toward luminal surfaces when located within epithelia, the alignment of RCs can be more haphazard in connective tissues (Morrison and Odense, 1978). The basic morphological appearance of RCs in haematoxylin and eosin (HE)-stained sections is that of an approximately 10–15 μm long by 7–10 μm wide oval or oblong cell that has a relatively thick, slightly refractile eosinophilic limiting membrane, eccentric basal nucleus, clear or translucent cytoplasm and several or more slender eosinophilic cytoplasmic spicules called rodlets. The rodlets are typically aligned with the longitudinal axis of the cell and taper as they extend toward the apex. Visualized ultrastructurally, the RC capsule is thick and fibrillar and covered externally by a thin plasmalemma. The capsule is interrupted by a single apical pore, through which tufts of cytoplasmic material may protrude and adjacent to which desmosomal attachments to neighbouring cells (but not other RCs) may be present. The oval to irregular basal nucleus features internal and peripheralized heterochromatin, and the cytoplasm contains mitochondria that are concentrated apically, in addition to small vesicles that are scattered throughout. Each rodlet itself consists of a dense core surrounded by a bulbous sac, the greatest diameter of which is oriented toward the basal pole of the RC. The authors of this paper and others have observed that the light microscopical appearance of RCs varies among fish species and anatomical locations; this manifests chiefly as differences in size (especially length) and overall shape and in the thickness of the fibrillar coat and rodlets (Morrison and Odense, 1978; Manera *et al.*, 2009). Identification of RCs is usually based on their characteristic appearance in routine HE-stained sections, but a few histochemical and immunohistochemical biomarkers have been reported. For example, cytoplasmic granules (rodlets) of RCs are consistently highlighted by the periodic