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Neoplastic diseases of marine bivalves

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ABSTRACT

Two types of prevalent neoplastic diseases have been described in marine bivalves of commercial interest: disseminated neoplasia (DN) and gonadal neoplasia. The first involves the excessive proliferation of abnormal cells with unknown origin (probably of hemic source in some cases/species), disseminating through the circulatory system and infiltrating the connective tissue of various organs; the second consists of an abnormal proliferation of undifferentiated germinal cells of the gonad. These two types of bivalve neoplasia fit the criteria of malignant tumors: pleomorphic and undifferentiated cells, rapid and invasive growth, abundance of mitotic figures, metastasis and progressive development often resulting in the death of the affected individual. Different causes have been suggested regarding etiology: genetic alterations, virus, retrotranspons, and contaminants, although it could depend on the mollusk species; evidence of horizontal transmission of clonal cancer cells as the cause of DN spreading in clam *Mya arenaria* populations has been recently reported. In some species and populations, the neoplastic disorders affect only a few individuals, but in others reach high prevalence. Among the diagnostic methods, DN has been detected by histology and cytologic examination of hemolymph, and with developed specific antibodies. Recently, flow cytometry has also been applied, allowing detecting DNA quantity alteration. Several studies reported many genes and pathways critically involved in neoplastic transformation in *Mya arenaria*, *Mytilus* spp. and *Ostrea edulis*. These genetic studies will allow the development of diagnosis by PCR which can be used in biomonitoring studies.

1. Introduction

Neoplasms are growth disturbances characterized by excessive, abnormal proliferation of cells, independent of normal-regulating mechanisms of the animal, and persisting after termination of the stimulus that initiated growth (Sparks, 1985). Neoplasms are either malignant (progressive growth spread to distant sites, terminating in death) or benign. The list of different types of benign and malignant neoplasia described in bivalve mollusks is long (Pauley, 1969; Sparks, 1985; Sindermann, 1990), but just some malignant types have raised mollusk industry concern. Two predominant types of malignant neoplasia have been described in marine bivalves: disseminated neoplasia (DN) and gonadal neoplasia. Disseminated neoplasia consists of proliferation of abnormal circulating cells of unknown origin but probably with hemic origin in some cases/species; gonadal neoplasia consists of proliferation of undifferentiated germ cells (Barber, 2004). Since the initial description of a proliferative neoplastic disease in oysters *Crassostrea virginica* and *Crassostrea gigas* by Farley (1969a), similar diseases have been reported in other species of marine bivalves from 4 continents and all oceans except the Artic (Peters, 1988; Elston et al., 1992; Landsberg, 1996). In some species and populations DN is detected in few individuals but in others reach high prevalence associated with significant mortalities, as in mussels *Mytilus trossulus* from the northwestern Pacific coast of America, in cockles *Cerastoderma edule* from the western Atlantic coast of Europe, in softshell clams *Mya arenaria* from the northeastern Atlantic coast of America (Elston et al., 1992), and in Baltic clams *Macoma balthica* from the Chesapeake Bay (Christensen et al., 1974) and the Baltic Sea (Pekkarinen, 1993; Thiriot-Quiévreux and Wolowicz, 1996). Disseminated neoplasias are characterized by the presence of large, anaplastic cells in the connective tissue, blood vessels, and sinuses of multiple organs (Peters, 1988; Elston et al., 1992; Barber, 2004). The idea that these defining characters are shared by neoplasias with different tissue origins (different neoplasia types) should not be discarded. The disease is progressive and the replacement of normal cells by the neoplastic cells (involving loss of the normal architecture of tissues and organs) often cause the death. However, remission of neoplasia, at least temporally, was detected in some mussels *Mytilus edulis*, which developed a host response against disease (Elston et al.,

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1988a), and also in some clams *M. arenaria* (Brousseau and Baglivo, 1991).

Gonadal neoplasia or germinoma was first described in clams, *Mercenaria mercenaria*, by Yevich and Barry (1969), and further in clams, *M. arenaria* (Brown et al., 1977). The highest prevalence was found in these two species, although the disease was reported in 14 species of bivalves including bay scallops *Argopecten irradians* (Peters et al., 1994). More recently, gonadal neoplasias have been described in mussels, *Mytilus galloprovincialis* (Alonso et al., 2001), razor clams, *Ensis magnus* (=*arcuatus*) (Darriba et al., 2006) and *Ensis siliqua* (Ruiz et al., 2013a), and the invasive mussel, *Xenostrobus securis* (Pascual et al., 2010).

Reviews of disseminated neoplasia (Peters, 1988; Elston et al., 1992) and gonadal neoplasia (Peters et al., 1994) in bivalves were published before. Barber (2004) revised both neoplasia types. In recent years, there have been more reports on disseminated neoplasia than gonadal neoplasia; most studies have focused on the etiology and genetic basis of the disease while others on the diagnosis of disseminated neoplasia based on ploidy alterations, using flow cytometry. The aim of this review is to provide an ovewrview of the knowledge on these two predominant neoplastic diseases affecting mollusks of commercial interest, with emphasis in literature published after the review by Barber (2004).

2. Disseminated neoplasia (DN)

2.1. Description

2.1.1. Morphological characteristics

Neoplastic cells of bivalves affected by DN share morphological characteristics. They are large cells with a rounded or oval shape and a high nucleus: cytoplasm ratio; their nuclei are round or pleomorphic with patent nucleoli and a high frequency of mitotic figures. Neoplastic cells are observed in the connective tissue of multiple organs and in vessels and sinuses of the circulatory system (Fig. 1). These cells showed absence of pseudopodia and minimal cohesion in fresh preparations (Elston et al., 1992). Some ultrastructural characteristics are also shared among affected bivalve species; they are anaplastic cells with similar characteristics to vertebrate tumor cells; the diversity of cellular organelles is poor in the cytoplasm, which contains swollen mitochondria, ribosomes, vesicles and cisternae of endoplasmic reticulum, small vacuoles and altered Golgi complexes (Fig. 2) (Farley, 1976; Lowe and Moore, 1978; Mix et al., 1979; Auffret and Poder, 1986; Smolarz et al., 2006; Díaz et al., 2011; Carballal et al., 2013).

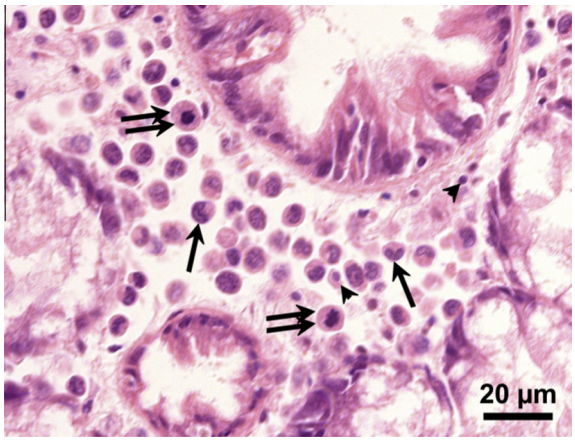

Fig. 1. Light micrograph of a histological section through the digestive gland of a cockle *Cerastoderma edule* showing neoplastic cells (arrows) in the connective tissue. Note mitotic figures (double arrow) and normal haemocytes (arrowheads).

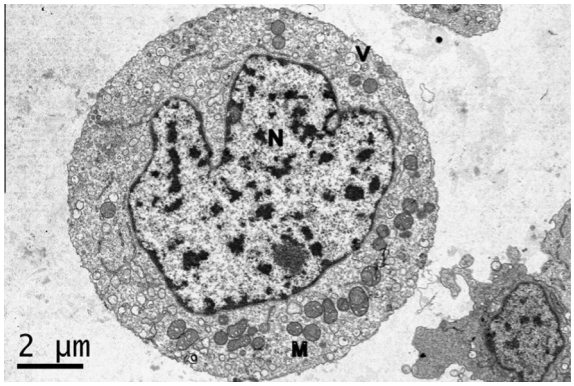

Fig. 2. Transmission electron micrograph of a neoplastic cell of *Venerupis aurea*. N: nucleus; M: mitochondria; V: vesicles.

Several researchers denominated atypical hemocytes to the neoplastic cells based on the probable hemocytic origin of DN. Two morphologically different types of neoplastic cells were distinguished in mussels *Mytilus* spp. (Lowe and Moore, 1978; Mix et al., 1979; Moore et al., 1991), in the softshell clam, *M. arenaria* (Brown et al., 1976) and in the Olympia oyster, *Ostrea lurida* (Mix, 1975). Similarities and differences in neoplastic cell characteristics of these bivalves were reviewed by Elston et al. (1992). Mix et al. (1979) and Lowe and Moore (1978) suggested that the two cell types represent the two extreme morphological expressions of a single cell line. Later, Moore et al. (1991) suggested that the two cell types could represent two distinct cell lines as demonstrated by the pentaploid and tetraploid forms of neoplastic cells observed in *Mytilus* from Puget Sound. Carballal et al. (2001) also reported two types of neoplastic cells in cockles *C. edule* affected by DN. Most of the affected cockles had neoplastic cells similar to those previously described (Twomey and Mulcahy, 1984; Poder and Auffret, 1986) but another type of neoplastic cell was seen in a few cockles. The latter were smaller and had round to oval nuclei with a single nucleolus; they were more tightly packed in the connective tissue than the former neoplastic cells. These two different neoplastic cells could represent two types of DN. Recently, Carella et al. (2013) studied the cytomorphology and the proliferative behavior, assessed by the proliferating cell nuclear antigen (PCNA) of atypical hemocytes in *M. galloprovincialis* and *C. edule*. Five morphological types of atypical hemocytes were observed in neoplastic mussels. Two major types correspond to the previously described A and B neoplastic cell types (Lowe and Moore, 1978; Green and Alderman, 1983), and the other three minority types (indented/cleaved cells, binucleated cells and multinucleate giant cells) were considered A or B cell subtypes, according to the nomenclature used for similar cells detected in some lymphoproliferative disorders of higher vertebrates. The type A was predominant in early stages of the disease, and the type B in advanced neoplasias. PCNA in A cells was mostly confined to the cytoplasm (early neoplasia), whereas B cells had mostly nuclear positivity (advanced neoplasia). Cellular morphology was different in diseased cockles, with only one major type of neoplastic cells, although multinucleated giant cells, binucleated cells and indented cells were also recognized. Cockle neoplastic cells showed predominantly a nuclear PCNA positivity in the early phases of disease, with cytoplasm positivity in more advanced stages. These findings suggest distinct histo-pathogenetic pathways for DN in mussels and cockles. The results of this study on *M. galloprovincialis* support the hypothesis of a single neoplastic cell line based on the disease staging and the different PCNA staining of neoplastic cells.