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LETTER TO EDITOR

Tumors in invertebrates: molluscs as an emerging animal model for human cancer

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To the Editor

We read with interest the recent paper by Tascedda and Ottaviani (2014) about the occurrence of tumors in invertebrates. In order to further reinforce your statements that "histological and molecular biology studies have proved the existence of tumors in invertebrates" we would like to add to some insights focusing on molluscan neoplasia, an emerging animal model for human cancer (Walker, 2011; Carella *et al*., 2013).

It is known that neoplasia is a pathological process characterized by an overgrowth of a new tissue in the context of a pre-existing one, and consists of atypical cells, a term which incorporates the sum of the differences in morphological, biochemical and functional features of cancer cells relative to normal cells (Carella *et al*., 2013). Furthermore, neoplastic tissue is characterized by a self-growing, progressive, irreversible and non-finalistic behavior (Dianzani, 2005; De Vico and Carella, 2012).

Two predominant types of neoplasia have been reported in marine molluscs, viz. disseminated neoplasia, also called leukaemia or hemic neoplasia (HN), and gonadal neoplasia (Carella *et al.,* 2009).

In HN neoplastic cells are represented by atypical hemocytes, which display high nucleus to cytoplasm ratios, diffuse chromatin patterns and pleomorphic nuclei, and usually infiltrate tissues and organs of affected individuals (Auffret and Poder, 1986; Villalba *et al*., 2001). Since the initial description of the disease (Farley, 1969), its cause has not been clearly defined (Barber, 2004). Viral infection, genetic profile, environmental changes and anthropogenic pollution have been proposed as the causative factors. The prevalence of the disease ranges from 0.5 to 73.3 % , according to the species considered (*e.g., Crassostrea virginica*, *Mytilus* spp. and *Ostrea edulis*) and geographical origins of molluscs. In the affected bivalves HN have been for a long time considered a phenotypically similar proliferative disease in the different shellfish species involved (Walker *et al*., 2011). However, differences in neoplastic cell morphology, along with descriptions of neoplastic hemocyte subtypes, have frequently contradicted this assumption (Lowe and Moore, 1978; Green and Alderman, 1983). Recently, we described at last two different types of leukemia in two different bivalve species (*Mytilus galloprovincialis* and *Cerastodema edule)*, showing distinctive morphological and histo-pathogenetic behaviour of cells (Carella *et al*., 2013). In particular, in mussels, two predominant types of neoplastic cells (A and B) have been described, differently to common cockle where one population of cells have been observed; atypical cells in the respective species also showed a distinct pattern of PCNA (Proliferating Cell Nucler Antigen) expression, nuclear or cytoplasmic (Carella *et al*., 2013). Such difference could be indicator of a different mechanism of neoplastic cells initiation/progression in early and advanced phase of the disease, respectively, as also strongly supported by Diaz *et al*. (2013).

Gonadal neoplasia is mainly represented by germinoma, which consists of a proliferation of atypical germ cells. Germinoma has been described in several species of marine bivalve molluscs, and most consistently in some populations of *Mercenaria mercenaria*, *Mya arenaria* and razor clam, *Ensis arcuatus* (Barber, 2004; Darriba *et al*., 2006). In the above species, the prevalence of the disease in a given population could remain underestimated particularly in early cases, where neoplastic cells may still go undetected if the tissue section examined does not happen to contain them (Barber, 2004). In fact, the probability of correctly diagnosing the presence of gonadal neoplasia in molluscs increases with disease progression (Carella *et al.,* 2009). Three evolutive stages of the disease could be observed in *M. arenaria* (Barber, 1996; 2004), and four stages in *Mercenaria* spp. (Bert *et al*., 1993), according to the percentage of gonadal follicles involved and the extent of tissue invasion. Although the aetiology or causes of neoplasia remains unclear, pollution by carcinogenic agents is implicated in the heavily exploited littoral zones of coastal waters. Germinoma have been described also in the gastropod *Patella coerulea*, accompanied by other gonadal developmental disorders (Carella *et al*., 2009).

Many genes and pathways critically involved in neoplastic transformation and metastasis are

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evolutionarily conserved in molluscs. Some molecular evidence regarding stress biology and relationships to human biology is available in relation to the function of p53 superfamily members in bivalves. In particular, literature reports p53 (which is among the best known molecules involved in vertebrates carcinogenesis) is demonstrably involved in both bivalve HNs and germinoma (Olberding *et al.,* 2004; St-Jean *et al*., 2005; Walker *et al.,* 2011) Both structurally and functionally, bivalve p53 family proteins are the most highly conserved members of this gene superfamily so far identified outside of higher vertebrates and invertebrate chordates (protein sequences are 67 - 69 % conserved with human p53). However, while in vertebrates p53, p63 and p73 originating from different genes, isoforms of p53 of bivalve molluscs are splice variants of a single gene (Van Beneden *et al.,* 1997; Kelley *et al*., 2001; Muttray *et al.,* 2005, 2007; Goodson *et al*., 2006). The p53 protein was detected in tumor cells of molluscs *Mytilus edulis, Mytilus trossulus, M. arenaria, Spisula solidissima, Crassostrea rhizophoae and Crassostrea gigas.* In particular, a homologue of the human Hsp53 protein was cloned and characterized in *M. arenaria* affected by HN (Kelley *et al*., 2001) and presents a domain II-V DNA-ligand, a transactivation domain and a domain MDM2 stored for 73 % compared to the human p53, suggesting that the molecular mechanisms that regulate the transcription of the p53 gene in mollusks are similar to those involved in the human gene (Kelley *et al.,* 2001). Furthermore, in neoplastic hemocytes of *M. arenaria*, the mortalin (a member of the Hsp70 family whose expression is strongly correlated with the levels of expression of p53 in sick bivalve) (Wadhwa *et al.,* 2002; Siah *et al.,* 2008) sequesters inactivated p53 in the cytoplasm. A similar phenotype, characterized by Hsp70 cytoplasmic sequestration of p53 protein, has been observed in several human cancers (undifferentiated neuroblastoma, retinoblastoma, colorectal and hepatocellular carcinomas, and glioblastoma). Moreover, clam hemocyte cancer is the only animal model thus far investigated where cytoplasmically sequestered wild-type p53 can be reactivated both *in vitro* and *in vivo* using both genotoxic and non-genotoxic therapies. Results suggest that mortalin-based cytoplasmic sequestration of wild-type p53 in cancerous clam hemocytes can be reversed by treatment with antineoplastic drugs also employed against similar human diseases and will result either in transcription based apoptosis when the nucleus is accessible or non-transcription-based apoptosis when nuclear access is blocked (Walker *et al.*, 2012).

Based on these data, leukemic clam hemocytes is regarded as novel and easily accessible *in vivo* and *in vitro* models for human cancers displaying a mortalin-based phenotype, and marine bivalves as the most relevant and best understood model currently available for experimental studies by biomedical and marine environmental researchers.

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