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Neoplasia in Mollusks: What Does it Tell us about Cancer in Humans? – A Review

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Abstract

Neoplasia – the abnormal growth of cells – is associated mainly with higher vertebrates (e.g., humans and mammals). However, neoplastic processes or cancers are ubiquitous among living organisms, with a high incidence of reported neoplasms in mollusks. Both benign and malignant cancers have been described in mollusks, but just two malignant neoplasms have raised scientific and industry concerns: gonadal neoplasia and disseminated or leukemia-like neoplasia. These cancers have been reported in either wildlife or captive populations; and as in humans, different causes have been suggested including genetic alterations, virus, retrotransposons, and pollutants. In this review, I give a general overview on neoplasia in mollusks with a focus on genes and molecular pathways involved in gonadal and disseminated neoplasia. Subsequently, I highlight some similarities between disseminated neoplasia and human cancers, particularly with leukemia, as well as the advantages of using mollusks affected by this disease as a model system to better understand cancer in humans. Finally, I discuss the feasibility of using mollusks to investigate tumorigenesis. As the field of marine genomics advances, I predict that comparative oncology will gain more attention in the years to come.

Keywords: Neoplasia; Bivalve mollusks; Gonadal neoplasia; Disseminated neoplasia; Transmissible cancer; Comparative oncology; Marine genomics

Introduction

Neoplasm is defined as the abnormal division of genetically modified cells that evade the normal regulatory controls of cell growth, and sometimes, have the capacity of invading and destroying other tissues or organs. Neoplasia or cancer can be either benign, which means that altered cells remain localized in a given organ and do not conquer other tissues; or malignant, which means that abnormally proliferating cells spread over other tissues, changing their normal function and terminating often in the organism's death [1]. Cancer arises from genetic alterations that produce dysregulated gene expression programs [2]; these alterations can be spontaneous, hereditary, or acquired as a result of exposure to mechanical (e.g., radiation), chemical (e.g., carcinogens) or infectious (e.g., oncoviral) agents [1,3].

After decades of rapid advances, cancer research has generated a rich and complex body of knowledge revealing that cancer is a disease involving dynamic changes in the genome. Several lines of evidence indicate that cancer in humans is a multistep disease comprising: sustaining proliferative signaling, evading growth suppressors, resisting cell death, enabling replicative immortality, inducing angiogenesis, activating invasion and metastasis [4]; more recently, two more hallmarks were added to define human cancers: reprogramming energy metabolism and evading immune destruction [5]. These "hallmarks of cancer" drive the progressive transformation of normal cells into highly malignant cells [4,5], and their holistic understanding have allowed tremendous advances in cancer diagnosis and treatment during the past decades. However, great efforts made worldwide to find effective drugs to eradicate cancer have not been successful, and this disease remains a major cause of morbidity and mortality in humans [6], but also in several higher vertebrate species [7].

The hallmarks used to define cancer in mammals [4,5] are difficult to recognize in non-mammalian animals (i.e., lower vertebrates and invertebrates) because tissues, organ system, cell types and metabolic reactions are different from their vertebrate counterparts [8]. For example, invertebrates possess a variety of blood vascular systems ranging from "open" to "closed" circulatory systems [9], as well as some animal groups, lack specialized cardiovascular systems (e.g., flatworms) [9], making extremely difficult to identify the induction of angiogenesis [8]. In addition, effective diagnostic tools to identify and confirm neoplastic diseases are lacking in non-mammalian animals [8], and also sometimes, pathologists are unable to discriminate between pseudo-neoplasia, neoplastic diseases, and other types of deregulated proliferation resulting from wound repair or infection (e.g., granuloma caused by mycobacterial or mucosal infection) [1].

It is clear that exist many challenges to report properly neoplasms in non-mammalian animals; nonetheless, increasing evidence suggests that neoplasms or cancers are widely spread through lower vertebrates and invertebrates [1,8,10-13]. The incidence of cancers across non-mammalian animals is not distributed equally among taxonomic groups. For instance, there are no reports of neoplastic diseases in poriferans and echinoderms, while reports of neoplasia are low in cnidarians and crustaceans, but are quite frequent in insects and mollusks [1,8,13]. Though this distribution is biased undoubtedly to animal groups with economic and scientific relevance (reviewed in [8]), the study of neoplasms in non-mammalian animals has revealed that many genes and molecular pathways involved in neoplastic diseases and metastasis are evolutionarily conserved with mammals and humans. Indeed, non-mammalian model organisms such as *Xenopus, Drosophila,* and *Caenorhabditis elegans* have contributed significantly to understand cancer in humans [14-19].

In this review, I present a general overview of the evidence and causes of neoplasia in mollusks and I review our current understanding of the molecular components underlying gonadal and disseminated neoplasia, highlighting examples of cancer-related genes between mollusks and humans. Subsequently, I describe similarities between disseminated neoplasia and leukemia, as an example of human cancer, and the advantages of using mollusks as sentinels for better understanding cancer in humans. Finally, I discuss the feasibility of using mollusks and comparative oncology approaches to understand tumorigenesis.

Neoplasia in Mollusks: Occurrence, Type and Etiology

Mollusca is the second most species-rich animal phylum with over 100,000 recognized species, including many species of valuable importance as food sources, disease vectors, and jewelry resources [20]. Since the first report of an abnormal cell proliferation and probable neoplastic disease of the hematopoietic system in *Crassostrea gigas* and *C. virginica* in 1969 [21], similar cases of neoplasia have been reported in other mollusks, including bivalves, gastropods, cephalopods and polyplacophorans [8]. External neoplasms can be easily recognized, but internal or systemic neoplasms are not. Histopathology has been the main approach for documenting and understanding neoplastic diseases [8], although modern methodologies, such as immunoassays, have also been used [22]. However given that neoplastic cells contain more DNA than normal cells, flow cytometry, ploidy and karyotype analyses have been recognized as important and accurate methods for identifying neoplastic cells in mollusks [23-25]. As reviewed by Newton and Lewbart [8], more than 400 cases of cancers in mollusks have been registered by the Registry of Tumors in Lower Animals (RTLA), with a high incidence in bivalve mollusks. Unfortunately, the RTLA database was closed in 2007 and we have no up-to-date reports of neoplasia in lower vertebrates and invertebrates. However, the incidence of neoplasia in these animal groups should have grown during the last decade.

In mollusks, most neoplasms have been reported as benign, largely due to the lack of evidence of anaplasia or mitotic figures, as well as metastasis (reviewed in [1,8]). Some example include adenomas [26,27], polypoid tumors [28], papillomas [29], mesenchymal tumors [30,31], and tumors located in pulmonary and pericardial cavities [32-35]. Nonetheless, a number of tumors with malignancy have been documented, for example, epithelial carcinomas [36,37], gill carcinomas [38,39], gliomas [40,41], gonadal neoplasia [42,43] and disseminated neoplasia [44,45]. These neoplasms are considered malignant tumors because show many of the "hallmarks of cancer" [4,5], including pleomorphic and undifferentiated cells; abundant mitotic figures; rapid and invasive growth; metastasis; and proliferative growth resulting in the death of the individual. In particular, gonadal and disseminated neoplasia is the most widespread molluscan cancers present in four continents and all oceans except the Arctic [46]. This paper does not pretend to give a comprehensive review on gonadal and disseminated neoplasms, which are the most widely studied neoplastic diseases in mollusks; however, some reviews on these diseases have been published elsewhere [47-49].

These two predominant neoplastic diseases occur commonly in bivalve mollusks (e.g., clams, mussels, cockles, and oysters) and affect several economically important species, sometimes at epizootic levels (reviewed in [48]). Gonadal neoplasia consists of small, basophilic, undifferentiated germ cells that multiply to completely fill most – if not all – gonadal follicles producing gonad atrophy and degeneration. Sometimes, the proliferation is so rapid and invasive that neoplastic cells are capable of metastasis after invading interfollicular connective tissue, body wall, epibranchial chamber, and genital ducts [49]. On the other hand, disseminated neoplasia is characterized by amplification of large, pleomorphic cells in the hemolymph – the circulatory fluid of mollusks [48]. These abnormal cells show a high frequency of mitotic figures and are anaplastic with similar features to vertebrate tumor cells [44,50]. These cells have been observed in the connective tissue of multiple organs, as well as in vessels and sinuses of the circulatory system [48,49]. Neoplastic cells disturb phagocytosis and apoptosis abilities causing displacement, compression, and necrosis, which altogether produce organ disruption, dysfunction of the immune system and systemic failure [48,49]. Despite the significant body of knowledge accumulated regarding disseminated neoplasia in bivalve mollusks [46,48,49], a clear consensus has not reached on the origin of the neoplastic cells. Some researchers argue that undifferentiated mesenchymal cells and differentiated vesicular connective tissue cells might be the source of origin, whereas others think that neoplastic cells have a hemic origin because they possess similar characteristics to granular or hyaline hemocytes, and there are first observed within the hemolymph (reviewed in [48,49] and references therein).These observations have led to researchers to call disseminated neoplasia as leukemia or hemic.

As cancer in humans, several etiological factors are postulated as responsible agents for neoplasia in mollusks. An infectious etiology has been proposed since neoplasia can be transmitted either by inoculation of cell-free hemolymph from neoplastic mollusks into healthy individuals [51-53] or by moving diseased animals from infected areas to diseases-free locations (i.e., co-habitation) [53-55], but these studies have not been reproducible and no infectious agents have been confirmed [55]. Another possible cause proposed has been retroviruses; this cause has been postulated based on electron microscopic observation of retroviral-like particles and the detection of reverse transcriptase activity (RT) in neoplastic cells [56-58]. However, virus isolate or retroviral sequences have not been obtained and although some studies have found high levels of RT activity in neoplastic cells, electron microscopy observations have failed to show the presence of viral particles (i.e., retroviruses) in the affected cells [59-61]. Given that RT assays are not specific for detecting retroviruses [62], the RT activity cannot be considered as conclusive of infection by a retrovirus and an endogenous source of reverse transcriptase, such as endogenous retroviruses or mobile DNA elements, rather than exogenous retroviruses, has also been proposed [60,61].

The first clues on the participation of DNA mobile elements in the development of neoplastic diseases, particularly in disseminated neoplasia, was based on the up-regulation of transposase (DNA transposon) and polyprotein (LTR retrotransposon) genes in hemolymph cells from clams with high levels of the leukemia-like neoplasia [63]. In another study, the Piggy/Bac transposable element was found to be over-expressed in neoplastic hemocytes in oysters [64]. Mobile elements are DNA repeat sequences that can move through the genome and are classified into two classes according to whether their transposition intermediate is DNA (i.e., transposons) or RNA (i.e., retrotransposons) [65]. Sometimes retrotransposons carry along adjacent DNA sequences in a process called 3' transduction that can scatter genes and regulatory sequences across the genome, representing another mechanism by which cells acquire new mutations during tumorigenesis in a wide range of human cancers [66]. More recently, a novel retrotransposon – named Steamer – was identified from neoplastic hemocytes with high RT activity from the soft-shell clam (*Mya arenaria*) [67]. The Steamer element belongs to the gypsy/Ty3 retrotransposon family, encodes a single protein with similarity to retroviral Gag-Pol proteins, and interestingly, shows high levels of mRNA expression in diseased hemocytes and its expression is strongly correlated with the status of the disseminated neoplasia [67]. Furthermore, the DNA copy number is extremely high in neoplastic hemocytes, suggesting that Steamer is an extraordinary active retrotransposon that undergoes massive transcription and retrotransposition in neoplastic cells, and may initiate or accelerate the course of disseminated neoplasia in clams [67]. Steamer was originally found in the soft-shell clam (*M. arenaria*); however, this retrotransposon has also been identified in other clam species, mussels and cockles [68,69].

In addition to infectious, viral and DNA mobile element causes, environmental carcinogens were also proclaimed as trigger factor of neoplasia. This was postulated based on the high prevalence of infected mollusks in areas with high concentrations of pollutants in comparison to less or non-polluted areas [48,70-72], but this trend is not a golden rule and a lack of correlation between disease prevalence and environmental contamination has also been reported (reviewed in [48]). Similar conclusions can be made based on lab-experiments involving exposure of mollusks to pollutants. These studies have shown a positive correlation between carcinogens and the prevalence of diseases [73], but also a low level of neoplastic cells and no difference between treatments and control groups (reviewed in [48]). The potential role of biotoxins associated with harmful algal blooms [46] and environmental stressors, other than chemical pollutants [48], have also been postulated as etiological factors; however, as environmental contaminants, there is no clear correlation between these factors and the exacerbation of neoplasia in mollusks. As cancer in humans, a single etiological cause for neoplastic diseases has not been identified, and a relationship between the prevalence of neoplasia and infectious agents, environmental carcinogens, biotoxins, and other environmental stressors has not been proved. Therefore, the occurrence of neoplasia in mollusks seems to be a complex multifactorial process.

Genes, Molecular Pathways and Genetic Basis of Neoplasia in Mollusks

Regardless of the possible etiological factors, neoplasm formation ultimately has a genetic basis that can be either direct or indirect in nature. Several studies have investigated possible molecular mechanisms that underlie gonadal and disseminated neoplasia. However, most studies have been focused on disseminated neoplasia, and gonadal neoplasia has been neglected, with very few available studies. These studies have confirmed the presence of activated oncogenes in gonadal neoplasms [74], have identified differentially expressed genes in gonadal tumors that are involved in biosynthetic activities related to cell growth and proliferation [75], and have shown aberrant gene expression of ribosomal protein S19 in germinomas [76]. These results suggest that several molecular and genetic mechanisms are involved in this disease, but more studies are needed to better understand the molecular basis of gonadal neoplasia.

Many genes and molecular pathways involved in human cancers are evolutionarily conserved in mollusks. These genes have been investigated in order to understand their association with neoplasia in mollusks, particularly in disseminated neoplasia. Most studies have focused on the p53 superfamily. In humans, p53 superfamily comprises three members (p53, p63 and p73), being the tumor-suppressor p53 gene of which has received considerable attention because of the fact that it is mutated in approximately 50% of all cancers and plays important role in protecting cells against DNA damage and cellular stressors [77]. In addition, each member of the human p53 superfamily produces different protein

isoforms that play critical roles in the regulation of biological processes and their abnormal expression contributes to tumorigenesis [78]. In mollusks, p53 superfamily coding sequences have been characterized for clams [79-81], mussels [82-84], cockles [85], oysters [86], and gastropods [87]. Interestingly, in some species, various isoforms have been detected, and these isoforms seem to arise from alternative splicing, as regions that overlap between different proteins from the same species are 100% identical [79,80,83,84]. Examination of neoplastic hemocytes, using expression profiles and antibodies to p53 family members, has shown differential up-regulation of p53 gene in neoplastic cells compared with healthy ones [22,79,85,88-90]. Likewise, p53 and the isoform called ΔNp63/p73-like, due to the similarity with ΔNp73 of mammals, were also up-regulated in neoplastic cells compared to normal cells in the mussel *Mytilus trossulus* [22]. Furthermore, single nucleotide polymorphisms (SNPs) analysis in a fragment of the p53 gene has shown specific genotypes that are in strong association with late-states of the disease in mussels [91,92].

The functional inactivation of p53, which is a frequent event in human cancer cells, involves the interaction between p53 and mortalin (a Hsp70 family member) through a phenomenon known as cytoplasmic sequestration. This phenomenon contributes significantly to tumorigenesis, and the up-regulation of mortalin is responsible for tethering p53 protein in the cytoplasm in many human carcinomas [93]. In a similar way in *M. arenaria*, mortalin is over-expressed in neoplastic hemocytes [89,90,94], and the binding between mortalin and p53 produces the accumulation of p53 protein in the cytoplasm instead of the nucleus, as occurs in normal hemocytes, suggesting the inactivation of p53 transcriptional activation and apoptotic functions [79,95,96]. It has been shown that the p53-mortalin complex can be disrupted by the inhibition with MKT-077 (a cationic mortalin inhibitor), which resulting in translocation of some p53 molecules to the nucleus [95] and ultimately in apoptosis of the neoplastic cells [94]. These results suggest that molluscan mortalin-based cytoplasmic sequestration of wild-type p53 protein in neoplastic cells employ similar molecular mechanisms as in human cancer cells [97]. In vertebrates, p53 is regulated by a negative auto-regulatory feedback with MDM2 (a proto-oncogene) in order to avoid excessive p53 activity [98], and the over-expression of MDM2 gene produces the inhibition of p53 function causing the development of many human carcinomas [99]. Although the participation of the *M. arenaria* p53 gene in a regulatory feedback loop with human MDM2 gene has been supported by structural comparisons under non-stressed conditions, the *M. arenaria* p53 gene is unable to rescue completely p53 function in the human p53-null cell line likely due to primary structure and/or post-translational modifications [100], Interestingly, a protein homologous to MDM2 that bind p53 has been identified in *M. trossulus*, and its expression level was directly correlated with those of the p53 gene in hemocytes from diseased and healthy mussels [101], suggesting that the regulatory mechanism between p53 and MDM2 is also present in mollusks. Despite these promising results, future studies are needed to better understand whether the regulation between p53 and MDM2 in neoplastic diseases in mollusks is similar to humans.

In addition to p53 superfamily, proto-oncogenes of the RAS family have been intensively studied because they are often implicated in many human cancers [102]. The RAS proteins are involved in signaling cascades responsible for cell growth, differentiation, and survival, and have been reported that activating mutations in the RAS genes themselves or the alterations in upstream or downstream signaling components contribute significantly to several aspects of the hallmarks of cancer [103]. Given that RAS genes are evolutionarily conserved over animals (including mollusks), RAS homologous genes have been characterized in some molluscan species [82,85,104,105]. In addition, RAS gene over-expression has been observed in disseminated-affected animals compared to non-affected ones [85,104], and gene mutation have also been detected [85,105], which may indicate genomic instability in molluscan populations with high prevalence of neoplasia. Other members of the RAS family, including GTPase, RAS-Rho, RAS C3, c-jun and c-myc, have also been characterized in *M. arenaria*, but only up-regulation of c-myc gene has been observed in neoplastic hemocytes [106].

Recent studies involving the use of suppressive subtracted hybridization (SSH) and transcriptomics have identified many genes expressed preferentially in neoplastic cells in oysters *(Ostrea edulis*) [64] and clams *(M. arenaria*) [107]. As expected, most of those genes have not been reported in association with cancer, but some of those genes are in agreement with observation in human cancers. In particular, molecular pathways involved in regulation of cell cycle and genes related to human leukemia, among others, are the most highly expressed during development of disseminated neoplasia [64,107], which give some support to the link between these two diseases.

In the last decade, tremendous advances have been performed to understand the molecular basis of molluscan neoplasia, especially in disseminated neoplasia. Remarkably, these advances have revealed similar molecular mechanisms that underlie leukemia and other human cancers. These results make bivalve mollusks an emerging animal model for understanding human cancer.

Neoplasia in Mollusks: What Can Teach us About Cancer in Humans?

Remarkable similarities have been found between disseminated neoplasia and leukemia. These similarities rely on the fact that disseminated neoplasia is characterized by rapidly dividing circulating tumor cells that ultimately invade connective tissue and can kill the host [108], and also on the fact that neoplastic cells resemble human leukemia cells because show a variable number of chromosomes, alteration of apoptosis, abnormal expression of cancer-related genes (i.e., CDC42, CDC7, SMC3, PPA2, THAP, TRIB2, cyclin A), and similar molecular mechanisms involving the participation of RAS genes [49,64,107,109]. Furthermore, neoplastic cells (in disseminated neoplasia) are an example of transplantable

tumor that is not hampered by two of the limiting factor for the applicability of mouse transplantable tumor models: (i) histocompatibility is not an issue because mollusks have only innate immunity [110]; and (ii) tumor cell inoculation is not subcutaneous as it is in mice, but occurs in an anatomically appropriate site for the tumor, which is the mollusk circulatory system. These characteristics – just to name a few – make disseminated neoplasia and mollusks an excellent *in vivo* and *in vitro* model system for understanding human cancers.

One striking difference between human cancer and disseminated neoplasia is that the latter is transmissible. Transmissible tumors are extremely rare and – by definition – are those that have evolved the ability to infect other individuals through direct transfer of cancer cells, suggesting that they are clonal in origin [111]. Direct transmission of cancer cells is quite rare and the phenomenon has only been observed in two mammal species: domestic dogs and Tasmanian devil. The best well-known examples of transmissible tumors are the canine transmissible venereal tumor (CTVT) and the Tasmanian devil facial tumor disease (DFTD), being the former not necessarily lethal, while the latter has driven the Tasmanian devil population near to extinction (reviewed in [111]).

As mentioned above, analysis of neoplastic cells in *M. arenaria* has revealed a dramatic amplification in the DNA copy number of the Steamer retrotransposon [67]. Strikingly, analysis of the Steamer integration sites, mtDNA, SNPs, and microsatellites have demonstrated that the genotypes of neoplastic cells collected from multiple locations, along 1,000 km extension, were nearly identical to one another, and all cases differed considerably from their transient host, which make concluding that disseminated neoplasia is spreading as a clonal, transmissible cell derived from a single original clam [112]. Recently, transmission of disseminated neoplasia was investigated in three other bivalve species, including mussels *(M. trossulus)*, cockles (*Cerastoderma edule*), and golden carpet-shell clam (*Polititapes aureus*), and neoplasias in all three species were attributable to independent transmissible cancer lineages [69]. In mussels and cockles, the cancer lineages are derived from their respective host species, as has been shown in *M. arenaria* [112]; however, unexpectedly, cancer cells in the golden carpet-shell clam are all derived from *Venerupis corrugata* – another clam species living in the same geographical area – suggesting cancer cells can also be transmitted between different species [69]. This study suggests that direct transmission of cancer among marine animals may be much common that previously thought.

These results are astonishing and suggest that neoplastic cells are contagious themselves with the capacity of spreading through seawater from one bivalve species to another. The lack of an adaptive immune system in mollusks to recognize foreign intruders might explain why transmissible cancer cells are able to spread within and across species. Transmission of cancer cells in the ocean might be seen as an extreme version of metastasis – one of the hallmarks of cancers [4,5], and the example of cross-species transmission constitutes a distinct class of infectious agent that shows the remarkable ability of cancer tumors to acquire new phenotypes to promote their own survival and propagation.

The fact that cancer cells may be able to cross the bounds of their incipient host to infect another species makes it a greater medical threat, raising questions about the implications for cancer transmission in humans. Human-to-human transmission of cancer is exceedingly rare; however, transmission and survival of cancer cells has been reported during organ transplantation, pregnancy, and parasitic infections, when the human host immune system has been heavily compromised [113,114]. Therefore, this fact is also important giving that some of mollusk species that show disseminated neoplasia are a constant food source for humans [48]. The mechanisms of cancer cell transmission and metastasis remain unclear, and bivalve transmissible cancers (i.e., disseminated neoplasia) provide a new model system to understand cancer transmission and host response [114]. Thus, I can see a very promising research avenue using mollusks as models to investigate the clonal transmission of cancers and the metastasis process in general.

How Feasible are Mollusk as Naturally Occurring Model System for Cancer Research?

There is no discussion about the complexity of cancer and the development of cell and animal models that accurately depict human tumorigenesis remains a major goal of cancer research. Our understanding of this biological process has led to the development of increasingly comprehensive experimental models, from *in vitro* tumor cell lines to *in vivo* murine models, to non-mammalian model systems [14-19,115]. For example, one of them is mouse where tumors are induced or invasive human cell lines that are transferred to immune-deficient mice; however, the use of mice is strictly regulated and though cultured cancer cells often mirror their behavior *in vivo*, not always is the case [116]. Other model systems are *Drosophila* and *Caenorhabditis*, however, these are not good enough for studying cancers that involve mortalin-p53 complex due to mortalin does not exist in these models, adult somatic cells in these organisms do not divide, and furthermore, p53 homologs in these species are highly derived from their human counterparts proteins [96]. Although these experimental models have several advantages [14-19,115], also have limitations such as the absence of key molecular components associated with cancer in humans and also tumors are not spontaneously generated therefore must be induced.

In this context, we have to look for models where naturally occurring cancers are present, given that they grow in an intact immune system allowing the complex interactions between the tumor and the immune system to develop naturally. Thus, tumors are susceptible to the selective pressure of spontaneous immunity, leading to genetic instability that finally faithfully reproduces human cancers [115]. Here is where the comparative oncology approach offers a unique and strong opportunity to learn more about the basic cancer

mechanisms as well as their treatment. This approach has successfully been applied using dogs, as naturally-occurring cancer model system [117].

Some authors have postulated to mollusks as naturally occurring cancer model system for understanding human cancer, mainly because: (i) this new cancer model is more similar to an out-breeding human clinical population than are those generated from inbreed mouse strains that have intentionally exposed to known tumor viruses or cells; (ii) homologs for both human p53 and mortalin genes have been cloned; (iii) considerable information on the functions of the p53-mortalin complex and cytoplasmic sequestration phenomenon exists; (iv) protocols for maintaining neoplastic cells *in vitro* and for amplifying them *in vivo* have been developed; and finally, but most importantly, disseminated neoplasia is a naturally occurring cancer in mollusks [94-96,118]. These features give support for using mollusks and disseminated neoplasia not only to understand human cancers that involve cytoplasmic sequestration of wild-type p53 such as undifferentiated neuroblastoma and colorectal adenocarcinoma, but also other cancers, for example, leukemia [94-96].

Although growing evidence suggests the feasibility of using mollusks as model system to understand human cancer, now the question that arises is: are there enough resources to become mollusks as a naturally occurring cancer model system? As mentioned earlier, it is clear that disseminated neoplasia and human cancers share numerous features such as genetic characteristics, histopathological appearance, and biological behavior, to name just a few. Although we do not known much about molluscan immune system, the rate at which new, low-cost, and high-throughput –omic technologies are being developed has allowed an expansion in the number of studies aimed at gaining a better understanding of immune system and disease processes in mollusks [110,119]. Thanks for the tremendous advances in the marine genomics field; we have now not only valuable transcriptomic, but also genomic resources (reviewed in [119]) that can be applied to understanding human cancer. Indeed, draft genomes are already available (or in progress) for some of the species that disseminated neoplasia and other types of cancers have been described. For example, *Crassostrea gigas* [120], *Mytilus galloprovincialis* [121], *Mya arenaria* (ongoing genome project; GenBank: PRJNA221639), and *Haliotis discus hannai* [122]. Thus, in the near future, I hope to see more studies looking at differentially expressed genes between neoplastic and normal cells in different molluscan species. Also, it would be interesting to see how epigenetic marks in affected and healthy mollusks are decoupling the interactions between epigenetic modifications and environmental triggers. With the explosion of knowledge on genes and molecular pathways that will emerge in the coming years, I foresee that our understanding of cancer will be greatly improved and this will strength mollusks species affected by disseminated neoplasia as good naturally occurring model system for cancer research, drug design, and treatment.

Conclusion

Since the first report of a neoplastic disease in mollusks, our knowledge on molluscan neoplasia has increased mostly in the etiological, molecular and genetic aspects. Especially, the etiology and molecular mechanisms underlying disseminated neoplasia (or leukemia-like neoplasia) are now well known. Thus, bivalve neoplasia has been proposed as a naturally occurring model system that could be applied to understanding human cancers. Therefore, I predict that mollusk species affected by disseminated neoplasia would be a good as naturally occurring cancer model system for cancer research. In addition, thanks to advances in marine genomics and comparative oncology, the use of mollusks as sentinels for understanding cancer will be strengthened. I foresee that by working with mollusks and applying comparative oncology approaches, researchers from human and marine biology can combine scientific findings to understand cancer and translate them into novel therapies to benefit both human and mollusks.

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