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REVIEW

Tumors in invertebrates

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Abstract

Tumors are ectopic masses of tissue formed by due to an abnormal cell proliferation. In this review tumors of several invertebrate species are examined. The description of tumors in invertebrates may be a difficult task, because the pathologists are usually inexperienced with invertebrate tissues, and the experts in invertebrate biology are not familiar with the description of tumors. As a consequence, the terminology used in defining the tumor type is related to that used in mammalian pathology, which can create misunderstandings in some occasions.

Key Words: tumors; insects; molluscs; invertebrates

Introduction

In invertebrates, in addition to spontaneous tumors, there are those due to hereditary phenomena as well as those due to a wide range of environmental factors. Among the latter are those induced by chemical toxins, physical stress (heat, salinity, UV), biological infections (bacteria, virus, parasites), and potentially carcinogenic substances.

The literature in this area has been discontinuous. In the early twentieth century it was thought that tumors were present only in vertebrates (Teutschlaender, 1920; Engel, 1930). Today, the situation has changed and morphological and molecular biology investigations have provided a better understanding of the nature of these abnormal growths. The data obtained from these studies were mainly obtained from insects such as *Drosophila*, and molluscs.

Insect tumors

As far as *Drosophila* is concern, Harshbarger and Taylor (1968) summarized the studies performed on tumors in the fruit fly and other insect species.

In *Drosophila,* tumors are classified as malignant or benign. More than 50 % of the proteins that provoke human diseases including, tumors, have orthologues in *Drosophila* (Gonzales, 2013). Mutations that promote excessive and non- controlled growth in tissues and cells of the flies that provoke their death are considered as tumor mutants. By studying recessive-lethal tumor mutations six non-allelic mutants were found that develop malignant neuroblastomas in the adult opitc centers called *Drosophila lethal giant larvae (l(2)gl)*. Three malignant blood tumor mutants were described from defects in the hematopoiesis and signed as *l(1)mbn, l(2)mbn* and *l(3)mbn.* The hemocyte mutant population *l(1)mbn* is characterized by the same cell types present in the wild-type and having the same functions, but in the mutant strain, the number of hemocytes is larger and has a different differentiated cell types themselves. This last phenomenon is not present in the mutants *l(2)mbn* and *l(3)mbn*. In addition, the blood-forming organs, while not differing morphologically from the wild type, greatly increase up to 300 - 400 times (see for review, Gateff, 1978). The fruit-fly melanotic tumor mutants show a neoplastic growth at different times during larval devolpment (Woodhouse *et al*., 1998).

In *Drosophila* and many other arthropods a characteristic response against aberrant tissues and parasitic challenges is melanotic encapsulation. The pigment appears on the surface of the cuticle where the pathogen breaches the integument, as well as on and very near the surfaces of organisms that have invaded the hemocel of the host (Fig. 1) (Christensen *et al*., 2005). Melanin is derived from the oxidation of monophenols and diphenols and the ensuing polymerization of their respective orthoquinones, a cascade of reactions initiated by tyrosinase or phenoloxidase (PO) and frequently involve the participation of blood cells (hemocytes).

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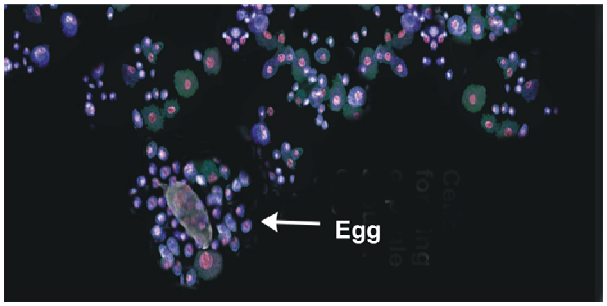
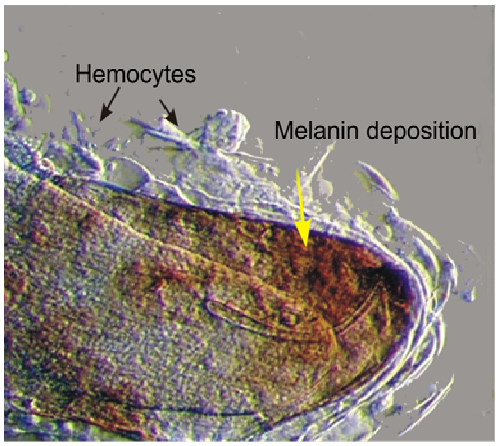
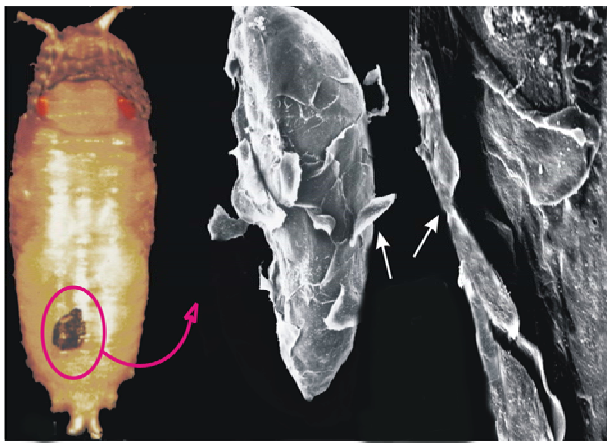
Ovipositor of parasitoid inserted into Drosohila larva. Wasp egg then passes into the body cavity of the host.



Parasitoid egg detected as foreign, causing hemocyte proliferation, aggregation, and melanotic encapsulation



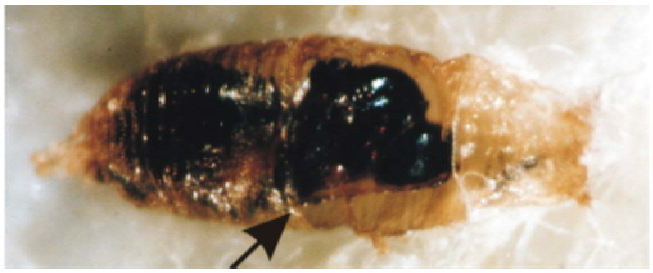
Early stage of melanotic encapsulation of parasitoid egg following it's detection as foreign.



Melanotic capsule in host pupar.

Fully formed melanotic capsule showing layers of adhering hemocytes.

Host develops with melanotic capsule containing dead parasitoid within abdomen.



Developing parasitoid

If host response is unsuccessful, or suppressed, wasp consumes host tissues and develops to become an adult.

Fig. 1 Formation of melanotic encapsulations in *Drosophila* in response to infection. In some genetic strains, aberrant tissues apparently provoke similar responses.

In insects, activation of PO requires a limited proteolysis by a serum protease that itself is activation by upstream proteases (Fig. 2). The polymerization of melanin proceeds via repeated electron transfer reactions involving redox-active melanogenic intermediates that, alone or in combination with reactives intermediats of oxygen (ROI) and nitrogen (RNI), constitute a potentially formidable cytotoxic system. Melanotic responses generally are very site-specific, and do not provoke undesirable systemic activation in the host's open circulatory system. Substances other that tyrosine

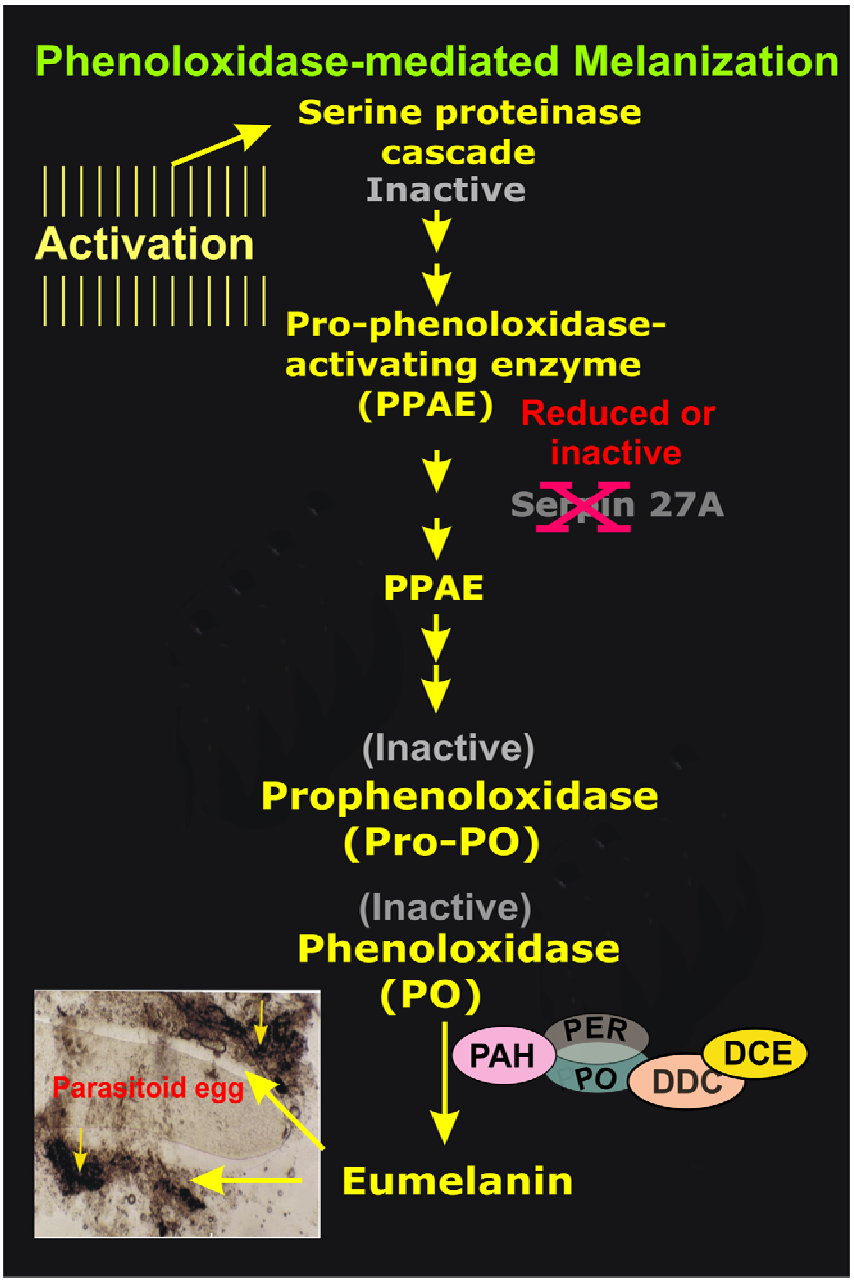


Fig. 2 Early stage in melanotic encapsulation in *Drosophila* involves conversion of inactive prophenoloxidase to phenoloxidase, a cascade of responses mediated by serine specific proteinases, and by the diminished or inactivation of Serin 27A. Some enzymes involved include the following: DCE, dopachrome conversion enzyme; DCT, dopachrome tautomerase; DDC, dopa decarboxylase; MPO, myeloperoxidase; PAH, phenylalnine hydroxylase; PER, peroxidase; PO, phenoloxidase.

and dopa that can lead to melanin precursors include dopamine and tryptophan (Sugumaran, 2002). Thus, the polymerization of melanin proceeds via repeated electron transfer reactions involving redox-active melanogenic intermediates that, alone or in combination ROI and RNI, constitute a potentially formidable cytotoxic system.

Melanotic tumors are heritable, benign growths which result from encapsulation and melanization of certain host tissues by hemocytes (Sparrow, 1978). In homozygous Black Cell/Modulo mutants tumors appear in late second-stage tumors larvae, several days before the flies die as prepupae. The melanotic material was found either in the

hemolymph or attached to the viscera (Nappi *et al*., 1994). Mutations of *wizard* and *dappled* genes are involved in the melanotic tumor phenotype (Rodriguez *et al*., 1996). Furthermore, the overgrowth of neurogenic mutants such as *Notch* shows the conversion of epidermal cells to neuroblasts leading to a phenotype in which there is an increase of nerve cells. The same situation was observed for tumor mutants of the ovary. A higher cell proliferation was detected in comparison to the female germ line than in the male germ line (Watson *et al.,* 1994).

The intracellular JAK/STAT pathway plays a crucial role in the *Drosophila* immune responses (Lemaintre and Hoffmann, 2007). Hyperactivation of this pathway provokes epithelial and hematopoietic tumors in these flies (Harrison *et al.*, 1995; Amoyel *et al*., 2014), a situation referred to by some investigators as fly leukemia (Corwin and Hanratty, 1976). The *Drosophila* JAK/STAT signaling has a reduced genetic complexity with a single JAK gene called *hop-scotch* (*hop*, similar to *Jak2*) and a single STAT gene called Stat92E, homologous to *Stat3* and *Stat5* (reviewed by Arbouzova *et al.,* 2006; Amoyel *et al*., 2014). This relative reduced complexity has led to *Drosophila* being adopted as a useful model for studying the role of JAK/STAT signaling in tumorigenesis. Two major transforming mutations were identified in the hop locus. Tumorous-lethal (*Tum-l*) is a dominant, temperature-sensitive mutation that leads to overproliferation of hemocytes and formation of melanotic tumors (Luo *et al.,* 1997). A second mutation of the hop locus, caused by E695K substitution, was demonstrated and well characterized (Hanratty and Dearolf*,* 1993; Minakhina and Steward*,* 2011; Amoyel *et al*., 2014). Several independent groups have shown that the hop Tum-l melanotic phenotype is due to hyperactivation of JAK/STAT signaling as heterozygosity of *Stat92E* suppresses the lethality and the tumorigenic phenotype associated with both *hopTum-l* and *hopT42*. These melanotic tumors with dysregulated JAK/STAT signaling are invasive and correlate with lethality (Luo *et al*., 1997; Amoyel *et al*., 2014). Both hop locus mutations result in a hyperphosphoryation of Stat92E, leading to increased association of Stat92E with specific DNA promoter sequences (Amoyel *et al*., 2014).

Although the role of JAK/STAT activation in oncogenesis has been well defined, the molecular targets that mediate transformation still need to be identified. The simplified structure of the JAK/STAT pathway in *Drosophila* makes this fly a useful model for understanding the mechanism in tumor formation, and for the identification of relevant targets of JAK/STAT transcriptional activity.

With regard to the possible presence of tumors in other insects, Brun (1925) shows the presence of a brain tumor in an ant and suggested that it was due to a brain injury. In addition, spontaneous tumors were reported in *Leucophaea maderae* (Sharrer, 1945; Matz, 1961) and *Locusta migratoria* (Matz, 1961). In both species the experimental separation of a nerve induces tumors in organs innervated by this nerve. Tumors in the salivary glands of *Periplaneta america* were also observed by either by tying or removing the salivary duct (Sutherland, 1963, 1964).

As reported before chemicals, physical stress, hormones and biological infections can cause tumors. In insects various experiments are performed (see for review, Harshbarger and Taylor, 1968). In *Drosophila,* several juvenile hormones, such as farnesyl methyl ether-FMS, dodecyl methyl ether derivative and farnesoate provoke an increase in the melanotic tumor (Bryant and Sang, 1969). Furthermore, thymidine analogues cause tumors in the somatic cells of *Drosophila* (Rizki and Rizki, 1973).

Molluscan tumors

Various authors have studied tumors or tumor-like growths in molluscs mainly in shellfish, such as Gastropoda, Bivalvia and Cehalopoda (Pauley, 1969; Michelson and Richards, 1975).

In Gastropoda, a benign tumor of epithelial origin was described in the slug *Limax flavus* (Szabò and Szabò, 1934). In *Biomphalaria glabrata* tumors were formed in the lung cavity (Richarrds, 1973), buccal gland, and mantle cavity (Michelson and Richards, 1975). The tumors of the lung cavity were observed in the walls presenting variable dimensions to fill the cavity. The histology of the second tumor revealed that it emerges from the buccal gland region and then expanded. The glandular cells appeared densely packed of granules, and numerous mitotic figures were observed. Ultimately, there was a reduction of the cavity with a lining epithelium rich in mitotic figures. In *Helix pomatia* two tumors were describe, one on the dart sac and the other on the lower part of albumin gland (Nolte, 1962). In *Achatina fulica,* a pedunculate tumor was found on the dorsal surface of the head near the inner side of the right tentacle (Michelson, 1972).

In Bivalvia the first studies were performed on the freshwater mussel *Anodonta cygnaea* where a tumor load of the mantle was described (Willians 1890; Collinge, 1891). A benign tumor of mesenchymal character which had its origin in the pericardium was found in the *Ostrea virginica* (Smith, 1934). In the freshwater mussel *A. californiensis* a tumor-like was found in the foot (Pauley, 1967). In hard shell clams *Mecenaria* spp. a gonadal tumor was detected and was seen as proliferations of atypical germ arising from the germinal epithelium of the follicles (Hesselmann *et al*., 1988). A gonadal tumor was also provoked by herbicides in the softshell clam (*Mya arenaria*) and in *Mecenaria* spp. (Van Beneden, 1997). In *Mytilus galloprovincialis* it has been observed that the development of gonadal tumor is related to the months of the year. Indeed, this tumor is present between april and June, while during the rest of the year the gonads show a normal tissue (Alonso *et al*., 2001). A mesenchymal tumor characterized by a disorganized structure, with cells in mitotic activity and other in necrotic state, was observed in the mantle of the mussel *Modiolus difficilis* (Usheva and Odintsova, 1997).

Tumors in other group of invertebrates
Porifera

As far as we know, there are no data on tumors in sponges, but many anti-tumor active compounds have been found, including sesquiterpene quinones, hydroquinones, and the ctenotoxin-2. These compounds present in the sponges of the order Dictyoceratida possess cytotoxic and antiproliferative properties (Gordaliza, 2010) on human cancer cells inhibiting the mitotic separation and provoking the depolymerization of actin filaments (Kim *et al*., 2013).

Platelminta

Sponteneous tumors were described in three planarian species: *Dugesia tigrina*, *D. etrusca* and *D. ilvana* (Goldsmith, 1939; Stephan, 1962; Lange, 1966). In *D. dorotocephala,* Hansen *et al.* (1993) considered the injuries to be caused by polychlorinated biphenyls of cadmium. The tumor protein 53, which is a transcription factor that regulates the cell cycle, plays a role in tumor suppression. It is interesting to note that Planaria have a single *p53* family member, *Smed-p53* (Pearson and Sánchez Alvarado, 2010).

Arthropoda (excluding insect)

As for other arthropods the data relating to the tumors are scarce. A tumor-like mass was found in *Limulus polyphemus* (Hanstrom, 1926). The growth looked like a chitinous foreign body located near the anterior end of the brain. By examining the presence of tumors in decapod crustaceans Vogt (2008), noted the low incidence of this event, particularly when compared with other invertebrates, fish and mammals. According to the author this behavior seems to be due to some peculiarity of these animals related to their detoxification pathways, their immune system and specific mechanisms to ensure that their integrity of stem cells during their life.

Concluding remarks

Despite the difficulties of diagnosis and terminology, histological and molecular biology studies have proved the existence of tumors in invertebrates. Tumor mutant genes have been detected and the pathways, such as the JAK/STAT, defined in *Drosophila*, provide a framework comparable to that seen in mammals. Invertebrates also possess tumor suppressor genes. Three in *D. melanogaster*: *lgl* involved in the suppression of the development of neuroblastomas and imaginal disc tumors (Opper *et al*., 1987), *scribble* whose mutation causes aberrant cell shapes and loss of the monolayer organization of embryonic epithelia (Bilder and Perrimon, 2000) and the QM homolog (pDQM pDQM-7A1 and-2B1) (Nguyen-Yue 1997). Other two tumor suppressor genes were found. One, in the shrimp *Penaeus japonicus*, *i.e.,* the QM gene (designated as PjQM) that is involved in the up-regulation of virus-resistant shrimp (Xu *et al.*, 2008) and the other, the QM-like gene in disk abalone *Haliotis discus discus* plays a defensive role against pathogenic infections (Oh *et al*., 2010).

Overall, despite a simplified framework compared to that of mammals, invertebrate models of tumor development provide a valuable aid in our understanding of how various organisms successfully detect aberrant cells and prevent proliferation.

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